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The ameliorative effects of *Nigella sativa*, thymoquinone, and bentonite against aflatoxicosis in broilers via AFAR and Nrf2 signalling pathways, and down-regulation of caspase-3

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ABSTRACT

1. Aflatoxins (AFs) are metabolites which especially have toxic effects on proteins, and are detoxified by the aflatoxin-B_1 aldehyde reductase (AFAR) pathway. In this pathway, the aldo-keto reductase family 7, member A2 (AKR7A2) enzyme, which is controlled by nucleic-related erythroid factor 2 (Nrf2), plays an active role. However, data on the efficacy of this critical pathway in broilers is limited.
2. The aim of the following study was to investigate the changes in the expression levels of AKR7A2, Nrf2, and caspase-3, and the effects of *Nigella sativa* seeds (NS), thymoquinone (TMQ), and bentonite (BNT) in broilers exposed to AFs.
3. One-hundred broilers were divided into ten groups (control (CNT); AF; NS; TMQ; BNT; AF+TMQ; AF +NS; AF+BNT; AF+BNT+NS; AF+BNT+TMQ) and fed for 28 d. AF, TMQ, NS and BNT were added to diets at levels of 2 mg/kg, 300 mg/kg, 50 g/kg and 10 g/kg respectively.
4. The addition of AF to the diet decreased AKR7A2 and Nrf2 levels dramatically, but increased caspase-3 ($P < 0.01$). TMQ, NS and BNT additions to the diet eliminated all negative effects caused by AF ($P < 0.01$); and AKR7A2 and Nrf2 were further raised in TMQ and NS groups when compared to the control group. TMQ and NS showed a positive effect on detoxification parameters when given together with BNT.
5. Supplementation with NS and TMQ enhanced AF detoxification *via* the AFAR pathway, by increasing AKR7A2 and Nrf2 levels, in addition to reducing hepatocyte apoptosis.

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Mycotoxin; AKR7A2; apoptosis; Nrf2; black cumin; liver

Introduction

Aflatoxins (AFs) are a serious public health problem, due to their possible presence in animal product residues and contamination to some human food, apart from being an important problem in livestock, especially in the poultry industry. AFs, which are a Group I carcinogen in the International Agency for Research on Cancer classification, are bioactivated to form harmful metabolites that cause acute toxicity and mutations by binding to proteins and DNA as a result of phase I metabolism in the liver (Verma 2004). Aflatoxin-8,9-epoxide (AFBO), the most harmful metabolite of AFB_1, can react rapidly with DNA and cause carcinogenic effects (Deng et al. 2018). The toxic metabolites of AF resulting from phase I metabolism are firstly conjugated with glutathione (GSH) by glutathione S-transf erases (GST) and undergo phase II enzymatic metabolism to be rendered harmless (Bbosa et al. 2013). At the same time, some toxic metabolites that are toxic to proteins and are bioactivated by Phase I metabolism are detoxified through another mechanism, called the aflatoxin-B1 aldehyde reductase (AFAR) pathway (Kensler et al. 1999).

The conversion of the toxic AFB_1-dialdehyde to AFB_1-dialcohol is catalysed by AFB1 aldehyde reductase, a component of the aldo-keto reductase (AKR) superfamily (Hayes et al. 1993). Since the AFB_1 reduction pathway mediated by AFAR performs a critical role in the prevention of AF-induced cytotoxicity, it needs to be better studied to determine whether efficacy of these enzymes could be used for protective purposes against AF toxicity. In addition, although AFAR detoxification activity against AF in turkeys has been demonstrated (Klein et al. 2002, 2003), it has been noted that additional studies are still needed in other poultry (Deng et al. 2018).

AF intake with feed or food causes oxidative stress and liver damage (Karaman et al. 2010). Free radicals, AFB_1-DNA adducts and damage to cellular antioxidant systems generated during biotransformation of AFs dictate the severity and progression of hepatic injury induced by AFs (Ates and Ortatatli 2021b). Nucleic related erythroid factor 2 (Nrf2) is a transcription factor that controls the cellular antioxidant defence system by regulating genes in the various antioxidant proteins (GSH etc.), detoxifying enzymes (GST, catalase, haem oxygenase 1, and superoxide dismutase) and stress response protein pathways (Wang et al. 2018). Therefore, it has been reported that Nrf2 plays an important role in the host defence system against AF-induced inflammation, oxidative stress and toxicity (Liu and Wang 2016). It has been acknowledged that oxidative damage causes apoptosis (Chandra et al. 2000). Many substances, such as AFs that induce apoptosis, are both pro-oxidants and stimulants that increase cellular oxidative stress metabolism (Duan et al. 2005). In addition, defective proteins and mutations in the genes encoding them can cause apoptosis (Kumar et al. 2007).

