Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-020-09489-3

RESEARCH ARTICLE

Aflatoxicosis in cattle: clinical findings and biochemical alterations

Magdy M. Elgioushy[1] · Shimaa A. Elgaml[2] · Mohamed M. El-Adl[3] · Ahmed Medhat Hegazy[4] · Emad A. Hashish[2]

Received: 23 November 2019 / Accepted: 27 May 2020

Abstract

Aflatoxicosis is a serious health condition resulted from aflatoxin (AF)-producing fungi. Major health threats resulted from AFs and reflect on the livestock industry with great economic losses. There are limited scientific evidences concerning the AFs in ruminant, therefore it is important to evaluate AFs health hazards in cattle. Here, we investigate biochemical, oxidative stress, and postmortem changes associated with unexpected acute bovine aflatoxicosis. Seventy-two cattle were suffered from aflatoxisocis. Depression and inappetence were predominant clinical findings of the diseased animals. Analysis of feedstuffs revealed presence of aflatoxin B1 (AFB1). The AF-intoxicated animals showed a significant increase in alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), serum creatinine (SCr), catalase (CAT), and malondialdehyde (MDA) levels. Moreover, a significant decrease in total protein (TP), magnesium (Mg), and reduced glutathione (GSH) were also seen. Hepatomegaly, enlarged gallbladder as well as congestion of the intestine and kidney were observed. This study elucidates the critical and constructive measurements needed for the prevention of the AFs hazardous effects to livestock for the future control of AF outbreaks. Conducting series of diagnostic assays reflect the marked health condition alterations in the biochemical and antioxidant status of the AF-intoxicated cattle.

Keywords Aflatoxicosis · Biochemistry · Cattle · Feed poisoning · Oxidative stress · Postmortem changes · Toxicity

Abbreviations

AFB1	Aflatoxin B1
AFs	Aflatoxins
A/G	Albumin/globulin ratio
ALT	Alanine amino transferase
ALP	Alkaline phosphatase
AST	Aspartate amino transferase
Ca	Calcium
CAT	Catalase
GSH	Reduced glutathione
MDA	Malondialdehyde
Mg	Magnesium
NO	Nitric oxide
P	Phosphorus
SCr	Serum creatinine
TLC	Thin layer chromatography
TP	Total protein

Responsible editor: Lotfi Aleya

✉ Emad A. Hashish
emadhashish@vet.zu.edu.eg

[1] Department of Animal Medicine, Faculty of Veterinary Medicine, Aswan University, Aswan 37916, Egypt

[2] Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

[3] Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

[4] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Aswan University, Sahari, Airport Way, Aswan 81528, Egypt

Introduction

The incidence of contaminated food by aflatoxins (AFs) is reported to occur in African and European countries affecting both of the animal and human food stuff (Battilani et al. 2016). It is considered as a serious problem in Africa due to the lack of some control measures during several steps of handling, transportation, and processing of the agricultural stuff. These information were supported by a research in Tanzania about sunflower seeds and another study in Egypt which reported AF contamination in sugarcane (Stepman 2018). In this regard, the adverse effect on the human and animal health is reported with a significant impact on the economy and society (Sarma et al. 2017). Social scientists play a great role to scale-up the effect of research in Africa aiming to afford possible

Published online: 27 June 2020

solutions to reduce the health and food safety impact and thus ensure enough awareness, plus the affordability, and accessibility to be systematically assessed (Stepman 2018).

Although there is an improvement in the awareness of the occurrences of mycotoxin contamination in silages and the other forage crops, still there are limited information which hinder the proper assessing of the hazardous effect due to the exposure to mycotoxin in cattle and other ruminant species. Several overlapping factors play a major role behind the limited availability of information concerning mycotoxin and their impact on ruminants; these factors incriminated to the complexity of designing specific animal clinical trials due to the confounding effects of the toxin itself, animal species, the type of food, plus the related environmental factors, and the presence of modified mycotoxins that might exists (Gallo et al. 2015). There are several factors that might enhance the incidence of aflatoxicosis. Generally, the temperature and relative humidity will build a suitable atmosphere which is favorable for the proliferation and growth of *Aspergillus* spp. (Kamika and Tekere 2016). Beside the previously mentioned climatic conditions, other socioeconomic factors might enhance the incidence of AF contamination; these factors include (a) wide spread of the informal marketing systems, (b) limited availability of the required tools and equipments, (c) the lack of the safety information and the inappropriate knowledge in regard to the harvest management, plus (d) in some countries, poor governmental regulations have great impact (Udomkun et al. 2018).

Generally, mycotoxins are considered a serious problem which resulted from feeding unconditioned storage of foodstuff. They could be found as unpredictable food contaminants which is difficult to be avoided and contribute to a massive economic losses in the agriculture industry worldwide (Alshannaq and Yu 2017). In spite of, there are twenty AFs that have been identified, aflatoxin B1 (AFB1) is reported to be the highly toxic and carcinogenic one from the *Aspergillus* species that might predispose to serious health consequences in human and animals such as hepatocellular carcinoma (HCC) (Rushing and Selim 2019; Mahato et al. 2019). Dairy and beef cattle are more susceptible to aflatoxicosis than sheep or horses (Constable et al. 2016). Several outbreaks of aflatoxicosis had been previously recorded in cattle (Sohooa et al. 2015) and horse (Caloni and Cortinovis 2011). Nonetheless, still there is a limited amount of the available information concerning mycotoxins and their negative effects in ruminants in comparison with non-ruminants.

