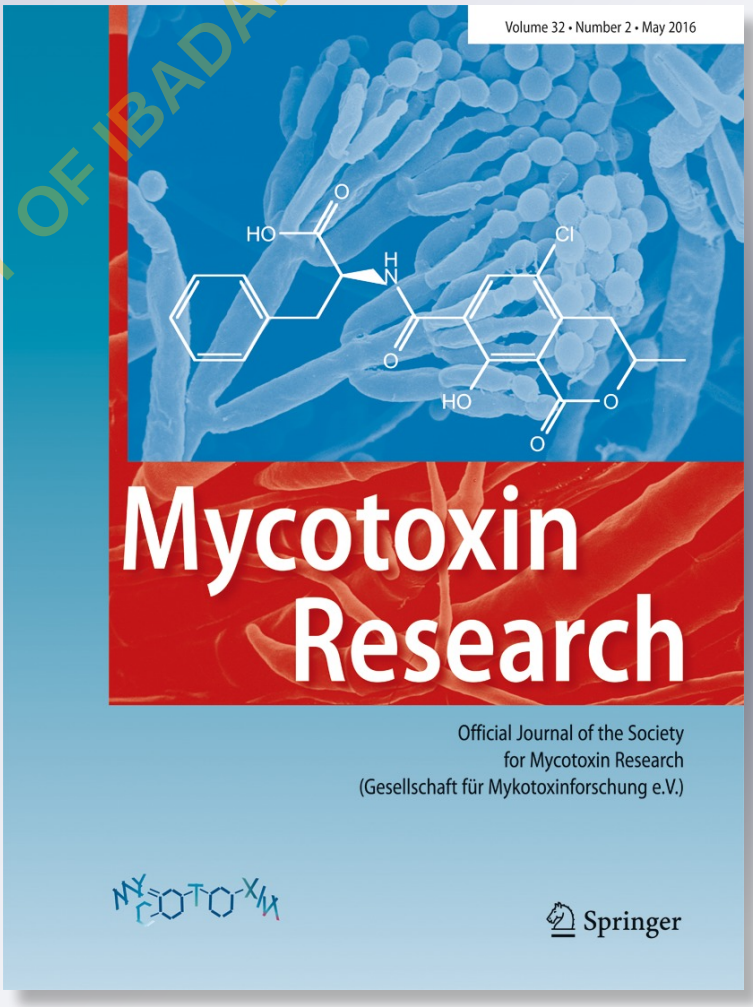
Changes in serum cytokine levels, hepatic and intestinal morphology in aflatoxin B1-induced injury: modulatory roles of melatonin and flavonoid-rich fractions from Chromolena odorata

Fadeyemi Joseph Akinrinmade, Akinleye Stephen Akinrinde & Adetayo Amid

Mycotoxin Research

ISSN 0178-7888
Volume 32
Number 2

Mycotoxin Res (2016) 32:53-60
DOI 10.1007/s12550-016-0239-9



Springer

Mycotoxin Res (2016) 32:53–60
DOI 10.1007/s12550-016-0239-9

ORIGINAL ARTICLE

Changes in serum cytokine levels, hepatic and intestinal morphology in aflatoxin B1-induced injury: modulatory roles of melatonin and flavonoid-rich fractions from *Chromolena odorata*

Fadeyemi Joseph Akinrinmade[1] · **Akinleye Stephen Akinrinde**[2] · **Adetayo Amid**[1]

Received: 21 August 2015 / Revised: 1 January 2016 / Accepted: 14 January 2016 / Published online: 22 January 2016