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Supplemental data for this article can be accessed here.

Nigella sativa L. (NS) and thymoquinone (TMQ), which is considered the bioactive component of NS, have many pharmacological effects beside its antioxidant and hepatoprotective effects (Woo et al. 2012). The expression of nuclear receptors CAR, PXR and AhR, which are crucial in the phase-I metabolism of AFs, as well as cytochrome P450 enzymes (CYP450s) released under their control, have been found to be reduced by NS and TMQ. Thus, it has been demonstrated that the toxic effect of AF is alleviated, and the hepatobiliary serum parameters and histological appearance are ameliorated in broilers (Ates and Ortatatli 2021b). It has been reported that NS and TMQ boost AF conjugation by increasing GSH and GSTA3 levels, which play an important role in the phase-II detoxification mechanism, formation of AFB_1-DNA adducts and the toxic effects of AFs (Ates and Ortatatli 2021a). However, it is not known whether these substances affect the AFAR pathway, which is another AF detoxification stage.

Bentonites and other aluminosilicates are clays that bind the mycotoxins in feed in the gastrointestinal system, preventing their absorption from the intestines, thus reducing their toxic effects (Kiran et al. 1998). Clay-based toxin binders are added to feeds alone or in combination with other clays and yeast-based products (glucomannan etc.), especially for their effects on AFs (Oguz 2012; Oguz et al. 2018; Yalcin 2018).

The primary purpose of the following study was to determine the detoxification capacity and efficiency of the AKR7A2 enzyme belonging to the AFAR pathway. This has an important place in the detoxification mechanisms of AF in broiler chickens. In addition, the effectiveness of NS and TMQ in this pathway was examined by comparing with sodium bentonite (BNT), which is currently used as a toxin binder in feeds. Thirdly, the trial investigated the effects of AF, NS, TMQ, and BNT on Nrf2, which is a critical factor in protecting against oxidative damage, and elucidated the effects of these substances on AF metabolism. In addition, these substances were used to assess if they had a positive or negative effect on caspase-3, which is critical in the apoptosis pathway that increases due to the effect of AF.

Material and methods

Chickens and treatments

The experimental stages were approved by the Selcuk University Faculty of Veterinary Ethics Committee before the start of the study (No: 2017/58). In the study, 100-one day old male Ross broiler chicks (unvaccinated) were randomly divided into ten groups: control (basal diet); AF (2 mg/kg AF); TMQ (300 mg/kg TMQ); NS (50 g /kg NS); BNT (10 g/kg BNT); AF+BNT (2 mg/kg AF and 10 g/kg BNT); AF+TMQ (2 mg/kg AF and 300 mg TMQ); AF+NS (2 mg/kg AF and 50 g/kg NS); AF+BNT+NS (2 mg/kg AF, 10 g/kg BNT and 50 g/kg NS); AF+BNT+TMQ (2 mg/kg AF, 10 g/kg BNT and 300 mg/kg TMQ) and the trial was completed in four weeks (Table 1). The broilers were fed with a basal diet composed of starter feed (1–10 d, 22% crude protein and 3100 kcal/kg ME) and broiler grower feed (11–28 d, 20% crude protein and 3100 kcal/kg ME; Table S1). The basal diet was provided daily to meet the nutritional requirements (NRC 1994), and no anticoccidial, antibiotic, or similar additives were included. During the trial, 2 mg/kg AF as administered to cause symptoms of acute aflatoxicosis (Karaman et al. 2005) and 10 g/kg BNT was included at a generally accepted rate in broiler farming (Pappas et al. 2016) and mixed daily with the feed. In total, 5 g of pure crystalline TMQ was first homogenised with 95 g of milled rice. Then, 6 g of this mix was added to 1 kg of feed to produce a homogeneous mixture (300 mg TMQ/kg feed). To compare the effectiveness of NS and TMQ, the feeds were supplemented with NS containing the same quantity of TMQ. Because 1 g of NS contains an average of 6 mg of TMQ (Ali and Blunden 2003), the feeds were supplemented with 50 g of NS (containing approximately 300 mg of TMQ).

Chemicals

The strain of *Aspergillus parasiticus NRRL 2999* from the Agricultural Research Service Culture Collection (USDA, Agricultural Research Service, Peoria, IL, USA) was collected for the preparation of AF, while potato dextrose agar was provided from Merck Biomaterial GmbH (Darmstadt, Germany) and rice from Duru Food Co. (Karaman, Turkey). The TQ and NS seeds were purchased from Santa Cruz® (purity >98%, CAS 490–91-5, USA), and Bağdat Spices Company (Pururity: 100%, Ankara, Turkey), respectively. Sodium bentonite was obtained from Karben AŞ (Ankara, Turkey). Chemicals used in tissue processing in immunohistochemical (IHC) experiments (formaldehyde, alcohol, xylene, and paraffin) were acquired from Sigma-Aldrich (St. Louis MO., USA), and the immunostaining kits were purchased from Bond™ Polymer Refine Detection (Leica, DS9800 Buffalo Grove, IL, USA).