Their mode of action is still poorly understood, although there are several studies reported that they might exert various interactions with the physiological functions inside the body. Generally, protein adducts was reported to be most commonly associated with the incidence of acute intoxication, where it blocks the protein synthesis, especially those enzymes which enrolled in the vital functions, including metabolic pathways, protein synthesis, and immune response. It is also reported that phospholipid adducts with the reactive oxygen species (ROS) predisposing to lipid peroxidation (LPO) as the major cause behind the disruption of the cell membrane integrity and function (Benkerroum 2020). AFs might predispose to a decrease in the resistance to diseases, interfere with vaccine-induced immunity, and also decrease the animal growth and reproduction with serious economic losses (Richard et al. 2003). They reported to cause hazardous toxic effects, predisposing to several changes in the plasma biochemical parameters (Vaziriyan et al. 2018). An induction of several alterations such as the release of the ROS which predispose to oxidative stress with subsequent damages to proteins, lipids, RNA, and DNA was also reported (Mohajeri et al. 2018).

This study was done to provide adequate information describing a toxicological outbreak in a cattle farm due to an accidental ingestion of AFB1 contaminated food. Monitoring the livestock health condition in a context of protecting the livestock and their populations utilizes multi-biomarker approach through the evaluation of several biochemical, electrolyte, antioxidant status, and postmortem lesions of the intoxicated animals.

Materials and methods

Animals and feed

This study was carried out during December 2018 on a native breed beef cattle farm in Aswan Governorate, southern Egypt. Seventy-two cattle, aged from 1 to 2 years and weighed 250 to 350 kg, suffered from diarrhea with a history of feeding unconditioned stored feed pellet were investigated. Another twenty apparently healthy cattle fed on properly stored food pellet were used as a normal control. All the institutional and national guidelines needed for the animal care and use were followed. All approaches and procedures involving animals followed the guideline for the use and care of animals of the National Institutes of Health (NIH publication No.85–23, revised 1996). These guidelines of the protection of the animals which used for scientific research followed the ethical guidelines provided by the institutional committee of Aswan University, Egypt.

Determination of AFB1 in feed

Sampling

Five hundred grams of the livestock feeding pellet samples were collected from each of the control feed pellets which is free from AFs and the other feed pellets collected from the intoxicated farm. The total amount of 500 g of feeding pellet sample was collected into dried, cleaned, sterilized

polyethylene bag. All the collected samples were directly sent to the toxicology laboratory immediately for the analytical process and the identification of AFB1.

Analytical assays

Qualitative identification of AFB1 in the feeding pellets of the collected samples was performed according to the method of Braicu et al. (2008) with some modifactions as follows:

Extraction of AFB1 Extraction from AFB1 from each homogenized sample (50 g) was done using 100 mL chloroform then filtered by filter paper with 2 g anhydrous sodium sulfate. The extract was reduced to around 3 mL using rotary evaporator. The extract was then quantitatively transferred to a separatory funnel where methanol/distilled water (1:1 V/V) and 40 mL n-pentane was added. The aqueous methanolic layer was then drained into a beaker, while pentane fraction collected into another one. The aqueous methanolic layer was repeated twice with chloroform (50 mL). Then, the aqueous layer was discarded and the chloroform layer was evaporated in a rotary evaporator (3 mL).

Adsorption column chromatography Ten grams of silica gel was weighed to pack glass column (22 × 1.5 ID). Before the sample was loaded, the column topped with 2 g of anhydrous sodium sulfate then filed with 20 mL chloroform. The flow rate was 10 mL/min where the AFB1 was eluted with 150 mL chloroform-methanol mixture (97:3 V/V). The extract was then reduced to 1 mL and used for thin layer chromatography (TLC) analysis.

TLC analysis The TLC plates were activated at 80 °C for 1 h in a hot air oven before use, then samples were spotted on TLC plates. Different concentrations of standard solution ranged from 5 to 20 ng/ml were used for the identification of AFB1. The TLC plates were placed in TLC tank containing chloroform and acetone in the ratio of 90:10 (v/v) for 30 min at room temperature and then they were observed under UV light for the presence of AFs by their characteristic fluorescence properties. The blue fluorescence corresponding to the authentic AFB1 indicates the presence of AFB1 in the sample.

Clinical examination

Intoxicated cattle suffered from diarrhea and depression after the exposure to AF-contaminated feed by 18–36 h. Both aflatoxicosis and control cattle were exposed to a thorough clinical examination via the methods described in cattle (Rosenberger 1990). In brief, vital signs (rectal temperature, pulse rate, respiratory rate) and auscultation of the digestive, the respiratory tract, and the heart were recorded.

Postmortem examination

Postmortem examination was done on five cattle which were found to be dead in the farm out of the seventy-two cattle to clarify and report the postmortem lesions associated with the death. Intestinal congestion, enlarged gallbladder, and hepatomegaly were observed.

Collection of blood samples

Blood samples were collected from all animals enrolled in this study. Five milliliters of venous blood were collected from the jugular vein and added to tubes without anticoagulant for serum separation. Centrifugation of samples was done at 3000 rpm for 20 min at 25 °C within 2 h of collection, and then the clear serum was collected from the supernatant via sterile disposable Pasteur pipettes. The collected serum was then transferred to 1.5 mL dry sterile Eppendorf tubes.

Biochemical analysis

The biochemical parameters were measured through the commercial kits (BioMérieux, Marcy, L'Etoile, France) using Spectrophotometer 5010 v5+, (RIELE GmbH, Germany). Colorimetrical determination of the serum total protein (TP) (Lowry et al. 1951) and albumin were carried out (Doumas and Biggs 1972). Simply, 20 μL serum sample and 1000 μL chromagen reagent (100 mM/L phosphotungestic acid and 50 mM/L of phosphomolybidic acid, 0.5 M $CuSo_4$) were mixed together, and a color reagent was added (0.12 mM/L bromocresol green and 50 mM/L citrate buffer, pH 3.8) for the estimation of serum TP and albumin concentration. This mixture was incubated at room temperature for 10 min then spectrophotometerically measured against blank at wave length 550 and 630 nm, respectively. Linearity of serum TP is up to 10 g/dL, while linearity of serum albumin is up to 6 g/dL. The globulin was then calculated via the subtraction of the serum albumin from the total protein. The albumin/globulin ratio (A/G) is also calculated.

