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Original article

The effects of dexamethasone and minocycline alone and combined with N-acetylcysteine and vitamin E on serum matrix metalloproteinase-9 and coenzyme Q10 levels in aflatoxin B1 administered rats

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Abstract

This study aimed to determine the effects of dexamethasone and minocycline alone and combined treatment with N-acetylcysteine (NAC) and vitamin E on serum coenzyme Q10 (CoQ10) and matrix metalloproteinase-9 (MMP-9) levels in rats administered aflatoxin B1 (AFB1). The study was carried out on 66 male Wistar rats. Following the intraperitoneal (IP) administration of AFB1 at dose of 2 mg/kg, minocycline (45 and 90 mg/kg, IP) and dexamethasone (5 and 20 mg/kg, IP) were administered alone and combined with NAC (200 mg/kg, IP) and vitamin E (600 mg/kg, IP). CoQ10 and MMP-9 levels were analyzed using the HPLC-UV method and a commercial kit by ELISA, respectively. AFB1 increased MMP-9 level and decreased CoQ10 level compared to the control group. After dexamethasone and minocycline administration, there is no increase in CoQ10 level, which is caused by AFB1. However, dexamethasone and minocycline combined with NAC+vitamin E caused significant increases in CoQ10 levels. Dexamethasone and minocycline alone and combined with NAC+vitamin E decreased MMP-9 levels compared to the single AFB1 treated group. The use of MMPs inhibitors and oxidative stress-reducing agents is anticipated to be beneficial in the poisoning with AFB1.

Key words: Aflatoxin B1, coenzyme Q10, matrix metalloproteinase-9, rat, treatment

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Introduction

Aflatoxin B1 (AFB1), which is a well- known hepatic carcinogen, is one of the most toxic mycotoxins. AFB1, a common food contaminant, causes immunodeficiency, reproductive problems, growth impairment, acute toxication, economic losses, and damage to organs such as the heart, kidney, brain (Tras et al. 2017, Shan 2020) It was reported that AFB1 passes into the brain and has a toxic effect on the central nervous system with its effect on both nerve cells and the blood-brain barrier (Qureshi et al. 2015, Mehrzad et al. 2017, Tras et al. 2017). In addition, AFB1 has destructive effects on subcellular organelles such as mitochondria and molecules such as DNA (Ge et al. 2017, Shan 2020).

Matrix metalloproteinases (MMPs), which are a zinc--linked endopeptidases and break down most extracellular matrix proteins, are considered to play a major role in the etiology of tissue injury, inflammation, brain edema, metastasis, and neurodegenerative diseases. It is reported that MMP9 and 2 isoforms found in vessels, especially MMP9, are activated by pathophysiological events caused by hemorrhages and traumas of the brain, stroke and impairment of blood-tissue barriers such as the blood brain barrier (Swann et al. 2007, Bolognin et al. 2009, Hayashi et al. 2009, Tianand Kyriakides 2009, Li et al. 2013, Yang et al. 2013). Several toxic agents, including some mycotoxins, activate MMPs by increase in the efficiency of tyrosine kinase mediated by oxidative stress and cytokine secretion (Haorah et al. 2008, Jayaraj et al. 2011, Weinberger et al. 2011, Agrawal et al. 2012, Chen et al. 2013, Wang et al. 2014). It is stated that the acute intoxication with mycotoxins may cause brain hemorrhages and sudden death due to it (Bergmann et al. 1988, Sabater-Vilar et al. 2004, Puel et al. 2010, Jayaraj et al. 2011, Agrawal et al. 2012). Free oxygen radicals (ROS) are important factor in the MMPs activation and the disruption of the blood-brain-barrier (BBB) (Kamada et al. 2007). Mycotoxins such as trichothecene, zearalonon and fusarium impair the blood-brain barrier by increasing cytokine and ROS production and MMP activity (Jayaraj et al. 2011, Wan et al. 2013, Jia et al. 2014).

