Heliyon 9 (2023) e21837

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Acute aflatoxin B1-induced hepatic and cardiac oxidative damage in rats: Ameliorative effects of morin

Ahmed E. Altyar [a,b,**], Osama A. Kensara [c], Amany A. Sayed [d], Lotfi Aleya [e], Mikhlid H. Almutairi [f], Mohamed Sayed Zaazouee [g], Alaa Ahmed Elshanbary [h], Fatma M. El-Demerdash [i], Mohamed M. Abdel-Daim [j,k,*]

[a] Department of Pharmacy Practice, Faculty of Pharmacy, King Abdulaziz University, Jeddah, 21589, Saudi Arabia
[b] Pharmacy Program, Batterjee Medical College, P.O. Box 6231, Jeddah, 21442, Saudi Arabia
[c] Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, P.O. Box 7067, Makkah, 21955, Saudi Arabia
[d] Zoology Department, Faculty of Science, Cairo University, Giza, 12613, Egypt
[e] Chrono-Environnement Laboratory, UMR, CNRS 6249, Franche-Comté University, CEDEX, F-25030, Besançon, Bourgogne, France
[f] Zoology Department, College of Science, King Saud University, P.O. Box: 2455, 11451, Riyadh, Saudi Arabia
[g] Faculty of Medicine, Al-Azhar University, Assiut, Egypt
[h] Faculty of Medicine, Alexandria University, Alexandria, Egypt
[i] Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt
[j] Department of Pharmaceutical Sciences, Pharmacy Program, Batterjee Medical College, P.O. Box 6231, Jeddah, 21442, Saudi Arabia
[k] Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt

ARTICLE INFO

Keywords:
Mycotoxin
Flavonoids
Morin
Oxidative stress
Inflammation
Liver
Heart

ABSTRACT

Aflatoxins (AFs) are secondary metabolites produced by the fungus *Aspergillus flavus*, of which Aflatoxin-B1 (AFB1) appears to be the most cancerogenic and of the highest toxicity. AFB1 causes serious effects on several organs including the liver. Morin is a flavonol that exists in many fruits and plants and has diverse biological properties including anticancer, anti-atherosclerotic, anti-oxidant, anti-inflammatory, immunomodulatory, and multi-organ protective activities. The present study aims to evaluate the potential protective effects of morin against acute AFB1-induced hepatic and cardiac toxicity in rats. Forty rats were divided into five groups (n = 8) as follows: control received the vehicle, morin was orally administered 30/mg/kg body weight (MRN_{30}), the AFB1 was administered orally at a dose of 2.5 mg/kg, twice on days 12 and 14 of the experiment for the 3rd, 4th, and 5th groups., AFB1-MRN15 was orally given morin at a dose of 15 mg/kg body weight, and AFB1-MRN30 orally received morin at 30 mg/kg body weight. The results indicated a significant decrease in serum AST, ALP, LDH, GGT, CK, CK-MB, 8-OHdG, IL-1β, IL-6, TNF-a levels in MRN30 compared to AFB1, and AFB1-MRN15 groups. However, the results indicated non-significant differences in the serum levels between MRN30, control, and AFB1-MRN30 groups. Meanwhile, regarding the hepatic and cardiac parameters, there were significant differences in the levels of MDA, NO, GSH, GSH-Px, SOD, and CAT in MRN30 compared to AFB1, and AFB1-MRN15 groups, overall implying the protective effects of morin. To conclude, morin at a dose of 30 mg/kg b. wt. showed significant enhancements in acute AFB1-induced

* Corresponding author. Department of Pharmaceutical Sciences, Pharmacy Program, Batterjee Medical College, P.O. Box 6231 Jeddah 21442, Saudi Arabia. Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt.
** Corresponding author. Department of Pharmacy Practice, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, Jeddah 21589, Saudi Arabia.
E-mail addresses: aealtyar@kau.edu.sa (A.E. Altyar), abdeldaim.m@vet.suez.edu.eg (M.M. Abdel-Daim).

https://doi.org/10.1016/j.heliyon.2023.e21837
Received 10 July 2023; Received in revised form 25 October 2023; Accepted 30 October 2023
Available online 4 November 2023

hepatic and cardiac toxicity in rats, which could play a role in limiting the public health hazards of AFs.

1. Introduction

Aflatoxins (AFs) are a type of secondary metabolite having high toxicity and low molecular weight [1]. AFs were first discovered in 1960 after the "Turkey X disease" epidemic when about 100,000 turkeys in England died after being fed groundnut meal from Brazil on a poultry farm in London [2,3]. AFs are produced by *Aspergillus flavus, and Aspergillus parasiticus* [1]. Aspergillus flavus produces four compounds belonging to the AF class, which are aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2 [4,5]. As these compounds significantly contaminate these animals' food supply, there is a major concern regarding food contamination worldwide, particularly for human-consumed food sources [5]. According to the Food and Drug Administration (FDA), the action levels of AF range between 20 and 300 ppb, apart from AFM1, which is as low as 0.5 ppb in milk for human consumption. This range depends on the intended use and the grain, grain by-product, feed, or other products [6].

Generally, the isolated and identified AFs now exceed 20, out of which AFB1 appears to be of the highest toxicity, and the International Agency for Cancer Research listed it as a human class I carcinogen [7,8]. AFB1 gets through humans via the respiratory tract and mouth, leading to the weakening of the body's antioxidant capacity [9], immunity [10], and internal organs damage, particularly the liver [11]. AFB1 induced liver damage through an imbalance between the body's antioxidant defense system and reactive oxygen species (ROS), leading to the liver's hydropic degeneration, fatty vacuolar degeneration, and bile duct proliferation. Moreover, death receptors FAS, TNFR1, and related genes are upregulated, while the inhibitory apoptotic proteins XIAP and BCL-2 are downregulated [12]. Moreover, the cardiac damage is presented as mitochondrial dysfunction, ROS generation, and apoptosis, likely involved in the nuclear factor erythroid 2-related factor 2 signal pathway in broiler cardiomyocytes [13,14].

Morin is a flavonol extracted as a yellow pigment from plants that belong to the Moraceae family [15]. Morin has anti-inflammatory [16], antibacterial [17], antioxidant [16], anti-atherosclerotic [18], and anti-stress effects [19]. Tian et al. showed that morin could suppress TLR4/NF-κB and activate Nrf2 and HO-signaling pathways, thereby protecting lipopolysaccharide-caused acute hepatic injury in mice [20]. Gao et al. investigated the protective role of morin in chicks for hepatic and renal injury with aflatoxicosis [21]. Morin ameliorated cardiac toxicity in bisphenol-S and diethyl Phthalate co-exposed rats through inhibitory activities on inflammation and oxidative stress [22]. However, there is no data regarding morin's role against acute AFB1-induced acute oxidative Hepato-and cardiotoxicity in mammals including rodents. Therefore, this study aims to investigate the role of morin in acute AFB1-induced hepatic and cardiac toxicity in rats.