Liver transaminases (aspartate amino transferase (AST) and alanine amino transferase (ALT)) (Reitman and Frankel 1957). A total volume of 500 μL of 100 mM/L phosphate buffer pH 7.5, 100 mM/L aspartate, 2 mM/L α-ketoglutrate or 100 mM phosphate buffer pH 7.5, 200 mM/L alanine, 2 mM/L a-ketoglutarate was mixed with almost 100 μL of serum that was previously incubated at 37 °C for 60 and 30 min for AST and ALT measurement, respectively. Then, such mixture was then mixed with 1 mM/L 2,4 dinitrophenylhydrazine which was incubated at 25 °C/20 min. Building a standard curve used pyruvate to evaluate the enzymatic activity of both enzymes. Linearity of serum ALT is up to 120 U/L, while linearity of AST is 150 U/L.

Alkaline amino transferase (ALP) (Hausamen et al. 1967) and serum creatinine (SCr) (Heinegård and Tiderström 1973) were also measured. ALP was calorimetrically measured where a mixture of 25 μL of serum sample mixed with 25 μL of both 50 mM/L of phosphate buffer (pH 7.5) and 500 μL enzyme inhibitor (100 mM/L EDTA and 50 mM/L aminophenazone) which was previously incubated at 37 °C for 20 min. A total volume of 250 μL of 200 Mm/L of potassium ferricyanide was added to the mixture and the measurement of enzyme activity was done against blank at 510 nm (linearity is up to 250 U/L). SCr was calorimetrically measured; briefly the serum sample was deproteinized with 20 mM/L of trichloroacetic acid, then 100 μL of deproteinized serum was mixed with 1000 μL working reagent (25 mM/L boric acid and 0.4 mM/L sodium dodecyl sulfate). The mixture was incubated at 25 °C for 20 s, then read the absorbance after 2 min with standard reagent against distilled water at 500 nm (linearity was up to 10 mg/dL).

The catalase (CAT) was determined (Aebi 1984). A mixture of 50 μL of serum was mixed with 100 mM/L phosphate buffer (pH 7) and 1000 times diluted 500 mM/L H_2O_2 that was incubated for 1 min at 25 °C, then was then mixed with catalase inhibitor (50 mM/L sodium cyanide) and enzyme mix containing peroxidase (1000 U/L) and aminoantipyrine (2 mM/L). Same conditions were done to sample blank and standard then further incubation was executed at 37 °C for 10 min, where sample and standard were read against sample and standard blank, respectively, and the catalase activity was inversely proportional to red colored complex intensity that was read at 510 nm. Calibration curve was executed for CAT activity in all samples, where linearity was up to 600 U/L.

The reduced glutathione (GSH) was calorimetrically measured via dithio dinitrobenzene (DTNB) derivatives (Beutler 1963). Briefly, 100 μL of serum was mixed with 500 μL of 500 mM/L trichloroacetic acid for deproteinization and centrifuged at 3000 rpm for 5 min, where 50 μL of supernatant was aliquoted and mixed with 1000 μL of 100 mM/L of phosphate buffer (pH 7.5) and 100 μL of 1 mM/L of DTNB. The absorbance was determined against reagent blank at 405 nm and the concentration of GSH was determined by multiplying the absorbance with 66.66, linearity was up to 120 mg/dL.

The malondialdehyde (MDA) and nitric oxide (NO) were also analyzed (Draper and Hadley 1990; Nims et al. 1995), respectively. MDA concentration was performed by adding 1000 μL of chromagen containing 25 mM/L of thiobarbituric acid and 50 mM/L of n-butanol with 200 μL of serum, and a further incubation process was done at 95 °C for 30 min for producing a characteristic pink color. The absorbance was measured against reagent blank, where linearity was up to 100 nmol/L. Nitric oxide was measured by adding 100 μL serum to 1000 μL of 10 mM/L sulfanilamide that was vortexed and allowed to settle for 5 min, and 1 mM/L of N-naphthanyl-ethylenediamine was added to the mixture and a final incubation step at 37 °C was done for 5 min, and the absorbance was read against reagent blank at 540 nm, where linearity was up to 200 μmol/L.

Serum electrolyte measurement

Serum were analyzed for calcium (Ca) (Trinder 1960), phosphorus (P) (Daly and Ertingshausen 1972), and magnesium (Mg) concentrations (Ising et al. 1995). Calcium was measured in serum sample, where 20 μL of serum was mixed with working reagent containing (0.2 mM/L methylthymol blue, 140 mM/L hydroxyquinoline, 200 mM/L hydrochloric acid, 6 M/L ethanolamine). The mixture was then incubated at 25 °C for 5 min. Then, absorbance of sample and standard were read at 585 nm against blank (linearity up to 20 mg/dL). Phosphorus was determined, where 25 μL of the sample was mixed with 1000 μL of color reagent (2 M/L formic acid, 0.6 M/L glycerol, and 0.32 mM/L ammonium molybdate), and then diluted stannous chloride (2 mM/L) was added. The intensity of blue color was measured at 640 nm against blank (linearity was up to 15 mg/dL). The determination of magnesium was done, where 1000 μL of working reagent (0.2 mM/L calmagite, 1 M/L diethanolamine, Ph 12.5) was mixed with 20 μL of serum that was incubated at 25 °C for 2 min and measured against reagent blank with standard reagent at 520 nm (linearity was up to 6.49 mg/dL).

Statistical analysis

Differences between AF-intoxicated cattle and the control one were measured by *t* test. The results were presented as mean (x) ± standard deviation (SD). The significance was determined at $P < 0.05$. All statistical analysis was performed using the statistical software (SPSS Inc., Chicago, IL, USA).