Aflatoxin analysis

AF was manufactured in rice by fermentation utilising the *Aspergillus parasiticus* NRRL 2999 strain (Shotwell et al. 1966) with slight modification by Demet et al. (1995). The fermented rice was dried, ground and analysed, by extraction using the HPLC method (Shimadzu, Tokyo, Japan) through

Table 1. Treatments according to trial groups.

Groups	Treatments
Control (CNT)	Standard broiler feed (Basal diet) and clean *ad-libitum* water were given
Aflatoxin group (AF)	2 mg/kg AF was added to the basal diet
Thymoquinone group (TMQ)	300 mg/kg TMQ was added to the basal diet
Nigella sativa group (NS)	50 g/kg black cumin seed (equivalent to approximately 300 mg/kg TMQ in feed) was added to the basal diet
Na bentonite group (BNT)	10 g/kg Na bentonite (BNT) was added to the basal diet
AF + BNT group	2 mg/kg AF and 10 g/kg BNT were added to the basal diet
AF + TMQ group	2 mg/kg AF and 300 mg/kg TMQ were added to the basal diet
AF + NS group	2 mg/kg AF and 50 g/kg NS were added to the basal diet
AF + BNT + NS group	2 mg/kg AF, 10 g/kg BNT and 50 g/kg NS were added to the basal diet
AF + BNT + TMQ group	2 mg/kg AF, 10 g/kg BNT and 300 mg/kg TMQ were added to the basal diet

the RF-20A fluorescence detector with 360 nm excitation and 430 nm emission wavelengths. AF analysis was carried out at the Pharmacology and Toxicology Laboratory, Faculty of Veterinary Medicine, Selcuk University, Turkey. The total AF was determined to be 41.8 mg/kg of rice powder (83.36% AFB_1: 34.84 mg/kg; 9.29% AFB_2: 3.88 mg/kg; 5.35% AFG_1: 2.24 mg/kg; and 2% AFG_2: 0.84 mg/kg). The rice powder was added to the basal diet to meet the 2 mg total AF/kg diet requirement.

Euthanasia and immunohistochemical method

At the end of the four-week study, chicks from all the groups were euthanised with sodium pentobarbital injection and systemic necropsy was performed. After removal, the liver was fixed within 10% neutral buffered formalin solution for 24 h, and then routine tissue processing procedures were applied (Leica, TP1020, Leica Microsystems, Wetzlar, Germany). A microtome was used to cut paraffin-embedded tissues into five thick slices (Leica RM 2125RT, Leica Microsystems, Wetzlar, Germany). The Bond™ Polymer Refine Detection kit protocol (Leica DS9800, Newcastle, UK) was used to stain the adhesive slides using the IHC staining machine (Leica Bond Max, Wetzlar, Germany). Primary antibodies were applied in accordance with the manufacturer's manual (3 antibodies; Aldo-Keto Reductase Family 7 Member A2 (AKR7A2, Abcam, ab97458, 1: 750); Nucleic related erythroid factor 2 antibody (Nrf2, Abcam, ab31163,1:200); Caspase-3 antibody (Abcam, ab44976, 1:100)). The Allred method was used to evaluate the results of IHC staining with AKR7A2 and Nrf2 primary antibodies (Choudhury et al. 2010). In this method, the staining intensity (0 no staining; 1 weak; 2 moderate; 3 intensive) and the staining diffuseness (0 no staining; 1 (> 0–1/100); 2 (> 1/100-1/10); 3 (> 1/10-1/3); 4 (> 1/3-2/3), 5 (> 2/3-1)) values were summed and the scores obtained for each case were used. To evaluate caspase-3, the method reported by O'Neill et al. (2001) was used with the following intensity scores: 0, no staining; 1, weak; 2, moderate; 3, strong, and where the percentage value of positive cells (0, no stain; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100%) were multiplied and the values obtained were recorded as the score of each case.

Statistical analyses

The Statistical Package for the Social Sciences program(SPSS Inc. for Windows® version 25.0, Chicago, IL, USA) was used. The Kolmogrov-Smirnov test was used to check the variation of the data, and the Levene test validated the homogeneity of the variances. The one-way ANOVA *post-hoc* Duncan test was used to analyse normally distributed data. The results were presented as mean ± standard error of mean (SEM), with $P < 0.05$ being significant.