2. Materials and methods

2.1. Chemicals

We purchased the pure AFB1 and morin powder from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All kits that tested serum biochemistry, oxidative stress indicators, and antioxidant status were supplied by Biodiagnostics Co. (Cairo, Egypt), except the kits used to assess DNA damage, proinflammatory cytokines, and lactate dehydrogenase (LDH). Cayman Chemical (Co., MI, USA) supplied the kits for measuring 8-hydroxy-2′-deoxyguanosine (8-OHdG), R&D (Mannheim, Germany) supplied ELISA kits for measuring interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-alpha (TNF-α), while Randox Laboratories (Ltd., Crumlin, UK) supplied the kits for assessing LDH.

2.2. Animals and experimental design

All animal treatments and experimental procedures were certified by the Faculty of Veterinary Medicine's Ethical Committee, University of Suez Canal, Ismailia, Egypt (Approval number 201936). A total of 40 mature male Wistar Albino rats (weighted 190 ± 10 g and aged 10 weeks) were obtained from the Egyptian Organization of Biological Products and Vaccines.

One week before the start of the experiment, rats were reared in cages with adequate ventilation at a temperature of 25 ± 2 °C, a range of relative humidity between 40 and 50 %, and 12 h of light and dark cycle. A nutritionally complete commercial pellet and running water were provided on demand.

After a week of acclimation, the rats were randomly allocated to one of five groups (eight in each one); (I) received saline as control, (II) received morin (30 mg/kg) orally for 14 days [23], (III) received AFB1 (2.5 mg/kg, orally) twice on days 12 and 14 [24], (IV) received AFB1 (2.5 mg/kg, orally) twice on days 12 and 14, and morin (15 mg/kg) orally for 14 days, and (V) received AFB1 (2.5 mg/kg, orally) twice on days 12 and 14, and morin (30 mg/kg) orally for 14 days [23].

2.3. Blood collection and serum and tissue preparation

On day 15, retro-orbital venous plexus blood was drawn under the effect of isoflurane inhalation then all rats were sacrificed for further hepatic and cardiac tissue collections. After centrifuging blood at 3000×*g* for 15 min, sera were kept at 20 °C for biochemical analysis of hepatic and cardiac function enzymes as well as pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) analysis, in addition to

DNA damage biomarker (8-OHdG) product. For additional investigations of tissue oxidative biomarkers, the heart and liver were dissected and washed thoroughly with saline to remove blood clots and RBCs. After that, we homogenized the tissues in 5–10 mL of ice-cold buffer per gram tissue and centrifuged these tissues at 5000 rpm for 30 min. The supernatant was tubed and stored at −80 °C for spectrophotometric analysis.

2.4. Serum biochemistry

Serum liver damage indicators (gamma-glutamyl transferase [GGT], alkaline phosphatase [ALP], aspartate transaminase [AST], and alanine transaminase [ALT]) were measured according to the techniques reported by Vazquez-Medina et al. [25], Tietz et al. [26] and Reitman et al. [27], respectively. Serum concentrations of creatine kinase (CK), CK-MB, and lactate dehydrogenase (LDH) were determined using the techniques of Szasz et al. [28], Würzburg et al. [29], and Babson et al. [30], respectively.

2.5. Oxidative DNA damage markers and proinflammatory cytokines assessment

Following the instructions supplied by the manufacturer, we assessed DNA oxidation by the amount of 8-OHdG in the serum and the proinflammatory cytokines IL-6, IL-1β, and TNF-α using ELISA kits.

2.6. Tissue antioxidant status and oxidative stress markers

The levels of nitric oxide (NO) and malondialdehyde (MDA) -markers for lipid peroxidation-were measured spectrophotometrically according to Green et al. [31] and Mihara et al. [32], respectively. Other tissue markers, including glutathione (GSH), GSH peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) were determined based on the methods by Beutler et al. [33], Paglia et al. [34], Aebi [35], Nishikimi et al. [36], respectively.

2.7. Statistical analysis

SPSS 26.0 was used to conduct the analysis. One-way analysis of variance (ANOVA) was performed to figure out whether the results were statistically significant, and Tukey's multiple range test was utilized to compare individuals. Data were described as mean with standard error (SE), and statistical significance was determined by a p-value of less than 0.05.

3. Results

3.1. Role of MRN on tissue serum biochemical parameters in AFB1-intoxicated rats

The analysis revealed a significant decrease with MRN30 compared to AFB1 in the serum liver enzyme levels AST, ALT, ALP, and GGT (54.1 %, 48,77 %, 53.7 %, and 46.7 %, respectively), and in LDH, CK, and CK-MB (39.8 %, 43.7 and 35.9 %, respectively). Moreover, there was a significant decrease, with AFB1-MRN15 and AFB1-MRN30 compared to AFB1, in the levels of AST (77.9 % and 59.5 %, respectively), ALT (74.58 % and 54.15, respectively), ALP (75.3 % and 55.5 %, respectively), and GGT (73.8 %, and 52.4 %, respectively), LDH (70.3 % and 46.2 %, respectively), CK (68 % and 49 %, respectively), and CK-MB (60 %, and 40 %, respectively). The control group also showed a significant decrease in all serum parameters compared to AFB1. Also, there were insignificant differences between the control, MRN30, and AFB1-MRN30 groups with all serum parameters, but a significant difference in the control, as well as MRN30, when compared to AFB1-MRN15. Table 1.

Table 1
Role of morin on tissue serum biochemical parameters, DNA damage and proinflammatory cytokines levels in AFB1-intoxicated rats.