Results

Determination of AFB1

TLC analysis from the extract of the unconditioned stored feed samples identified the presence of AFB1 contamination in these samples. This was confirmed by the blue line fluorescence at UV transilluminator when compared with the AFB1 standard sample. The concentrations of AFs in the contaminated samples ranged from 5 to 20 ng/ml as it reveals positive with the standard used in the TLC assay. On the other side, AFB1 was not detected in the examined control samples.

Clinical signs

The clinical signs of aflatoxicosis-intoxicated cattle are listed in Table 1. All examined intoxicated animals showed normal

Table 1 Clinical findings in cattel suffered from acute aflatoxicosis in comparison with control

Clinical signs	Control cattle (n = 20) Number of animals and %	AF-intoxicated cattel (n = 69)
Vital parameters		
1. Body temperature		
38.2–39 °C	20 (100)	67 (97.1)
> 39 °C	0	0
< 38.2	0	2 (2.9)
2. Respiratory rate/minute		
10–30	20 (100)	67 (97.1)
> 30	0	0
< 10	0	2 (2.9)
3. Heart rate/minute		
60–80	20 (100)	67 (97.1)
> 80	0	0
< 60	0	2 (2.9)
4. Ruminal contractions		
2–3/2 min	20 (100)	0
Decreased	0	61 (88.4)
Absent	0	8 (11.6)
Inappetance	0	69 (100)
Depression	0	69 (100)
Recumbency	0	6 (8.7)
Fecal abnormalities		
1. Negative	20 (100)	53 (76.8)
2. Scanty feces	0	0
3. Diarrhea	0	16 (23.19)

*Clinical findings were documented on sixty nine (69) cattle, the total number of AF-intoxicated cattle was seventy-two (72) where three of them found dead in the morning, and later on, two more animals with subnormal temperature died. Twenty (20) control cattle were used in this study

body temperature except the two cattle which showed a subnormal body temperature. Ruminal contractions were decreased in 61 cases (88.4%) and ceased in 8 cases (11.6%) of AF-intoxicated cattle. Inappetence and depression were observed in all cases (100%); diarrhea (soft, pasty not watery diarrhea) was observed in 16 cases (23.19%) of AF-intoxicated cattle. Five dead animals (6.94%) had been reported 24 h from the ingestion of contaminated feedstuffs by AFs. Three of the dead animals were found dead early morning next day, and the other two died within 24 h from ingestion of the contaminated food.

Necropsy findings

The postmortem examination is listed in (Table 2). Results revealed the presence of hepatomegaly and greatly enlarged gallbladder in five cases; out of them, four cases with intestinal and kidney congestion was reported.

Table 2 Postmortem lesions in dead cattle

Postmortem lesion	Case number				
	1	2	3	4	5
Intestinal congestion	+	−	+	+	+
Hepatomegaly	+	+	+	+	+
Enlargement of gall bladder	+	+	+	+	+
Heart enlargement	−	−	−	−	−
Kidney congestion	+	−	+	+	+

Biochemical estimation

Serum TP showed a significant (P = 0.028) decrease, meanwhile serum albumin, globulin levels, and A/G ratio showed a non-significant (P = 0.065, 0.8, and 0.315, respectively) decrease in aflatoxicosis-intoxicated cattle when compared with control (Fig. 1). Serum AST, ALT, ALP, and SCr levels showed a significant (P = 0.008, 0.001, 0.006, and 0.017, respectively) increase in aflatoxicosis-intoxicated cattle when compared with control ones (Fig. 2).

Serum electrolyte analysis

The serum Ca and P levels showed a non-significant (P = 0.36 and 0.67, respectively) change; meanwhile, the level of serum Mg was significantly (P = 0.048) decreased in AF-intoxicated animals compared with the control cattle (Fig. 3).

Estimation of antioxidants and lipid peroxidation

Serum CAT and MDA showed a significant (P = 0.013 and 0.002, respectively) increase; meanwhile, GSH showed a significant (P = 0.001) decrease in aflatoxicosis-intoxicated cattle when compared with control ones. Serum NO showed a non-significant (P = 0.23) decrease in aflatoxicosis-intoxicated cattle when compared with control ones (Fig. 4).

Discussion

Although the systemic profound effect of certain mycotoxins on animal is not clear, it adversely effect the general health condition in cattle (Bodine and Mertens 1983). It is also known that mycotoxins, especially AFB1 is considered one of the most important threats in terms of their hazardous effect and risk to both animals and human health (Limaye et al. 2018). Nevertheless, there is a huge problem due to the lack

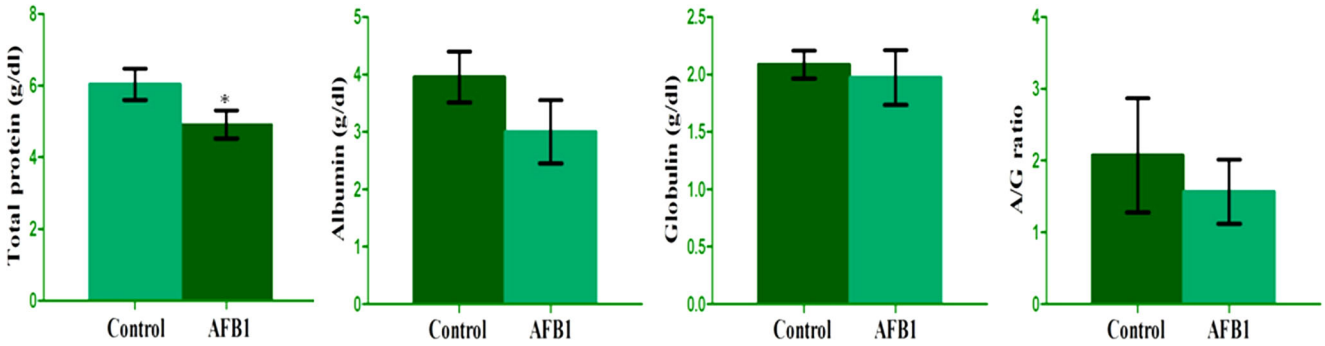

Fig. 1 Total protein (TP), albumin, globulin, and A/G ratio are presented. Values are presented as means ± SD. Statistically significant differences at ($P < 0.05$) are indicated by asterisk when compared with the control group

of knowledge and the continuous consumption of AF-contaminated food and feed which increase the need to be further investigated. The limited information and scientific evidences regarding the AF intoxication and ingestion in ruminants represents an obstacle in regard to the evaluation of the cattle performance and also the estimation of the real economic impact on the production system of ruminant livestock.