Results

Effects of AF and AF plus other treatments on AKR7A2

The average scores and statistical differences calculated according to the groups for the intensity and amount of AKR7A2 immunoreactivity determined in the liver of broiler chicks in the study are summarised in Table 2. The immunoreactivity of AKR7A2 in the AF group was significantly decreased compared to the other groups ($P < 0.01$). However, AKR7A2 levels were increased significantly in all groups fed NS and TMQ, and this increase was higher in groups with NS added. The addition of BNT to diets eliminated the negative effects of AF on AKR7A2.

Immunoreactivity was determined to be concentrated in the centrilobular and intermediate regions of the liver (Figure 1).

Table 2. IHC staining scores and evaluation in the liver.

	IHC staining scores		
Groups	AKR7A2	Nrf2	Caspase-3
CNT	3,30 ± 0,15^{b}	4,30 ± 0,30^{d}	0,20 ± 0,13^{a}
AF	2,50 ± 0,22^{a}	2,90 ± 0,23^{e}	2,80 ± 0,39 ^{c}
TMQ	5,00 ± 0,26 ^{cd}	6,90 ± 0,28^{a}	0,40 ± 0,27^{a}
NS	5,60 ± 0,27^{d}	6,00 ± 0,47^{b}	0,50 ± 0,34^{a}
BNT	3,20 ± 0,13^{b}	4,00 ± 0,21^{d}	0,40 ± 0,22^{a}
AF+BNT	3,40 ± 0,27^{b}	3,80 ± 0,20^{d}	1,80 ± 0,36^{b}
AF+TMQ	4,40 ± 0,16 ^{c}	5,30 ± 0,26^{bc}	0,90 ± 0,28^{a}
AF+NS	5,20 ± 0,25^{d}	5,50 ± 0,50^{bc}	1,00 ± 0,26^{ab}
AF+BNT+NS	5,10 ± 0,31 ^{cd}	5,00 ± 0,26 ^{cd}	1,10 ± 0,18^{ab}
AF+BNT+TMQ	3,70 ± 0,30^{b}	5,50 ± 0,31^{bc}	1,00 ± 0,26^{ab}

$^{a-d}$According to the One-way ANOVA and the post-hoc Duncan test performed afterwards, the difference between the values that do not have the common letter in the column is important ($P < 0.01$).

Evaluation of Nrf2 expression

The degree of immunoreactivity and statistical evaluations of Nrf2 observed in the liver are shown in Table 2. Exposure to AF significantly reduced the Nrf2 level, and the addition of BNT in feed corrected this. The addition of NS or TMQ the AF-containing diet significantly increased Nrf2 levels ($P < 0.01$). The difference between AF+BNT+NS/TMQ groups and AF+NS and AF+TMQ groups was not significant ($P > 0.05$), that is the addition of BNT, along with NS or TMQ in rations with AF, did not create any additional effect. Nrf2 immunoreactivity was seen in the cytoplasm and nuclei of hepatocytes (Figure 2).

Effects of AF and other dietary supplements on apoptosis

The immunoreactivity of caspase-3 is presented in Table 2. While the caspase-3 activity was significantly increased in AF group, this situation improved significantly in the other experimental groups and was determined to be closer to the CNT group ($P < 0.01$). In addition, caspase-3 immunoreactivity was mostly localised in the cell nucleus in the TMQ groups. While a cytoplasmic and membranous immunoreactivity was observed in the other groups, this was seen less in the nucleus (Figure 3).

Discussion

Despite numerous studies on the inactivation of aflatoxins in AF in feed, feedstuffs, and foods or the biotransformation stages after entering the body, results that can be transferred to routine practice have not yet been fully achieved. Most of the substances and methods used for this purpose are not practical and economical to apply, may cause toxicity in effective concentrations or leave residues in feeds and foods. In addition, in order to better resolve the issue, it is necessary to investigate and understand the effect of the