Parameters	Groups				
	Control	MRN30	AFB1	AFB1-MRN15	AFB1-MRN30
AST U/L	$50.18^{a} \pm 0.68$	$49.22^{a} \pm 1.08$	$90.94^{b} \pm 2.03$	$70.85^{c} \pm 1.75$	$54.12^{a} \pm 1.52$
ALT U/L	$27.04^{a} \pm 0.92$	$26.9^{a} \pm 0.84$	$55.16^{b} \pm 3.27$	$41.14^{c} \pm 0.81$	$29.87^{a} \pm 1.03$
ALP U/L	$56.5^{a} \pm 0.68$	$55.54^{a} \pm 1.08$	$103.51^{b} \pm 3.3$	$77.92^{c} \pm 1.75$	$57.41^{a} \pm 2.43$
LDH U/L	$191.86^{a} \pm 3.5$	$189.76^{a} \pm 3.64$	$476.99^{b} \pm 9.74$	$335.15^{c} \pm 12.34$	$220.32^{a} \pm 7.29$
GGT U/L	$3.03^{a} \pm 0.11$	$2.98^{a} \pm 0.08$	$6.38^{b} \pm 0.33$	$4.71^{c} \pm 0.13$	$3.34^{a} \pm 0.13$
CK U/L	$91.17^{a} \pm 4.25$	$88.76^{a} \pm 6.79$	$203.03^{b} \pm 4.89$	$137.43^{c} \pm 3.5$	$99.35^{a} \pm 2.46$
CK-MB U/L	$34.68^{a} \pm 0.9$	$33.55^{a} \pm 0.88$	$93.44^{b} \pm 2.81$	$55.81^{c} \pm 0.77$	$37.4^{a} \pm 0.9$

MRN30; morin 30 mg, MRN15; morin 15 mg, AFB1; aflatoxin B1, AST; aspartate transaminase, ALT; alanine transaminase, ALP; alkaline phosphatase, LDH; lactate dehydrogenase, GGT; gamma-glutamyl transferase, CK; creatine kinase, CK-MB; creatine kinase-MB.
Data are expressed as mean ± standard error (n = 8).
Values with different alphabetic superscripts within the same row differ significantly ($p < 0.05$).

3.2. Role of MRN on tissue serum DNA damage and proinflammatory cytokines levels in AFB1-intoxicated rats

Regarding oxidative DNA damage markers, there were significant decreases with MRN30, AFB1-MRN15, and AFB1-MRN30 compared to AFB1 in the levels of 8-OHdG (43.8 %, 76.3 %, and 51 %, respectively). Also, for the proinflammatory cytokines, a significant reduction was observed in MRN30, AFB1-MRN15, and AFB1-MRN30 compared to AFB1 in the levels of IL-1β (26.1 %, 46.2 %, 33.1 %, respectively), IL-6 (39.1 %, 67.9 %, and 48.6 %, respectively), and TNF-a (32 %, 60.2 %, and 39.3 %, respectively). Fig. 1 (A-D).

3.3. Effect of MRN on hepatic tissue oxidative stress and antioxidant status in AFB1-intoxicated rats

When comparing MRN30, AFB1-MRN15, and AFB1-MRN30 to AFB1, the results showed significantly lower levels of MDA (45.1 %, 79.3 %, and 51.9 %, respectively), NO (51.7 %, 69.7 %, and 56.7 %, respectively). On the other hand, they showed higher levels of GSH (234.2 %, 170 %, and 221.7 %, respectively), GSH-Px (243.4 %, 189.2 %, and 244.3 %, respectively), SOD (271.5 %, 171.6 %, and 220.3 %, respectively), and CAT (236.8 %, 164 %, and 204.4 %, respectively). The control group also showed similar patterns in these parameters compared to AFB1; but the results revealed non-significant differences between the control, MRN30, and AFB1-MRN30 groups, except in MDA, which showed a significant decrease with MRN30 than AFB1-MRN30 (87 %), and a significant higher CAT level (115.9 %). Moreover, there were significant differences in the control, as well as MRN30 compared to AFB1-MRN15. Fig. 2 (A-F).

3.4. Effect of MRN on cardiac tissue oxidative stress and antioxidant status in AFB1-intoxicated rats

When comparing MRN30, AFB1-MRN15, and AFB1-MRN30 to AFB1, the results indicated significantly lower levels of MDA (42 %, 60.9 %, and 47 %, respectively), NO (49.8 %, 65.6 %, and 53.5, respectively), while higher levels of GSH (176.3 %, 138.1 %, 167.8 %,