Abstract Aflatoxins are known to produce chronic carcinogenic, mutagenic, and teratogenic effects, as well as acute inflammatory effects, especially in the gastrointestinal tract. The potentials of the flavonoid-rich extract from *Chromolena odorata* (FCO) and melatonin (a standard anti-oxidant and anti-inflammatory agent) against aflatoxin B1 (AFB1)-induced alterations in pro-inflammatory cytokine levels and morphology of liver and small intestines were evaluated in this study. We utilized Wistar albino rats (200–230 g) randomly divided into five groups made up of group A, control rats; group B, rats given AFB1 (2.5 mg/kg, intraperitoneal) twice on days 5 and 7; rats in groups C, D, and E were treated with melatonin (10 mg/kg, intraperitoneal) or oral doses of FCO1 (50 mg/kg) and FCO2 (100 mg/kg) for 7 days, respectively, along with AFB1 injection on days 5 and 7. Serum levels of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) were determined using commercial ELISA kits and histopathological evaluation of the liver, duodenum, and ileum were also carried out. We observed significant elevation ($p < 0.05$) in serum IL-1β correlating with hemorrhages and leucocytic and lymphocytic infiltration in the liver and intestines as evidences of an acute inflammatory response to AFB1 administration. All treatments yielded significant reduction ($p < 0.05$) in IL-1β levels, although TNF-α levels were not significantly altered in all rats that received AFB1, irrespective of the treatments. Melatonin and FCO2 produced considerable protection of hepatic tissues, although melatonin was not quite effective in protecting the intestinal lesions. Our findings suggest a modulation of cytokine expression that may, in part, be responsible for the abilities of *C. odorata* or melatonin in amelioration of hepatic and intestinal lesions associated with aflatoxin B1 injury.

Keywords Aflatoxin B1 · Inflammation · *Chromolena odorata* · Melatonin · Liver · Gut

Introduction

Human and animal diets are often contaminated by toxic metabolic products of fungi called mycotoxins. Aflatoxins are highly potent mycotoxins produced by *Aspergillus* species. The most predominantly encountered aflatoxin is aflatoxin B1 (AFB1), which has been classified as a group 1 carcinogen (Eaton et al. 1994). Aflatoxin exposures occur mainly via ingestion of contaminated foodstuffs, in which case the absorption takes place through the gastrointestinal epithelium. However, blood-time concentration profiles of AFB1 in experimental animals have been obtained via intraperitoneal and intravenous routes (Hsieh and Wong 1994).

Absorbed AFB1 is translocated from the site of exposure to distant organs and tissues, the rate of organ perfusion being the primary factor determining the concentrations of the toxin reached in different tissues. Consequently, parenterally administered aflatoxin can reach substantial levels in highly perfused organs such as the liver. Bile produced in the liver is normally transported to the upper small intestine, where bile acids assist in digestion of lipids in the diet. In effect, xenobiotic metabolites that are secreted into bile can reach the small intestine,

✉ Akinleye Stephen Akinrinde
leyesteve2000@yahoo.com

[1] Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

[2] Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

where enterocytes are exposed to the toxic actions (Ito et al. 2001; Botta et al. 2004; Shibamoto and Bjeldanes 2009). Re-absorption and re-cycling of AFBI metabolites via entero-hepatic circulation also greatly contribute to a greater exposure and retention, and therefore, likelihood of toxicity of AFB1 to the intestinal epithelium (Grenier and Applegate 2013).

Much research on aflatoxin B1 transformation has focused on the chronic effects caused by formation of the toxic intermediate, exo-AFB1-8,9-epoxide (Gross-Steinmeyer and Eaton 2012), and other metabolites which can bind covalently to tissue macromolecules, forming lysyl adducts with proteins and guanine adducts with DNA (Bedard and Massey 2006). Biotransformation of AFB1 to this reactive epoxide occurs largely in the liver and also to some extent in extra-hepatic tissues (Sergent et al. 2008). Well characterized effects include the development of mutations of p53 and hepatocellular carcinoma (Ravinayagam et al. 2012). A number of reports in humans (Peraica et al. 1999) and animals (Gupta 2011) have, however, indicated symptoms of acute exposures to aflatoxins, including nausea, vomiting, diarrhea, abdominal pain, gastrointestinal erosions, etc. The exact mechanism by which AFB1 exerts acute effects on tissues, particularly the gastrointestinal tract is still largely unanswered. Effects on the cholinergic apparatus are thought to be implicated (Cometa et al. 2005).