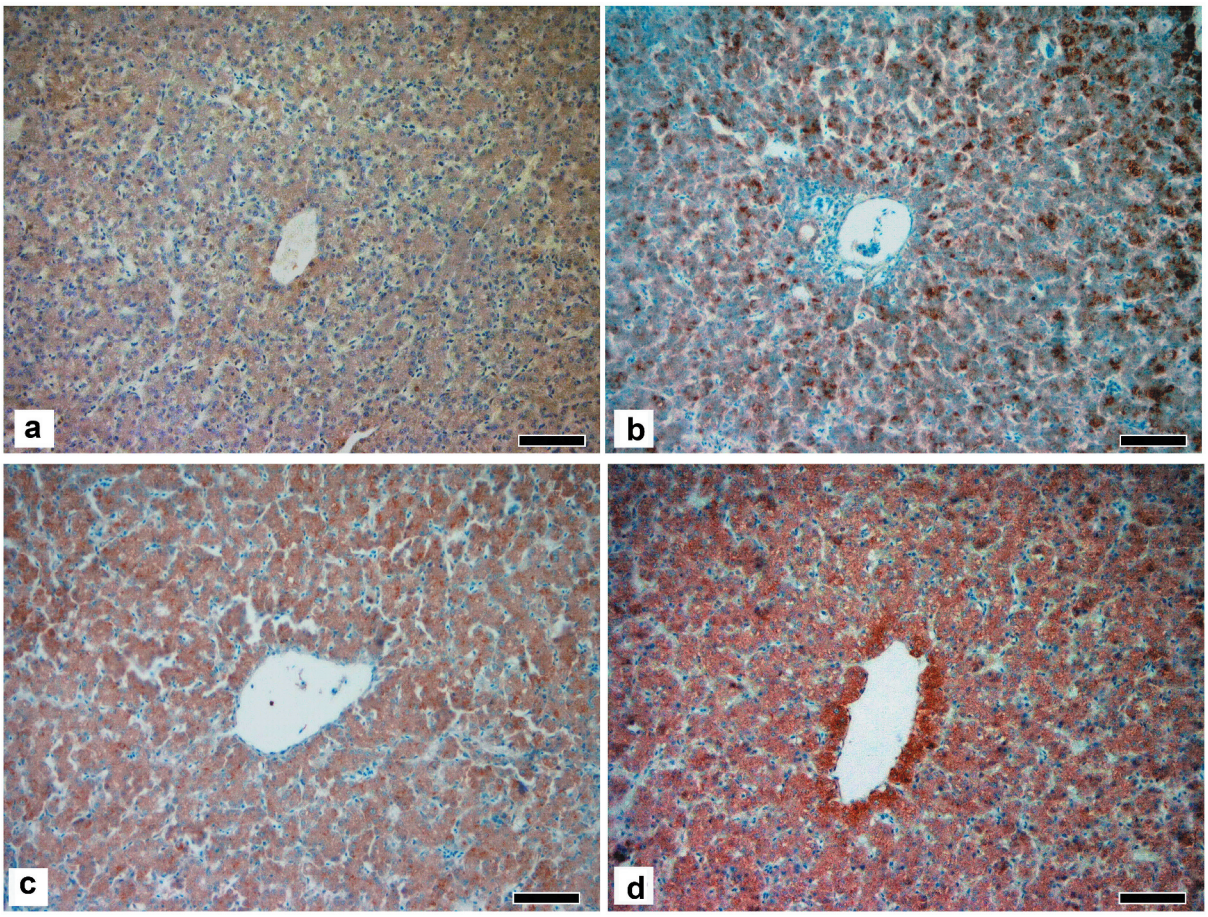

Figure 1. Immunoreactivity densities representing the amount of AKR7A2 in the liver tissue. (a) CNT group, moderate intensity positive immunoreactivity in hepatocytes; (b) AF group, mild positive immunoreactivity in hepatocytes; (c) AF+TMQ group, moderate and diffuse positive immunoreactivity in hepatocytes; (d) AF+NS group, intensive and widespread positive immunoreactivity in hepatocytes. IHC, DAB chromogen, Mayer's haematoxylin, Scale bar: 100 µm.

compounds used for protection on nuclear receptors, enzymes and some substances that play an important role in the prevention and control of AF cytotoxicity.

AFAR, a member of the aldo-keto reductase (AKR) enzyme family, has been shown to catalyse the reduction of AFB_1-dialdehyde, a toxic metabolite of AFs on proteins, to AFB_1-dialcohol (Hayes et al. 1993). The AFAR pathway has been shown to play a key role in phase-II detoxification of AFB_1 in humans, rats, and pigs (Ireland et al. 1998; Wu et al. 2013; Deng et al. 2018). Although a chicken DNA sequence corresponding to the *AKR7A2* gene is available in the National Biotechnology Information Center, it has been stated that additional studies are still needed (Klein et al. 2002, 2003; Deng et al. 2018; Murcia and Diaz 2020), although the presence of AKR7A2 has been demonstrated immunohistochemically in broiler liver. In the current study, the expression of AKR7A2 decreased in the group challenged AF, while those supplemented with NS, TMQ and BNT were shown to increase the quantity of *AKR7A2* in the liver by counteracting the detrimental effects of AF. A recent study has shown that AFAR activity is associated with resistance to the acute toxic effects of AFB_1 in chickens and ducks (Murcia and Diaz 2020). Therefore, NS and TMQ may have protective activity against the acute adverse effects of AF on the liver when compared with BNT supplemented group challenged with AF. The AKR7A2 enzyme level was found to be higher in the NS and TMQ groups challenged with AF. The use of NS and TMQ together with BNT has no positive or negative effects in terms of AKR7A2 levels.