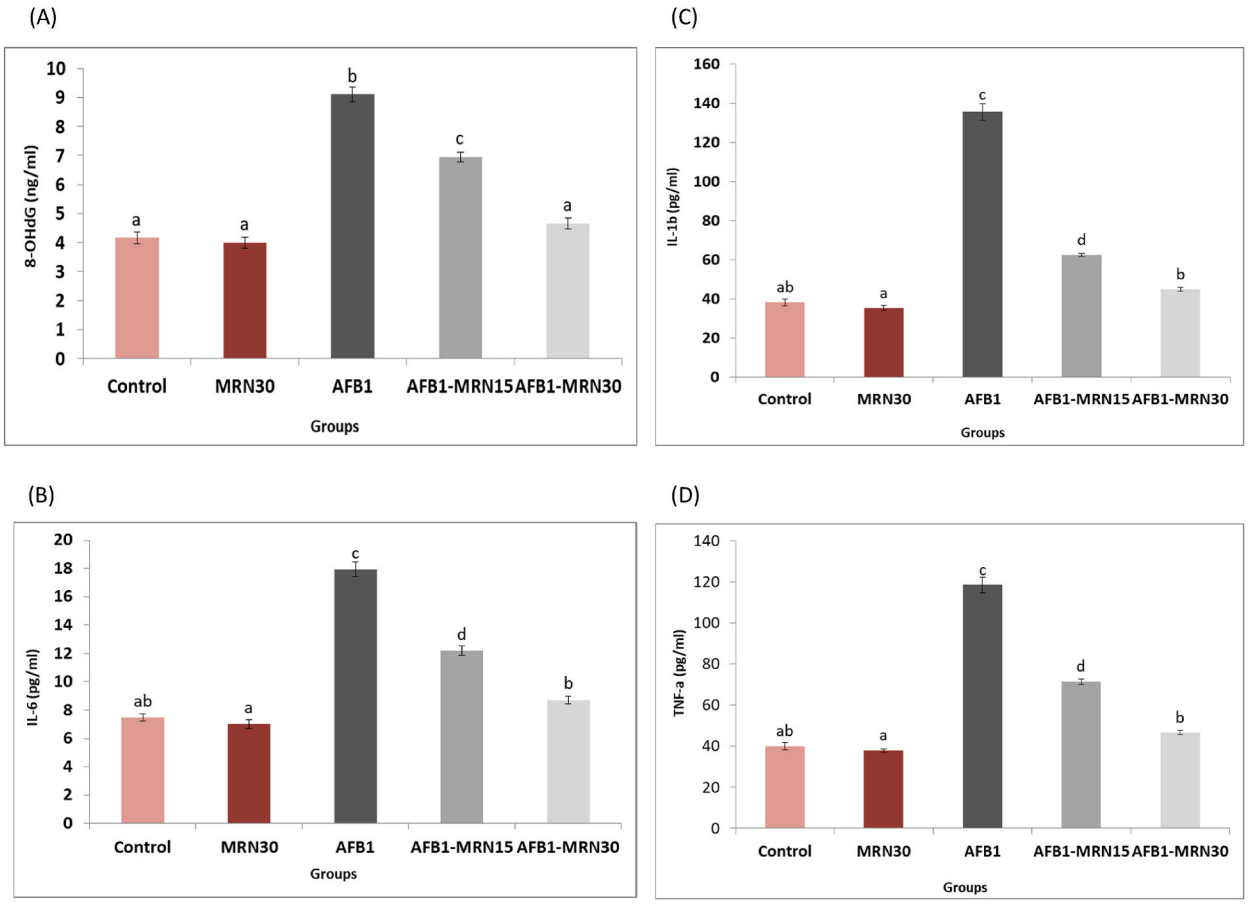

Fig. 1. Protective effects of morin on tissue serum DNA damage and proinflammatory cytokines levels in AFB1-intoxicated rats.
Legend:
A) 8-OHdG, 8-hydroxy-2′-deoxyguanosine; B) IL-1β, interleukin-1β; C) IL-6, interleukin 6; and D) TNF-a, tumor necrosis factor-alpha.
Data are presented as mean ± SE (number of each group = 8).
Columns labeled with different letters differ significantly ($p < 0.05$).

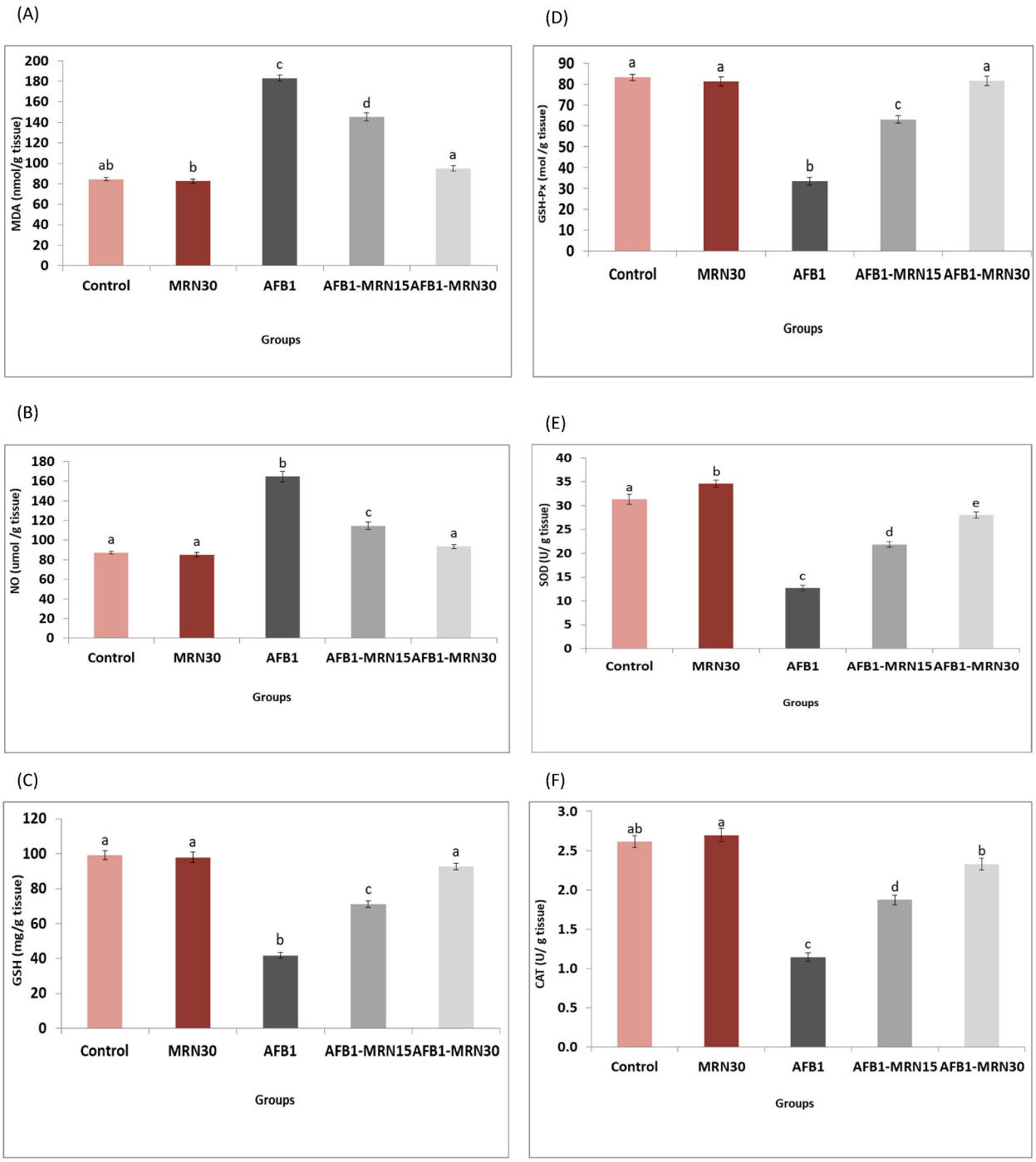

Fig. 2. Protective effects of morin on hepatic tissue oxidative stress and antioxidant status in AFB1-intoxicated rats.
Legend:
A) MDA, malondialdehyde concentration; B) NO, nitric oxide concentration; C) GSH, reduced glutathione concentration; D) GSH-Px, glutathione peroxidase activity; E) SOD, superoxide dismutase activity; and F) CAT, catalase activity.
Data are presented as mean ± SE (number of each group = 8).
Columns labeled with different letters differ significantly ($p < 0.05$).

respectively), GSH-Px (261.9 %, 162.7 %, 244 %, respectively), SOD (215.4 %, 153.6 %, and 188.5 %, respectively), and CAT (276.3 %, 194.9 %, and 250.8 %, respectively). Similarly, the control group had similar patterns in these parameters compared to AFB1. However, there were non-significant variations between the control, MRN30, and AFB1-MRN30 groups, except for the higher