Several AF outbreaks have been previously reported worldwide in animals and humans (Mahato et al. 2019; Pereira et al. 2019). Surprisingly, aflatoxicosis outbreaks resulted also in several cases of acute illness and deaths (Benkerroum 2020). Inappetence, depression, diarrhea, and acute death of 6.94% in AF-intoxicated cattle were documented in our investigation. Similar signs were also reported in aflatoxicosis-intoxicated ruminant (Dhanasekaran et al. 2011) which indicates that AFs might predispose to multiple health hazards in ruminant. Mortalities due to aflatoxicosis were previously seen in calves in the Republic of South Africa (Green et al. 1989). The congested intestine, liver, kidney, and enlarged gallbladder in necropized cattle are recorded in our examination, and coincided with the previously recorded AF-intoxicated cattle in Pakistan (Sohooa et al. 2015). The AFB1 is reported to be able to cross the rumen mucosa and appear in the blood at 30 min in intoxicated steers which may be the reason behind the early Peak of AF toxicity (Cook et al. 1986).

The liver is considered the target organ for toxic effects of AFB1 (Dhanasekaran et al. 2011), and the serum TP level is an indicator for the protein synthesis. Meanwhile, the increased activities of ALT, AST, and ALP are sensitive indicators of both hepatic tissues and biliary system impairment. The serum TP in the current study was significantly decreased, which might be due to the non-significant decrease in both of albumin and globulin levels. An increase in the serum ALT, AST, and ALP was also reported. The significant increase in the serum AST and ALT were also reported (Rastogi et al. 2001; Bintvihok and Kositcharoenkul 2006; Bingol et al. 2007) in AF-intoxicated animals. Aflatoxicosis is also reported to degenerate the hepatocytes leading to leakage of these hepatic enzymes into the circulation (Leeson 1995) which describe the reason behind the elevated levels of these enzymes. A reported increase in the serum aspartate transaminase enzyme was observed 24 to 48 h after AF intoxication in steers, where these changes are dose-related (Cook et al. 1986). The AFB1 has been reported to provoke the liver function through the inducing of apoptosis plus disturbing the cellular enzymatic activities (Mughal et al. 2017). Some researchers reported no significant changes in the TP and ALP after AF intoxication due to the fact that serum glycocholic acid level could be considered a sensitive indicator for biliary impairment or cholestasis than alkaline phosphatase or bilirubin (Cook et al. 1986).

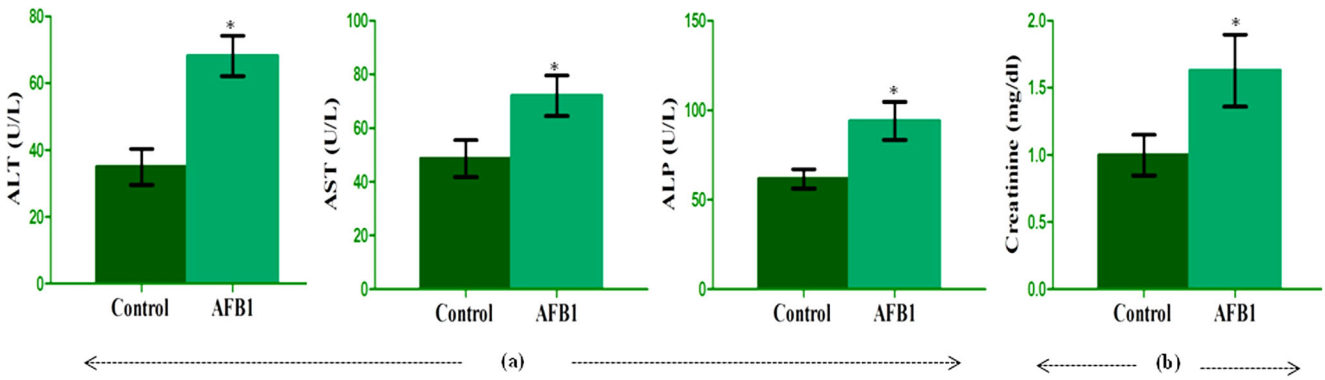

Fig. 2 AST, ALT, and ALP are presented on panel (**a**), while serum creatinine (Scr) on panel (**b**). Values are presented as means ± SD. Statistically significant differences at ($P < 0.05$) are indicated by asterisk when compared with the control group

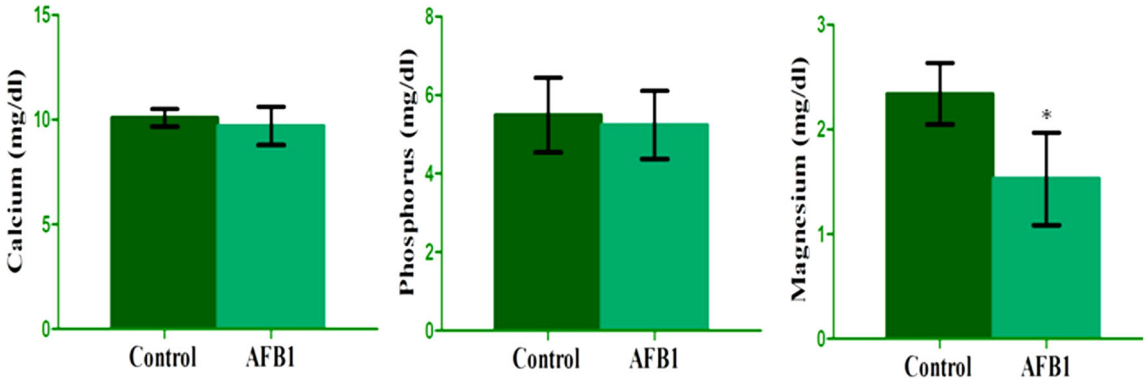

Fig. 3 Ca, P, and Mg are presented. Values are presented as means ± SD. Statistically significant differences at ($P < 0.05$) are indicated by asterisk when compared with the control group