Nucleic-related erythroid factor 2 (Nrf2) is considered as one of the most important defence systems in the antioxidant response elements pathway (Keap1-Nrf2-ARE) to protect against oxidative damage. It has been determined that high expression of Nrf2 protects the cell against such damage by increasing the level of antioxidant response element (ARE) dependent antioxidant enzymes (NAD(P)H quinone dehydrogenase, glutathione S-transferase, haem oxygenase-1, superoxide dismutase and glutathione peroxidase), which have important roles in cellular defence against reactive oxygen species. In the case of oxidative stress, Nrf2 translocalises into the nucleus after removing Keap1 suppression in the cytoplasm and stimulates the release of ARE-dependent antioxidant enzymes (Kensler et al. 2007; Sajadimajd and Khazaei 2018).

Previous studies have shown that Nrf2 activation enhances AF detoxification in rats, which is mediated by AKR7A2 and GSTA3 which are secreted in a Nrf2-dependent manner (Jowsey et al. 2003; Yates et al. 2006; Johnson et al. 2014; Liu and Wang 2016; Taguchi et al. 2016). In the current study, AF significantly decreased Nrf2 in the liver, like AKR7A2. It was thought that, with the administration of AF, Nrf2 expression in the cytoplasm would increase first, but the cell and DNA damage caused by oxidative stress (Ates and Ortatatli 2021a), which constantly increases due to the cumulative toxic effect of AF and cannot be adequately eliminated by the existing antioxidant systems, negatively affected the release of Nrf2. The decrease in ARE-dependent antioxidant enzyme levels, which are responsible for both AF detoxification and elimination of oxidative stress, in parallel with the decrease in Nrf2 release, may contribute to