The fragmentation of the basal membrane protein and the impairment of the BBB caused by the increase in MMP activity are inhibited by MMP inhibitors (Haorah et al. 2008). It is stated that the synthetic tetracyclines such as minocycline and doxycycline, which are defined as nonspecific MMP inhibitors, improve BBB function and are useful in the brain edema treatment (Amin et al. 1996, Yenari et al. 2006, Zhao et al. 2007, KaurandLing 2008, Homsi et al. 2009, Weinberger et al. 2011). The combination of minocycline, which is indicated to inhibit brain poly-ADP ribose polymerase-1, cyclooxygenase-2, inducible nitric oxide synthetase (INOS), metalloproteinase and NADPase, with N-acetylcysteine (NAC) has been shown to protect against the brain trauma-related perception and memory deficit in rats through the inhibition of neuroinflammation (Haber et al. 2013). The inhibitor effects of corticosteroids, which are used in the treatment of the brain edema and have been reported to restore BBB, on MMPs may be considered as the basis for the brain edema (Wolkowitz et al. 1990, Hoshino et al. 1999, Karssen et al. 2005, De Paiva et al. 2006, Green et al. 2009, Manzo-Avalosand Saavedra--Molina 2010, SandercockandSoane 2011, Wang et al. 2014). It has been shown that dexamethasone by decreasing the expression of MMP-2 and 9 improves the toxic effects of hydrogen sulfite causing acute lung injury (Wang et al. 2014). It has been experimentally proved that the antioxidant compounds such as Vitamins E and C, NAC and edaravon reduce MMP-9 activity and the damage of the BBB and have protective effects against the mitochondrial damage (Sokol et al. 1998, Yavuz et al. 2004, Manzo-Avalosand Saavedra-Molina 2010, Wang et al. 2012, Ashor et al. 2014, Miyamoto et al. 2014). Glutathione precursor NAC, which has thiol group and mucolytic, antioxidant and antiinflammatory effect, inhibits interleukin-8 and MMP-9 synthesis and supports mitochondrial ATP production at high doses (Kim et al. 2000, Woo et al. 2004, Radomska-Leśniewska et al. 2010, Saito et al. 2010, Tsai et al. 2010, McGill et al. 2014).

Coenzyme Q10 (CoQ10), also known as ubiquinone, is found especially in mitochondria and is one of the most important components of the electron transport chain and has protective properties on different membranes of the cell due to its antioxidant effect. The serum and tissue levels of CoQ10 decrease in metabolic and neurodegenerative diseases and poisonings (Miyake et al. 1999, Shults 2005, Abdallah et al. 2010, Coppadoro et al. 2013). The impairment of the BBB and increasing of mitochondrial ROS levels are observed in poisonings, hypoxia and chemical stress cases (Yeh et al. 2007, KaurandLing 2008, Zehendner et al. 2013, Ribas et al. 2014). No report is available about the effects of aflatoxins, which cause acute poisoning symptoms such as sudden death, bloody stool, epistaxis and convulsions on MMPs activity and CoQ10 level (Bortell et al. 1983, Chao et al. 1991).

This study is the first experimental research on the effect of AFB1 on MMP activity and CoQ10 levels in rat. Therefore, the aim of the present study was; 1) to investigate the effect of the AFB1 on MMP-9 and CoQ10 levels and 2) to evaluate the effect of combinations of MMP inhibitors (minocycline, dexamethasone) and antioxidant-effective agents

Table 1. Substances administered and their doses in groups of rats.

Group No	Group Name	Substances applied*	Dose
1	CNT	Corn oil	100 µL
2	AF	Aflatoxin B1	2 mg/kg
3	AF+LD	Dexamethasone	5 mg/kg
4	AF+HD	Dexamethasone	20 mg/kg
5	AF+LM	Minocycline	45 mg/kg
6	AF+HM	Minocycline	90 mg/kg
7	AF+LD+NE	Dexamethasone + N-acetylcysteine + Vitamin E	5 + 250 + 600 mg/kg
8	AF+HD+NE	Dexamethasone + N-acetylcysteine + Vitamin E	20 + 250 + 600 mg/kg
9	AF+LM+NE	Minocycline + N-acetylcysteine + Vitamin E	45 + 250 + 600 mg/kg
10	AF+HM+NE	Minocycline + N-acetylcysteine + Vitamin E	90+ 250 + 600 mg/kg
11	AF+HD+HM	Dexamethasone + Minocycline	20 + 90 mg/kg