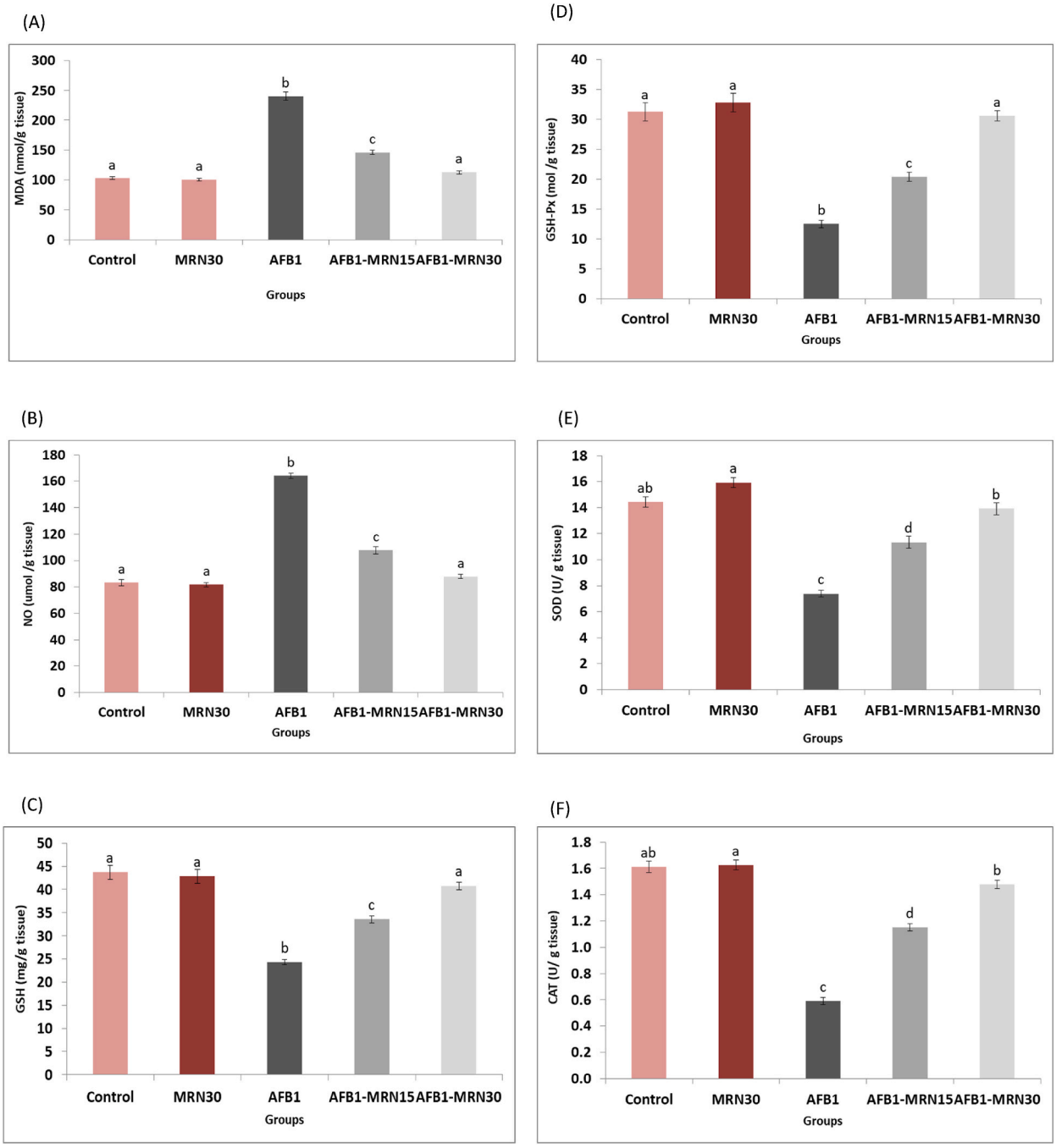

Fig. 3. Protective effects of morin on cardiac tissue oxidative stress and antioxidant status in AFB1-intoxicated rats.
Legend:
A) MDA, malondialdehyde concentration; B) NO, nitric oxide concentration; C) GSH, reduced glutathione concentration; D) GSH-Px, glutathione peroxidase activity; E) SOD, superoxide dismutase activity; and F) CAT, catalase activity.
Data are presented as mean ± SE (number of each group = 8).
Columns labeled with different letters differ significantly ($p < 0.05$).

significant levels of SOD (114.3 %) and a significantly higher CAT level (110.1 %) with MRN30 than AFB1-MRN30. Also, the results revealed significant differences with both the control and MRN30 groups compared to the AFB1-MRN15 group. Fig. 3 (A-F).

4. Discussion

The current study assessed the potential effect of morin against acute AFB1-induced hepatic and cardiac toxicity in rats. Our findings demonstrated that MRN at a dose of 30 mg/kg B. Wt. considerably enhanced the inflammatory response and oxidative damage and reduced antioxidant activities generated by AFB1 exposure. Notably, there were significant variations between the control and the AFB1-MRN15 groups.

Reactive oxygen species (ROS) is a normal product produced in cellular metabolism, but overproduction causes oxidative stress [37]. DNA damage and amino acid oxidation are possible results of this oxidative stress [38]. In the AFB1 group, the DNA oxidation marker (8-OHdG) was significantly elevated than in other groups, and the lowest marker level was observed in the AFB1-MRN15 group. The same results were observed in the proinflammatory cytokines, which confirmed that MRN at a dose of 30 mg/kg B. Wt.