The SCr is known as a marker of renal function. Current investigation showed a significant increase in creatinine level in AF-intoxicated cattle. The nephrotoxic effect of AFs may predispose to the decreased of the glomerular filtration rate (Grosman et al. 1983). Moreover, thickening of the glomerular basement membrane might occur following AFB1 exposure (Valdivia et al. 2001). AFB1 induce renal toxicity by the activation of oxidative stress which alter the expression of proline dehydrogenase and L-proline levels, and finally might induce downstream apoptosis (Li et al. 2018). This nephrotoxic effect predispose to an elevated SCr level.

The serum Ca and P are shown to be within the physiological range in the cattle affected with acute aflatoxicosis. Only the concentrations of serum Mg were significantly affected. Mg in ruminants is absorbed primarily in the forestomachs. The epithelium of rumen takes Mg up by both transcellular (active) and paracellular (passive) mechanisms (Urdaz et al. 2003). Unlike many other essential minerals, there is no specific hormonal system needed for the regulation of the homeostasis of Mg (Martens and Schweigel 2000). The kidney is also responsible for the excretion of the excess Mg. Meanwhile, in case of endogenous and exogenous losses of Mg, the extracellular concentration of Mg is decreased due to the continuous losses (Odette 2005).

The increased activities of CAT were reported. This enzyme has a protective function by destruction of hydrogen peroxide (Glorieux and Calderon 2017). Our finding showed that GSH is decreased significantly in the AF-intoxicated cattle, whereas the activity of NO was not significantly changed. The AF toxicity is manifested by the reduction of non-enzymatic and enzymatic antioxidants besides GSH depletion and increased hepatic lipid peroxide (Mohajeri et al. 2018). On bovine peripheral blood mononuclear cells, AFB1 showed an increase in the intracellular reactive oxygen metabolites (Bernabucci et al. 2011). It is also shown that some mycotoxins may induce the production of free radicals and/or the reduction of antioxidant defense during toxicity (Meissonnier et al. 2008). This leads to the induction of the oxidative stress, which has to be regarded as a cause or a consequence of the action of the toxicity on the cellular system and variable effects of mycotoxins on different cell systems.

Aspergillus spp. are widely spread in nature and considered the major source for environmental contamination. An increase in the outbreaks because of the limited information and the increase in the consumption of AF-contaminated crops, food, and animal feeds worldwide. They pose

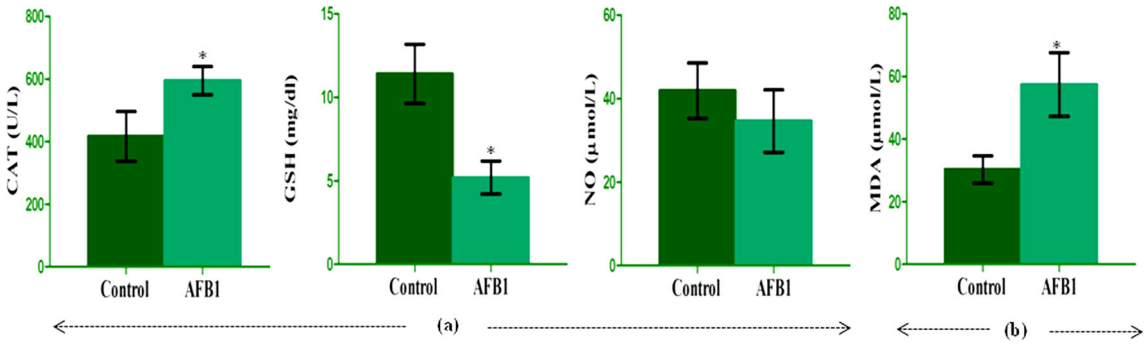

Fig. 4 CAT, GSH, and NO are presented on panel (**a**), while MDA on panel (**b**). Values are presented as means ± SD. Statistically significant differences at ($P < 0.05$) are indicated by asterisk when compared with the control group

enormous health hazardous concerns to both livestock as well as human that might predispose to even death. With the increase in the economic and the adverse health conditions, a huge demand is directed towards the *prevention of such threat through excessive AF research*. Meanwhile there are limited available data concerning the AF deposition inside the bovine edible tissues, it is expected that AF have low degradation rate in the rumen and rapidly metabolized in the liver (Peles et al. 2019). Residues of AFs were also reported to be in the liver and kidney tissues of broiler chicken; residues were also found in the reproductive organs, gizzard, and breast (Herzallah et al. 2014). These health risk reports incriminated behind AF contamination should be a strong message for the consumers to assess the health risk and a wake-up call for the farmers to convey practical control measures to prevent animal exposure to the contaminated feed for stronger economy and healthy community.

Conclusion

The present study demonstrates that AFB1 is a major challenge and highly toxic if it contaminate the feed stuff. AFs exert toxic effects and adverse health effects to livestock industry, consequently hazardous effect could be reported in people. We utilized several biochemical and electrolyte biomarkers in addition to evaluation of the oxidative stress and postmortem alterations to evaluate the adverse health effects from AFB1. Our results suggest that AFs are considered a health threat that could impair the animal health and productivity. Therefore, it is it is important to provide great attention for regular monitoring of the animal health to ensure the animal performance and save the economy. Great demand should be directed to focus on the occurrence of AF contamination in feedstuffs and immediately remove the contaminated feed if reported for the prevention of further consequences and restore the animal productivity.