CNT: Control, AF:Aflatoxin, AF+LD: Aflatoxin+Low Dose Dexamethasone, AF+HD: Aflatoxin+High Dose Dexamethasone, AF+LM: Aflatoxin+Low Dose Minocycline, AF+HM: Aflatoxin+High Dose Minocycline, AF+LD+NE: Aflatoxin+Low Dose Dexamethasone+N-acetylcysteine+Vitamin E, AF+HD+NE: Aflatoxin+High Dose Dexamethasone+N-acetylcysteine+Vitamin E, AF+LM+-NE: Aflatoxin+Low Dose Minocycline+N-acetylcysteine+Vitamin E, AF+HM+NE: Aflatoxin+High Dose Minocycline+N-acetylcysteine+Vitamin E, AF+HD+HM: Aflatoxin+High Dose Dexamethasone+High Dose Minocycline.

* All substances were administered intraperitoneally.

Drugs were administered at 3 h after administration of aflatoxin B1

(NAC, vitamin E) on plasma levels of MMP-9, which cause tissue damage and disruption of the BBB, and CoQ10, which is important for mitochondrial function, in AFB1 administered rats.

Materials and Methods

Chemicals and solutions

Dexamethasone (Decort, 4 mg/ml IM/IV, Injectable Solution, Deva Holding Inc., Turkey), Vitamin E (150 mg/ml, IM, Evigen, Aksu Farma Medicinal Products and Pharmaceuticals Co., Turkey), N-acetylcysteine (100 mg/ml, IV/IM, Hüsnü Arsan Drugs A.S., Turkey) and minocycline hydrochloride (Sigma-Aldrich Co. LLC, Darmstadt, Germany), AFB1 (Acros Organics, Thermo Fisher Scientifics, Germany). For animal applications, AFB1 (2 mg/ml) and minocycline hydrochloride (90 mg/ml) were dissolved in corn oil and sterile physiological saline, respectively. Chemicals with analytical purity were used in the study.

Experimental animals

A total of 66 Wistar male rats, 8-12 weeks old, obtained from Selcuk University Experimental Medical Application and Research Center were used in the study. During the study, animals were housed individually in polysulfone cages at 24±1°C, 60% atmospheric humidity and 12 hours of light/12 hours of darkness. Feed and water were provided ad libitum. The permit (2015/33) to the procedures to be performed on animals was obtained from Selcuk University Experimental Medical Application and Research Center Ethics Committee.

In the study, 66 rats were randomly divided into 11 groups with 6 animals in each group. The groupings, applied substances and their doses following AFB1 administration are presented in Table 1. A dose of 2 mg/kg of AFB1 was chosen to produce a non-lethal acute poisoning in rats. The doses of the drugs used in the study were determined according to the studies performed on rats in this area (Donald et al. 2003, Coskun et al. 2005, Golestani et al. 2006, Yenari et al. 2006, Zhao et al. 2007, Wrotek et al. 2011, Ekerbicer et al. 2016, Tripathi et al. 2020). Drugs were administered 3 h after administration of AFB1. In this study, blood samples from the heart of animals under thiopental sodium (40 mg/kg/bw, IP) anesthesia were taken 12 h following AFB1 administration. Then, the animals were sacrificed by cervical dislocation. The blood samples were placed into tubes containing gel to obtain serum and centrifuged at 5000 rpm for 10 min. Serum samples were stored at -70 °C until analysis.

CoQ10 analysis

The serum CoQ10 levels were determined by the HPLC-UV system using the commercial HPLC kit (Eagle Biosciences Inc., New Hampshire, USA) inclu-