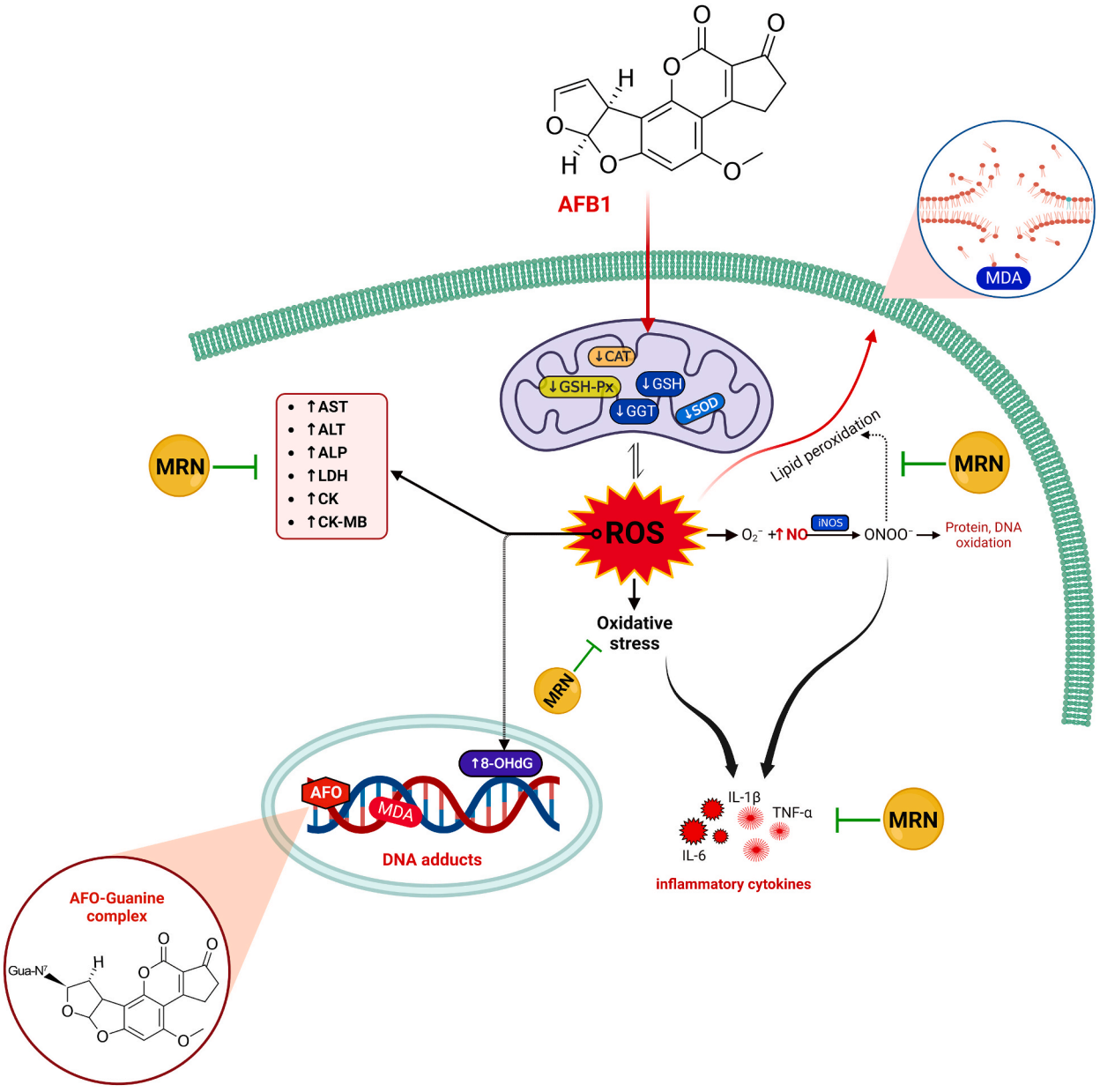

Fig. 4. Potential protective mechanisms of morin against AFB1-induced hepatic and cardiac toxicity in rats.

enhanced the inflammatory response and played a protective role against oxidative damage. Subash and Subramanian reported that in ammonium chloride (AC)- induced hyperammonaemia rats, morin significantly enhanced the antioxidants' status and lowered the liver markers' enzyme levels (serum AST, ALP, and ALT) when compared to the AC-treated group [39]. Rajput et al., in their recent comprehensive review of morin hydrate, reported morin's anti-inflammatory, antiapoptotic, and antioxidant effects [19]. Of the reported mechanisms, morin hugely decreased cyclooxygenase-2 (COX)-2 expression, 5-lipoxygenase (5-LOX), and inducible nitric oxide synthase (iNOS) genes. It also suppressed the phosphorylation pathway of the NF-ĸB (IĸB-α, P65) proteins and the MAPK (ERK and p38) [40]. Moreover, its pretreatment attenuated the pro-inflammatory cytokines' secretion. In addition, it diminished inflammation through NF-ĸB signaling and the NLRP3 inflammasome pathway [41].

Regarding hepatic and cardiac tissue oxidative stress and antioxidant status, there were significant differences in MDA, NO, GSH, GSH-Px, SOD, and CAT levels in the AFB1-MRN30 group compared to AFB1, and AFB1-MRN15 groups. Overall, our results highlight the antioxidant role of morin against AFB1-induced oxidative damage.

For morin's hepatic protective role, it up-regulated the expression of Nrf2 and its NQO1 and HO-1 downstream factors while decreasing AST, ALP, ALT, α-SMA, and collagen I&III in liver fibrosis induced by carbon tetrachloride [42]. For the cardioprotective role, Prahalathan et al. stated that, in rats, morin (50 mg/kg) supplementation enhanced DOCA-caused cardiac injury through lessening LPO and improving SOD, CAT, and GPx levels [43]. Several mechanisms were discussed [19], which mostly imply anti-inflammatory, antioxidant, and antiapoptotic morin effects [44]. Regarding the anti-inflammatory effect of morin, TNF- is the most significant proinflammatory cytokine involved in activating NF-κB and causing the expression of IL-1β, IL-6, COX-2, iNOS, and other downstream inflammatory mediators [45]. Gao et al. showed that TNF-, IL-1, IL-6, and inflammatory mediators COX-2 and iNOS were all significantly increased by AFB1. Still, their expression was significantly decreased by the morin group, suggesting that morin could effectively reduce the inflammatory response caused by AFB1 [46].

Feeding animals with AF-contaminated food led to oxidative stress, as shown by the significant lipid peroxidation increase and enzymatic antioxidant decrease like SOD and GSH-Px [47–49]. AFB1 promotes lipid peroxidation in rat liver, which is closely linked with hepatic cell injury [50]. AFB1 induced oxidative DNA damage in rat liver, revealed by a time-and dose-dependent 8-OHdG increase [50]. Moreover, AFs cause serious effects on the cardiovascular system, which could lead to cardiac damage [51]. In a study assessing morin's role in AFB1-caused hepatic and renal injury in chicks, the results demonstrated that morin could defend against AFB1-caused hepatic and renal damage through heterophil extracellular traps release inhibition, oxidative stress regulation, and inflammatory response inhibition [21].