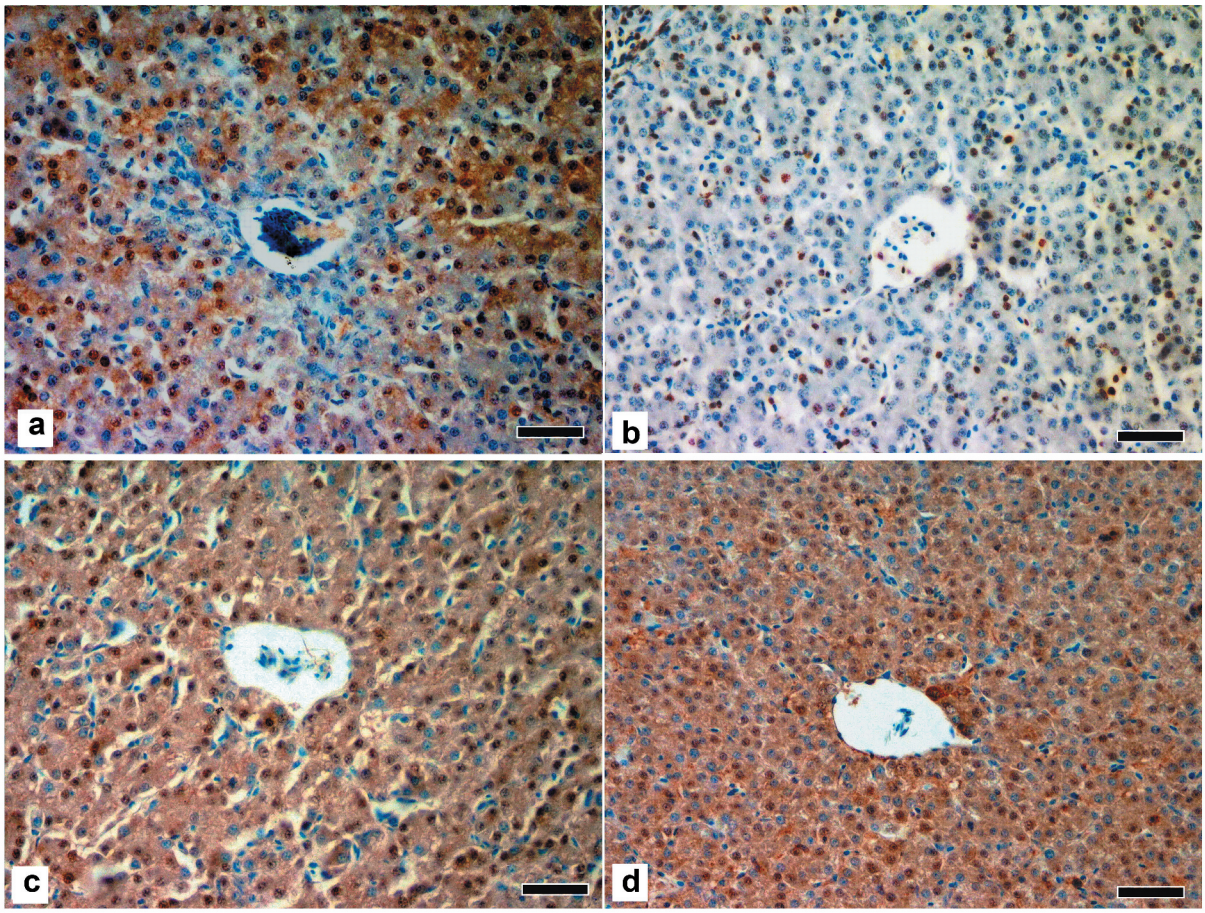

Figure 2. Nrf2 immunoreactivity densities in the liver tissue. (a) CNT group, moderate positive immunoreactivity in hepatocytes; (b) AF group, mild positive immunoreactivity in hepatocytes; (c) AF+TMQ group, moderate positive immunoreactivity in hepatocytes; (d) AF+NS group, intensive positive immunoreactivity in hepatocytes. IHC, DAB chromogen, Mayer's haematoxylin, Scale bar: 50 µm.

cellular damage in the liver (Keum et al. 2003; Lee and Johnson 2004; Chen et al. 2006). At the same time, it was revealed that the simultaneous decrease in AKR7A2 levels in the AF group was due to Nrf2, and this situation was consistent with other published trials (Jowsey et al. 2003; Yates et al. 2006; Taguchi et al. 2016). Thus, it was concluded that the Nrf2-dependent reduction of AKR7A2, may adversely affect AF detoxification.

The activation or blocking of the Nrf2 pathway has been proposed as a possible molecular basis for the toxicity of mycotoxins in humans and animals (Ulger et al. 2020; Kozieł et al. 2021) TMQ and NS activate the Nrf2 pathway by increasing Nrf2 levels, according to the current findings. Thus, TMQ and NS could have a hepatoprotective impact by detoxifying ROS and toxic metabolites that increased because of AF metabolism. While the effect of BNT on Nrf2 when used alone was insignificant compared to the CNT group, it returned to normal values in the presence of AF. This indicated that BNT bound AF in the intestines at certain rates which decreased their passage into blood from the GIT. The impact of BNT on Nrf2 was low compared to the NS and TMQ groups. The lack of antioxidant properties of BNT was considered to be the cause of this situation.

It has been stated that AF can cause apoptosis through the extrinsic mechanism by increasing the number of death receptors on the cell surface of hepatocytes, but the details of this mechanism are still unclear (Mughal et al. 2017). AFB_1 causes excessive expression of caspase-3 because of the increase of death receptors (FAS, TNF-R1) and related genes, and a decrease in anti-apoptotic proteins (BCL-2, XIAP) in chicken has been reported (Mughal et al. 2017). AFB_1 can directly or indirectly lead to caspase-3 activation and apoptosis in the liver, and this can be reduced by supporting the hepatic antioxidant/detoxification system (Meki et al. 2001). Ozen et al. (2009) found high levels of malondialdehyde (MDA) in the liver with many apoptotic cells in chicks exposed to AFB_1. There is no study investigating the effects of TMQ or NS on increased caspase-3 and apoptosis in aflatoxicosis situations. However, there are some studies investigating the effects of these substances on apoptosis, independently from AFs (Abd El-Ghany et al. 2009; Galaly et al. 2014; Pourbakhsh et al. 2014; Awad et al. 2016). The common result from these studies was that hepatic apoptosis was reduced by inhibiting the caspase-3 activity generated by the substances by TMQ or NS. In the current study, caspase-3 activity increased with the effects of AF. While this activity was decreased in all experimental groups, the decrease in caspase-3 expression was more pronounced in the groups given TMQ with AF challenge. It was thought that AF increased oxidative stress within the cell and caused apoptosis by inhibiting antioxidant mechanisms, while TMQ and NS prevented hepatic apoptosis by reducing the metabolism of AF due to their antioxidant properties. In addition, BNT may have decreased caspase-3 expression by reducing the amount of AF absorbed.