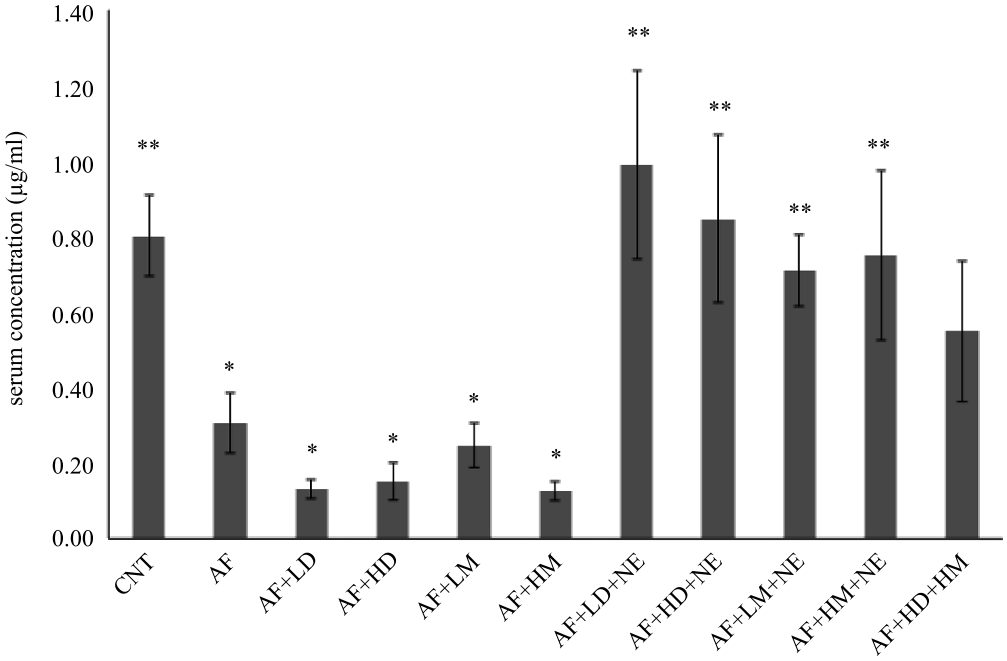

Fig. 1. The effects of administrations of dexamethasone and minocycline alone and in combination with N-acetylcysteine+vitamin E on the serum CoQ10 levels in aflatoxin B1 treated rats (n=6).

CNT: Control, AF:Aflatoxin, AF+LD: Aflatoxin+Low Dose Dexamethasone, AF+HD: Aflatoxin+High Dose Dexamethasone, AF+LM: Aflatoxin+Low Dose Minocycline, AF+HM: Aflatoxin+High Dose Minocycline, AF+LD+NE: Aflatoxin+Low Dose Dexamethasone+N-acetylcysteine + Vitamin E, AF+HD+NE: Aflatoxin+High Dose Dexamethasone+N-acetylcysteine+Vitamin E, AF+LM+NE: Aflatoxin+Low Dose Minocycline+N-acetylcysteine+Vitamin E, AF+HM+NE: Aflatoxin+High Dose Minocycline+N-acetylcysteine+Vitamin E, AF+HD+HM: Aflatoxin+High Dose Dexamethasone+ High Dose Minocycline.

Data are shown as the mean ± SD. Values with the different symbols indicate significant differences.

* Statistically different from the CNT group ($p<0.05$).

** Statistically different from the AF group ($p<0.05$).

ding calibrator and internal standard (IS). The HPLC system (Shimadzu, Tokyo, Japan) consisted of a pump (LC-20AT controlled by CBM-20A system), degasser (DGU-14A), autosampler (SIL-10AD), column oven (CTO-10A) and an SPD-20A UV detector. Column oven was set at 30°C. Autosampler was kept at room temperature. CoQ10 separation was performed with Ubichinon column (125x4 mm; ImmuChrom GmbH) and the wavelength was set at 275 nm. The flow rate of mobile phase in HPLC kit ingredient was 1 ml/min. Samples added IS were extracted according to HPLC kit procedure. A 100 µL of sample was injected to into HPLC system. HP PC-controlled LC solution software program (Shimadzu, Japan) was used for data analysis. The quantification is performed by following formula:

CoQ10 concentration in sample = (Peak area of CoQ10 in sample x Concentration of CoQ10 standard/ /Peak area of IS added to sample) x Peak area of IS added to calibrator/ Peak area of CoQ10 in calibrator.

The stock solution of CoQ10 (≥98%, Sigma, Aldrich) was prepared in 2-propanol to obtain a concentration of 1 mg/ml. Calibration standards (0.02-10 µg/ml) and quality control samples were prepared by adding standard solutions of CoQ10 and IS into the rat serum. Calibration standards were assayed in triplicate on 3 days to demonstrate the linearity of results. The calibration standards were linear with a correlation coefficient of >0.9993. The quality control samples, which were prepared in three replicate analyses of each level at the concentration of 0.04, 0.4 and 4 µg/ml within 1 day or on 3 consecutive days, were used to determine the recovery, precision, and accuracy. The recovery of CoQ10 from rat serum ranged from 89% to 103%. The low limit of quantification (LLOQ) was 0.02 µg/ml with an acceptable coefficient of variation (<20%) and bias (± 15%). The intraday and interday coefficients of variation for CoQ10 were ≤6.82% and ≤8.17%, respectively. The biases calculated for intraday and interday accuracy ranged from -5.92 to 6.12%.