In line with this study's results, it was suggested that morin might be of therapeutic value in preventing and treating several human disorders linked with oxidative stress [52]. This suggestion was built on morin showing protection of the lung fibroblast V79-4 cells from oxidative stress-caused DNA impairment and cell death through ROS generation suppression as well as malfunction of the mitochondria [52]. This mechanism was also connected with the activation of the Nrf2 the elevation of the expression of its downstream antioxidant gene, HO-1, which protects cells from the damaging effects of oxidative stress [52]. An in-vitro study reported morin's protection of pancreatic β-cells against oxidative stress-caused DNA damage through the enhancement of the intracellular antioxidants SOD, and CAT, and via Nrf2/ARE signaling pathway activation [53]. Caselli et al. [15] stated that morin's antioxidant activity is mainly because of the presence of a double bond between the carbon atoms (C2–C3) and the hydroxyl group that activates the double bond at the C3 position. Also, morin's anti-lipid-peroxidation activity appeared to be related to the two hydroxyl groups present on the B ring's 2′ and 4′ positions [54,55].

Depending on this study's results, morin showed significant improvements in acute AFB1-induced serum, liver, and heart toxicity in rats through its anti-inflammatory and antioxidant effects Fig. 4. This study could pave the way for future research to build on by providing an experimental basis and theoretical reference for the potential applications of morin. All in all, this could open doors for decreasing the public health concern of AFB1 hazards.

5. Conclusion

Morin showed significant protective potential in acute AFB1-induced hepatic and cardiac toxicity in rats, as oral morin (30 mg/kg) had significantly better results with inflammatory response, oxidative damage, and antioxidant activities in AFB1 exposure. We recommend applying morin in oxidative stress-related disorders. Further studies are needed to provide more information about the epigenetic alteration effect of AFB1 and the possible application of morin.

Data availability statement

The data are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Ahmed E. Altyar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. **Osama A. Kensara:** Formal analysis, Investigation, Methodology, Project administration, Software, Writing – original draft. **Amany A. Sayed:** Formal analysis, Investigation, Methodology, Project administration, Software, Writing – original draft. **Lotfi Aleya:** Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mikhlid H. Almutairi:** Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft. **Mohamed Sayed**

Zaazouee: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft. **Alaa Ahmed Elshanbary:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft. **Fatma M. El-Demerdash:** Data curation, Methodology, Project administration, Resources, Software, Supervision, Writing – original draft. **Mohamed M. Abdel-Daim:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors extend their appreciation to the Deputyship for Research and Innovation, “Ministry of Education” in Saudi Arabia for funding this research (IFKSUOR3-185–1).

References

[1] S. Rawal, J.E. Kim, R. Coulombe Jr., Aflatoxin B1 in poultry: toxicology, metabolism and prevention, Res. Vet. Sci. 89 (3) (2010) 325–331.
[2] W. Blount, Turkey" X" disease, J Br Turkey Fed 9 (1961) 52–77.
[3] D. Pickova, et al., Aflatoxins: history, significant milestones, recent data on their toxicity and ways to mitigation, Toxins 13 (6) (2021) 399.
[4] T. Asao, et al., Aflatoxins B and G, J. Am. Chem. Soc. 85 (11) (1963) 1706–1707.
[5] B.R. Rushing, M.I. Selim, Aflatoxin B1: a review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods, Food Chem. Toxicol. 124 (2019) 81–100.
[6] M.S. Uddin, et al., APOE and alzheimer’s disease: evidence mounts that targeting APOE4 may combat alzheimer’s pathogenesis, Mol. Neurobiol. 56 (4) (2019) 2450–2465.
[7] H. Jiang, et al., Inner-filter effect based fluorescence-quenching immunochromotographic assay for sensitive detection of aflatoxin B1 in soybean sauce, Food Control 94 (2018) 71–76.
[8] L. Xu, et al., Binding-induced DNA dissociation assay for small molecules: sensing aflatoxin B1, ACS Sens. 3 (12) (2018) 2590–2596.
[9] L. Liu, et al., Effects of selenium-enriched yeast improved aflatoxin B1-induced changes in growth performance, antioxidation capacity, IL-2 and IFN-γ contents, and gene expression in mice, Biol. Trace Elem. Res. 191 (1) (2019) 183–188.
[10] F.M. Reda, et al., Dietary supplementation of potassium sorbate, hydrated sodium calcium almuniosilicate and methionine enhances growth, antioxidant status and immunity in growing rabbits exposed to aflatoxin B1 in the diet, J. Anim. Physiol. Anim. Nutr. 104 (1) (2020) 196–203.
[11] S. Li, et al., Detection of Aflatoxin adducts as potential markers and the role of curcumin in alleviating AFB1-induced liver damage in chickens, Ecotoxicol. Environ. Saf. 176 (2019) 137–145.
[12] M.J. Mughal, et al., Aflatoxin B1 invokes apoptosis via death receptor pathway in hepatocytes, Oncotarget 8 (5) (2017) 8239–8249.
[13] J. Ge, et al., Assessment of aflatoxin B1 myocardial toxicity in rats: mitochondrial damage and cellular apoptosis in cardiomyocytes induced by aflatoxin B1, J. Int. Med. Res. 45 (3) (2017) 1015–1023.
[14] W.J. Wang, et al., Effects of aflatoxin B1 on mitochondrial respiration, ROS generation and apoptosis in broiler cardiomyocytes, Anim. Sci. J. 88 (10) (2017) 1561–1568.
[15] A. Caselli, et al., Morin: a promising natural Drug, Curr. Med. Chem. 23 (8) (2016) 774–791.
[16] Z. Wei, et al., Renoprotective mechanisms of morin in cisplatin-induced kidney injury, Int. Immunopharm. 28 (1) (2015) 500–506.
[17] G. Li, et al., Morin inhibits Listeria monocytogenes virulence in vivo and in vitro by targeting listeriolysin O and inflammation, BMC Microbiol. 20 (1) (2020) 112.
[18] Y. Zhou, et al., The anti-inflammatory effects of Morin hydrate in atherosclerosis is associated with autophagy induction through cAMP signaling, Mol. Nutr. Food Res. 61 (9) (2017).
[19] S.A. Rajput, X.-q. Wang, H.-C. Yan, Morin hydrate: a comprehensive review on novel natural dietary bioactive compound with versatile biological and pharmacological potential, Biomed. Pharmacother. 138 (2021), 111511.
[20] Y. Tian, et al., Protective effects of morin on lipopolysaccharide/d-galactosamine-induced acute liver injury by inhibiting TLR4/NF-κB and activating Nrf2/HO-1 signaling pathways, Int. Immunopharm. 45 (2017) 148–155.
[21] X. Gao, et al., Morin alleviates aflatoxin B1-induced liver and kidney injury by inhibiting heterophil extracellular traps release, oxidative stress and inflammatory responses in chicks, Poultry Sci. 100 (12) (2021), 101513.
[22] E.I. Ugwor, et al., Morin attenuates neurobehavioural deficits, hippocampal oxidative stress, inflammation, and apoptosis in rats co-exposed to bisphenol S and diethyl phthalate, Brain Res. 1794 (2022), 148068.
[23] S. Khamchai, et al., Morin attenuated cerebral ischemia/reperfusion injury through promoting angiogenesis mediated by angiopoietin-1-tie-2 Axis and wnt/β-catenin pathway, Neurotox. Res. 40 (1) (2022) 14–25.
[24] A.S. Akinrinde, T. Ogunbunmi, F.J. Akinrinmade, Acute aflatoxin B1-induced gastro-duodenal and hepatic oxidative damage is preceded by time-dependent hyperlactatemia in rats, Mycotoxin Res. 36 (4) (2020) 443–452.
[25] J.P. Vázquez-Medina, et al., Prolonged fasting increases glutathione biosynthesis in postweaned northern elephant seals, J. Exp. Biol. 214 (Pt 8) (2011) 1294–1299.
[26] N.W. Tietz, et al., A reference method for measurement of alkaline phosphatase activity in human serum, Clin. Chem. 29 (5) (1983) 751–761.
[27] S. Reitman, S. Frankel, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, Am. J. Clin. Pathol. 28 (1) (1957) 56–63.
[28] G. Szasz, W. Gruber, E. Bernt, Creatine kinase in serum: 1. Determination of optimum reaction conditions, Clin. Chem. 22 (5) (1976) 650–656.
[29] U. Würzburg, et al., [Determination of creatine kinase-MB in serum using inhibiting antibodies (author’s transl)], Klin. Wochenschr. 54 (8) (1976) 357–360.
[30] A.L. Babson, S.R. Babson, Kinetic colorimetric measurement of serum lactate dehydrogenase activity, Clin. Chem. 19 (7) (1973) 766–769.
[31] L.C. Green, et al., Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids, Anal. Biochem. 126 (1) (1982) 131–138.
[32] M. Mihara, M. Uchiyama, Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, Anal. Biochem. 86 (1) (1978) 271–278.
[33] E. Beutler, O. Duron, B.M. Kelly, Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61 (1963) 882–888.
[34] D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70 (1) (1967) 158–169.
[35] H. Aebi, Catalase in vitro, Methods Enzymol. 105 (1984) 121–126.