Author contributions Authors have equal contribution and responsibility for the content of such manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Animals studies All the institutional and national guidelines needed for the animal care and use were followed. All approaches and procedures involving animals followed the guideline for the use and care of animals of the National Institutes of Health (NIH publication No.85–23, revised 1996). These guidelines of the protection of the animals which used for scientific research followed the ethical guidelines provided by the institutional committee of Aswan University, Egypt.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

Alshannaq A, Yu JH (2017) Occurrence, toxicity, and analysis of major mycotoxins in food. Int J Environ Res Public Health 14(6):632. https://doi.org/10.3390/ijerph14060632

Battilani P, Toscano P, Van der Fels-Klerx H, Moretti A, Leggieri MC, Brera C et al (2016) Aflatoxin B1 contamination in maize in Europe increases due to climate change. Sci Rep 6:24328. https://doi.org/10.1038/srep24328

Benkerroum N (2020) Chronic and acute toxicities of aflatoxins: mechanisms of action. Int J Environ Res Public Health 17(2):423. https://doi.org/10.3390/ijerph17020423

Bernabucci U, Colavecchia L, Danieli PP, Basiricò L, Lacetera N, Nardone A, Ronchi B (2011) Aflatoxin B1 and fumonisin B1 affect the oxidative status of bovine peripheral blood mononuclear cells. Toxicol in Vitro 25:684–691. https://doi.org/10.1016/j.tiv.2011.01.009

Beutler E (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888

Bingol NT, Tanritanir P, Dede S, Ceylan E (2007) Influence of aflatoxin present in forages and concentrated feedingstuffs on milk and some serum biochemical parameters in goats. Bull Vet Inst Pulawy 51:65–69

Bintvihok A, Kositcharoenkul S (2006) Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon 47:41–46. https://doi.org/10.1016/j.toxicon.2005.09.009

Bodine A, Mertens D (1983) Toxicology, metabolism, and physiological effects of aflatoxin in the bovine. In: Diener UL, Asquith RL, Dickens JW (eds) Aflatoxin and Aspergillus tlavus in com. Auburn Univ, AL, Alabama Agric Exp Sta, pp 46–50

Braicu C, Puia C, Bodoki E, Socaciu C (2008) Screening and quantifcation of aflatoxins and ochratoxin in different cereals cultivated in Romania using thin-layer chromatography-densitometry. J Food Quality 31(1):108–120. https://doi.org/10.1111/j.1745-4557.2007.00187.x

Caloni F, Cortinovis C (2011) Toxicological effects of aflatoxins in horses. Vet J 188(3):270–273. https://doi.org/10.1016/j.tvjl.2010.06.002

Constable PD, Hinchcliff KW, Done SH, Grünberg W (2016) Veterinary medicine-e-book: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences

Cook W, Richard J, Osweiler G, Trampel D (1986) Clinical and pathologic changes in acute bovine aflatoxicosis: rumen motility and tissue and fluid concentrations of anatoxins B1 and M1. Am J Vet Res 47(8):1817–1825

Daly JA, Ertingshausen G (1972) Direct method for determining inorganic phosphate in serum with the "CentrifiChem". Clin Chem 18:263–265

Dhanasekaran D, Shanmugapriya S, Thajuddin N, Panneerselvam A (2011) Aflatoxins and aflatoxicosis in human and animals. In: Guevara-Gonzalez RG (ed) Aflatoxins-biochemistry and molecular biology. IntechOpen, https://doi.org/10.5772/22717

Doumas B, Biggs H (1972) Determination of serum albumin. In: Cooper GA (ed) Standard methods of clinical chemistry. Academic press Inc, New York, pp 175–188

Draper H, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186:421–431. https://doi.org/10.1016/0076-6879(90)86135-i

Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF (2015) Review on mycotoxin issues in ruminants: occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. Toxins 7(8):3057–3111. https://doi.org/10.3390/toxins7083057

Glorieux C, Calderon PB (2017) Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. Biol Chem 398:1095–1108. https://doi.org/10.1515/hsz-2017-0131

Green J, Marasas W, Thiel P, Stockenström S (1989) A field outbreak of chronic aflatoxicosis in dairy calves in the western Cape Province. J S Afr Vet Assoc 60:210–211

Grosman ME, Lías MM, Comin EJ, Garay EAR (1983) Alterations in renal function induced by aflatoxin B1 in the rat. Toxicol Appl Pharmacol 69(3):319–325. https://doi.org/10.1016/0041-008X(83)90255-7

Hausamen TU, Helger R, Rick W, Gross W (1967) Optimal conditions for the determination of serum alkaline phosphatase by a new kinetic method. Clinica Chimica Acta 15(2):241–245. https://doi.org/10.1016/0009-8981(67)90060-5

Heinegård D, Tiderström G (1973) Determination of serum creatinine by a direct colorimetric method. Clinica Chimica Acta 43:305–310. https://doi.org/10.1016/0009-8981(73)90466-x

Herzallah S, Al-Ameiri N, Al-Dmoor H, Masoud S, Shawabkeh K (2014) Meat and organs quality of broiler chickens fed diet contaminated with B1 aflatoxin. Glob Vet 12:376–380. https://doi.org/10.5829/idosi.gv.2014.12.03.82344

Ising H, Bertschat F, Gunther T, Jeremias E, Jeremias A (1995) Measurement of free magnesium in blood, serum and plasma with an ion-sensitive electrode. Eur J Clin Chem Clin Biochem 33:365–371. https://doi.org/10.1515/cclm.1995.33.6.365

Kamika I, Tekere M (2016) Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. Food Control 69:292–296. https://doi.org/10.1016/j.foodcont.2016.05.014