Matrix metalloproteinase-9 analysis

The serum MMP-9 levels were determined using the commercial ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, PRC). The standard curve range and sensitivity of the test were 7.81-500 ng/mL and 4.69 ng/mL, respectively. The correlation coefficient of the standard curve range was >0.9990. Intraday variability determined with three repetitive analyses

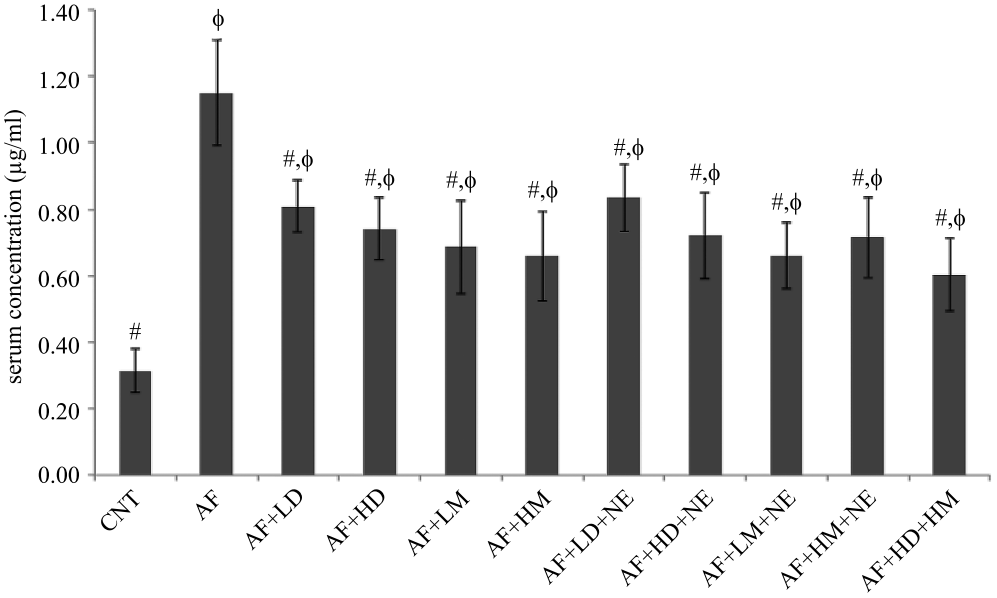

Fig. 2. The effects of administrations of dexamethasone and minocycline alone and in combination with N-acetylcysteine + vitamin E on the serum matrix metalloproteinase-9 in aflatoxin B1 treated rats (n=6).

CNT: Control, AF:Aflatoxin, AF+LD: Aflatoxin+Low Dose Dexamethasone, AF+HD: Aflatoxin+High Dose Dexamethasone, AF+LM: Aflatoxin+Low Dose Minocycline, AF+HM: Aflatoxin+High Dose Minocycline, AF+LD+NE: Aflatoxin+Low Dose Dexamethasone+N-acetylcysteine + Vitamin E, AF+HD+NE: Aflatoxin+High Dose Dexamethasone+N-acetylcysteine+Vitamin E, AF+LM+NE: Aflatoxin+Low Dose Minocycline+N-acetylcysteine+Vitamin E, AF+HM+NE: Aflatoxin+High Dose Minocycline+N-acetylcysteine+Vitamin E, AF+HD+HM: Aflatoxin+High Dose Dexamethasone+ High Dose Minocycline.

Data are shown as the mean ± SD. Values with the different symbols indicate significant differences.

[#] Statistically different from the CNT group ($p<0.05$).