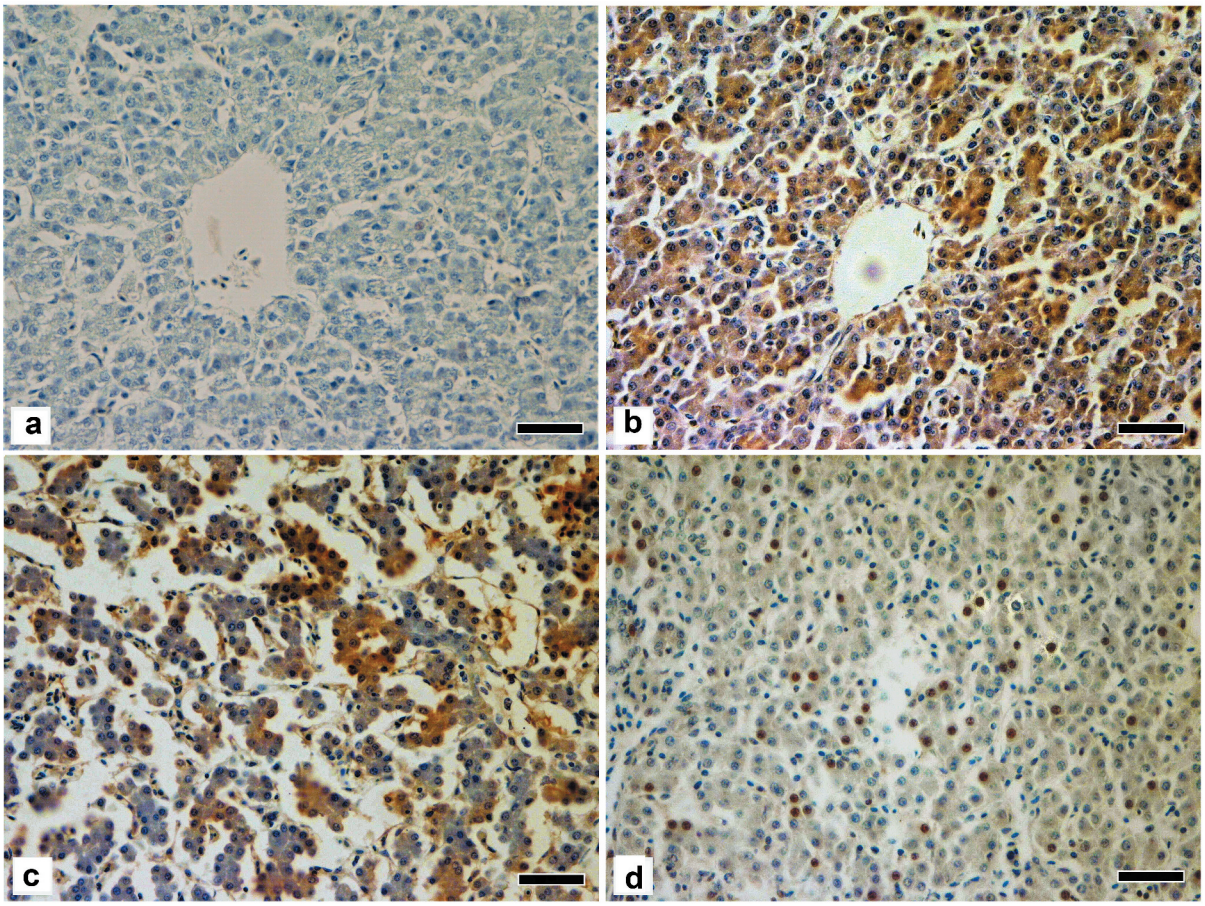

Figure 3. Caspase-3 immunoreactivity densities in the liver tissue, (a) CNT group, negative (no immunostaining); (b) AF group, diffuse and severe positive immunoreactivity in hepatocytes; (c) AF+BNT group, moderate positive immunoreactivity of hepatocytes; (d) AF+TMQ group, mild positive immunoreactivity in the nucleus of hepatocytes. IHC, DAB chromogen, Mayer's haematoxylin, Scale bar: 50 µm.

The addition of TMQ, NS, or BNT alone to the basal diet did not cause any differences from the CNT group in terms of the parameters examined or any toxic/harmful effects on the animal at the levels used. This indicated that the feed ingredients used in this study were inert and non-toxic to poultry according to EFSA (EFSA 2010).

NS and TMQ were shown to reduce phase-I bioactivation of AFs while improving phase-II detoxification capability, resulting in less liver damage and AFB1-DNA adducts (Ates and Ortatatli 2021a, 2021b). The current research found that NS and TMQ increased AKR7A2 enzyme levels in hepatocytes after Nrf2 activation, which inhibited apoptosis. When all these factors are evaluated, NS and TMQ were considered to have a complete hepatoprotective effect in aflatoxicosis.

In conclusion, the expression of Nrf2 was reduced considerably after AF administration, and the concomitant decrease in AKR7A2 levels was attributable to Nrf2 levels in broilers. TMQ and NS supported the hepatic antioxidant/detoxification system by increasing AKR7A2 and Nrf2 levels and minimised the negative effects of Afs, including hepatocyte apoptosis. Therefore, TMQ and NS can be used safely as a potential feed additive for the prevention of liver damage caused by AFs in broiler diets.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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