Leeson S (1995) Aflatoxins. In: Leeson S, Diaz G, Summers JD (eds) Poutry metabolic disorders and mycotoxins. University Books, Guelph, pp 248–279

Li H, Xing L, Zhang M, Wang J, Zheng N (2018, 2018) The toxic effects of aflatoxin B1 and aflatoxin M1 on kidney through regulating L-proline and downstream apoptosis. BioMed Research International. https://doi.org/10.1155/2018%2F9074861

Limaye A, Yu RC, Chou CC, Liu JR, Cheng KC (2018) Protective and detoxifying effects conferred by dietary selenium and curcumin against AFB1-mediated toxicity in livestock: a review. Toxins 10(1):25. https://doi.org/10.3390/toxins10010025

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Mahato DK, Lee KE, Kamle M, Devi S, Dewangan K, Kumar P et al (2019) Aflatoxins in food and feed: an overview on prevalence, detection and control strategies. Front Microbiol 10:2266. https://doi.org/10.3389/fmicb.2019.02266

Martens H, Schweigel M (2000) Pathophysiology of grass tetany and other hypomagnesemias. Implications for clinical management. Vet Clin North Am Food Anim Pract 16(2):339–368. https://doi.org/10.1016/s0749-0720(15)30109-2

Meissonnier GM, Pinton P, Laffitte J, Cossalter AM, Gong YY, Wild CP, Bertin G, Galtier P, Oswald IP (2008) Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol Appl Pharmacol 231:142–149. https://doi.org/10.1016/j.taap.2008.04.004

Mohajeri M, Behnam B, Cicero AFG, Sahebkar A (2018) Protective effects of curcumin against aflatoxicosis: a comprehensive review. J Cell Physiol 233(4):3552–3377. https://doi.org/10.1002/jcp.26212

Mughal MJ, Peng X, Zhou Y, Fang J (2017) Aflatoxin B1 invokes apoptosis via death receptor pathway in hepatocytes. Oncotarget 8(5):8239–8249. https://doi.org/10.18632/oncotarget.14158

Nims RW, Darbyshire JF, Saavedra JE, Christodoulou D, Hanbauer I, Cox GW, Grisham MB, Laval F, Cook JA, Krishna MC, Wink DA (1995) Colorimetric methods for the determination of nitric oxide concentration in neutral aqueous solutions. Methods 7(1):48–54. https://doi.org/10.1006/meth.1995.1007

Odette O (2005) Grass tetany in a herd of beef cows. Can Vet J 46(8):732–734

Peles F, Sipos P, Győri Z, Pfliegler WP, Giacometti F, Serraino A, Pagliuca G, Gazzotti T, Pócsi I (2019) Adverse effects, transformation and channeling of aflatoxins into food raw materials in livestock. Front Microbiol 10:2861. https://doi.org/10.3389/fmicb.2019.02861

Pereira CS, Cunha SC, Fernandes JO (2019) Prevalent mycotoxins in animal feed: occurrence and analytical methods. Toxins 11(5):290. https://doi.org/10.3390/toxins11050290

Rastogi R, Srivastava AK, Rastogi AK (2001) Biochemical changes induced in liver and serum of aflatoxin B1-treated male Wistar rats: preventive effect of picroliv. Pharmacol Toxicol 88:53–58. https://doi.org/10.1034/j.1600-0773.2001.088002053.x

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 8(1):56–63. https://doi.org/10.1093/ajcp/28.1.56

Richard JL, Payne GA, Desjardins AE, Maragos C, Norred W, Pestka J (2003) Mycotoxins: risks in plant, animal and human systems. CAST Task Force Report 139:101–103

Rosenberger G (1990) Die klinische Untersuchung des Rindes. In: Dirksen G, Gründer HD, Stöber M, (eds) 3rd edn. Verlag Paul Parey-Berlin und Hamburg

Rushing BR, Selim MI (2019) Aflatoxin B1: a review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem Toxicol 124:81–100. https://doi.org/10.1016/j.fct.2018.11.047

Sarma UP, Bhetaria PJ, Devi P, Varma A (2017) Aflatoxins: implications on health. Indian J Clin Biochem 32(2):124–133. https://doi.org/10.1007/s12291-017-0649-2

Sohooa R, Khana AU, Ameena K, Rafia-Munire A, Saleemb F (2015) Outbreak of aflatoxicosis on a local cattle farm in Pakistan. Veterinaria 3(1):13–17

Stepman F (2018) Scaling-up the impact of aflatoxin research in africa. The role of social sciences. Toxins 10(4):136. https://doi.org/10.3390/toxins10040136

Trinder PT (1960) Colorimetric micro-determination of calcium in serum. Analyst 85:889–894 10.1039AN9608500889

Udomkun P, Wossen T, Nabahungu NL, Mutegi C, Vanlauwe B, Bandyopadhyay R (2018) Incidence and farmers' knowledge of aflatoxin contamination and control in Eastern Democratic Republic of Congo. Food Sci Nutr 6(6):1607–1620. https://doi.org/10.1002/fsn3.735

Urdaz JH, Santos JE, Jardon P, Overton MW (2003) Importance of appropriate amounts of magnesium in rations for dairy cows. J Am Vet Med Assoc 222:1518–1523. https://doi.org/10.2460/javma.2003.222.1518

Valdivia AG, Martinez A, Damian FJ, Quezada T, Ortiz R, Martinez C et al (2001) Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B1 intoxication in broiler chickens. Poul Sci 80:727–734. https://doi.org/10.1093/ps/80.6.727

Vaziriyan M, Banaee M, Haghi BN, Mohiseni M (2018) Effects of dietary exposure to aflatoxins on some plasma biochemical indices of common carp (*Cyprinus carpio*). Iranian J Fish Sci 17(3):487–502. https://doi.org/10.22092/IJFS.2018.116552