[ϕ] Statistically different from the AF group ($p<0.05$).

of low (10 ng/mL), medium (100 ng/mL) and high (500 ng/mL) concentrations was accepted, with CVs (precision) of 4.37-12.05% and bias (accuracy) from -7.12% to 8.75%. Interday variability determined with three repetitive analyses of the each level on 3 different days was acceptable, with precision ranging from 5.64% to 13.92% and bias from -8.61% to 11.09%.

Statistical analysis

All data are presented as mean±SD. The Mann-Whitney U test was used to assess the group differences in the serum CoQ10 and MMP-9 levels. The serum CoQ10 and MMP-9 levels were compared between control and other groups or AF group and other groups. The SPSS 22.0 program (IBM Corp, Armonk, NY) was used for statistical analysis. Statistical significance was assigned at $p<0.05$.

Results

The effects of administrations of dexamethasone and minocycline as alone and in combination with NAC + vitamin E on the serum CoQ10 levels in rats receiving AFB1 are presented in Fig 1. AFB1 significantly decreased the serum level of CoQ10 compared to the control group. In the case of single dexamethasone and minocycline administration, there is no increase in the level of CoQ10, which is caused by AFB1 ($p>0.05$). However, dexamethasone and minocycline combined with NAC+vitamin E increased significantly in serum CoQ10 levels ($p<0.05$).

The effects of administrations of dexamethasone and minocycline as alone and in combination with NAC + vitamin E on the serum MMP-9 levels in rats receiving AFB1 are presented in Fig 2. AFB1 significantly increased the serum level of MMP-9 compared to the control group ($p<0.05$). The administration of the single and combined with NAC + vitamin E of dexamethasone and minocycline decreased MMP-9 levels compared to the single AFB1 treated group ($p<0.05$).

Discussion

In this study, AFB1 caused an increase in the serum MMP-9 levels at the dose administered. It is indicated that poisoning, trauma and various stress factors stimulate MMP-9 expression through ROS and inflammatory mediators, and increase the activity of the relevant enzyme (David et al. 2008, Haorah et al. 2008, Jayaraj et al. 2011, Weinberger et al. 2011, Agrawal et al. 2012, Chen et al. 2013, Wang et al. 2014, Yang et al. 2015,

Lundberg et al. 2016). Drug and drug combinations in all trial groups caused a statistically significant decrease in MMP-9 level compared to AF group. Previous studies have shown that the substances used in the trial lead to a decrease in MMP-9 levels and their effects are mediated by antiinflammatory and antioxidant activities (Zhang et al. 2008, Ramaesh et al. 2012, Sanches et al. 2013, Li et al. 2014, Lundberg et al. 2016). Similar to that reported by Lundberg et al, it was found in this study that the effects of minocycline and dexamethasone on MMP-9 were dose-related. We have observed that minocycline and dexamethasone, which have antiinflammatory and antioxidant effects, have a synergistic effect on MMP-9.

AFB1 caused a statistically significant decrease in serum CoQ10 level in this study. While single dexamethasone and minocycline treatment at the different doses decreased serum CoQ10 levels, the addition of NAC and vitamin E increased serum CoQ10 levels in all treatment groups. The increase provided by vitamin E and NAC may be related to the use of vitamin E and NAC instead of CoQ10 in oxidative mechanisms or the increase in CoQ10 synthesis. It has been indicated that vitamin E increases the tissue concentrations of CoQ10 and vitamin E-like tocotrienols increase CoQ10 synthesis (Bentinger et al. 2010). In addition, a positive correlation between the plasma concentrations of vitamin E and CoQ10 has been found (Gazdik et al. 2002). It is stated that the plasma concentration of CoQ10, expressed in organelles such as the mitochondria and peroxisomes of the cell, decreased in the patients receiving long-term corticosteroid therapy and this decrease was related to the disruption of the mitochondrial function by corticosteroids. In addition, it has been indicated that long-term use of corticosteroids causes dysfunction and oxidative damage in the skeletal muscle mitochondria (Mitsui et al. 2002). We found that the reducing effects of dexamethasone and minocycline on CoQ10 were reversed with NAC and vitamin E.

Conclusions

AFB1 caused increase in MMP activity and decrease in CoQ10 level. As oxidative stress and increasing in MMPs activity in the poisonings cause damage in tissues and impairment of the BBB, the use of MMPs inhibitors and oxidative stress-reducing agents is anticipated to be beneficial in the cases of exposure to AFB1.

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