[36] M. Nishikimi, N. Appaji, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem. Biophys. Res. Commun. 46 (2) (1972) 849–854.
[37] M.A. Babizhayev, Generation of reactive oxygen species in the anterior eye segment. Synergistic codrugs of N-acetylcarnosine lubricant eye drops and mitochondria-targeted antioxidant act as a powerful therapeutic platform for the treatment of cataracts and primary open-angle glaucoma, BBA Clin. 6 (2016) 49–68.
[38] M. Nita, A. Grzybowski, The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults, Oxid. Med. Cell. Longev. 2016 (2016), 3164734.
[39] S. Subash, P. Subramanian, Effect of morin on the levels of circulatory liver markers and redox status in experimental chronic hyperammonaemic rats, Singap. Med. J. 49 (8) (2008) 650–655.
[40] J.M. Kim, et al., Morin modulates the oxidative stress-induced NF-κB pathway through its anti-oxidant activity, Free Radic. Res. 44 (4) (2010) 454–461.
[41] S. Yu, et al., Morin protects LPS-induced mastitis via inhibiting NLRP3 inflammasome and NF-κB signaling pathways, Inflammation 43 (4) (2020) 1293–1303.
[42] L. Sang, et al., Morin enhances hepatic Nrf2 expression in a liver fibrosis rat model, World J. Gastroenterol. 23 (47) (2017) 8334.
[43] P. Prahalathan, S. Kumar, B. Raja, Morin attenuates blood pressure and oxidative stress in deoxycorticosterone acetate-salt hypertensive rats: a biochemical and histopathological evaluation, Metabolism 61 (8) (2012) 1087–1099.
[44] V.K. Verma, et al., Role of MAPK/NF-κB pathway in cardioprotective effect of Morin in isoproterenol induced myocardial injury in rats, Mol. Biol. Rep. 46 (1) (2019) 1139–1148.
[45] P. Rajendran, et al., The multifaceted link between inflammation and human diseases, J. Cell. Physiol. 233 (9) (2018) 6458–6471.
[46] X. Gao, et al., Morin alleviates aflatoxin B1-induced liver and kidney injury by inhibiting heterophil extracellular traps release, oxidative stress and inflammatory responses in chicks, Poultry Sci. 100 (12) (2021), 101513.
[47] S.H. Abdel-Aziem, A.M. Hassan, M.A. Abdel-Wahhab, Dietary supplementation with whey protein and ginseng extract counteracts oxidative stress and DNA damage in rats fed an aflatoxin-contaminated diet, Mutat. Res. 723 (1) (2011) 65–71.
[48] M.A. Abdel-Wahhab, et al., Red ginseng extract protects against aflatoxin B1 and fumonisins-induced hepatic pre-cancerous lesions in rats, Food Chem. Toxicol. 48 (2) (2010) 733–742.
[49] M. Kanbur, et al., The effects of evening primrose oil on lipid peroxidation induced by subacute aflatoxin exposure in mice, Food Chem. Toxicol. 49 (9) (2011) 1960–1964.
[50] H.M. Shen, et al., Aflatoxin B1-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA, Carcinogenesis 16 (2) (1995) 419–422.
[51] A.M. Kudayer, et al., Toxic effect of aflatoxin B1 on heart, lung, and testis of male Albino rats: histopathology study, IOP Conf. Ser. Mater. Sci. Eng. 571 (1) (2019), 012055.
[52] M.H. Lee, et al., Antioxidant and cytoprotective effects of morin against hydrogen peroxide-induced oxidative stress are associated with the induction of Nrf-2-mediated HO-1 expression in V79-4 Chinese hamster lung fibroblasts, Int. J. Mol. Med. 39 (3) (2017) 672–680.
[53] P. Vanitha, et al., Morin activates the Nrf2-ARE pathway and reduces oxidative stress-induced DNA damage in pancreatic beta cells, Eur. J. Pharmacol. 801 (2017) 9–18.
[54] O. Farkas, J. Jakus, K. Héberger, Quantitative structure – antioxidant activity relationships of flavonoid compounds, Molecules 9 (12) (2004) 1079–1088.
[55] K.L. Wolfe, R.H. Liu, Structure-activity relationships of flavonoids in the cellular antioxidant activity assay, J. Agric. Food Chem. 56 (18) (2008) 8404–8411.