More recently, the participation of inflammatory mediators in mycotoxin exposures have been evaluated. Available reports have suggested that low levels of mycotoxin exposure caused elevation in levels of some pro-inflammatory cytokines, while high concentrations induced down-regulation of some other cytokines (Grenier et al. 2012; Grenier and Applegate 2013; Singh et al. 2015).

Natural products from medicinal plants have been widely reported for their anti-fungal and anti-aflatoxigenic properties (Joseph et al. 2005; Kumar et al. 2007; Abdulmajeed 2011). *Chromolena odorata*, of the Asteraceae family, is a common perennial shrub that grows in many parts of the humid tropics and has been largely reported for its potent medicinal use due to its anti-inflammatory, anti-microbial, and cyto-protective properties (Owoyele et al. 2005; Nur Jannah et al. 2006; Ling et al. 2007, Hanh et al. 2011). A recent comprehensive review of the medicinal potentials of this plant is available (Omokhua et al. 2015). Phenolics from the plant have been found to exhibit potent protection against damage to cultured skin cells, with implications for cutaneous wound healing, a process with significant inflammatory component (Phan et al. 2001). We have also previously reported benefits of the methanol extracts of *C. odorata* in attenuation of intestinal injury in a model of ischemia-reperfusion injury in a previous study (Akinrinmade et al. 2015).

Aflatoxin-induced acute symptoms may involve inflammatory processes which may be related to the occurrence of some gastrointestinal symptoms earlier mentioned. In this study, we sought to investigate alterations in serum levels of the pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α), and interleukin 1 beta (IL-1β) following AFB1 administration in rats with a view to correlating these with lesions, if any, in the liver and intestinal tissues. As *C. odorata* has been reported to possess potent anti-inflammatory activities (Owoyele et al. 2005), we also investigated the effects of flavonoid-rich fractions from the plant on modulation of inflammatory status of the liver and intestines in rats exposed to AFB1.

Materials and methods

Chemicals and kits

Aflatoxin B1 was generously provided by Dr. Joseph Atehnkeng of the Aflasafe Laboratory at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Melatonin (5-methoxy-*N*-acetyltryptamine), TNF-α, and IL-1β kits were obtained from Abcam Biochemicals (Cambridge, UK). All other chemicals and solvents were of analytical grade and were purchased commercially.

Collection of plant materials and flavonoid extraction

Fresh *C. odorata* leaves were collected from the University of Ibadan campus. They were authenticated at the Department of Botany, University of Ibadan (voucher number UIH-22385). The leaves were air-dried at room temperature, after which they were milled into coarse powder with an electric blender. The plant materials were first de-fatted with *n*-hexane after which the flavonoid fraction was extracted using an ultrasonic method previously reported by Zhu et al. (2010), with slight modifications. A quantity of the de-fatted material was suspended in 70 % ethanol in the ratio of 1 g in 50 ml. This was placed in an ultrasonic bath (Supersonic X-3, Model DSD80A5QS) for flavonoid extraction for 1 h at room temperature (25 °C). The sample obtained was filtered, and the filtrate was concentrated using a rotary evaporator. The concentrated extract was suspended in distilled water for administration to animals.

Animals and experimental design

Thirty-five male Wistar rats (200–230 g) were obtained from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan. They were kept in plastic cages in a well-ventilated animal house and maintained on standard rat chow purchased commercially and clean water ad libitum. All animals received humane care in compliance with guidelines prepared by the National Academy of Science and published by the National Institutes of Health.

Institutional approval was obtained from the Animal Care and Use Research Ethics Committee of the University of Ibadan (Approval number: UI-ACUREC/App/2015/006). The animals were initially acclimatized for a period of 1 week after which they were divided randomly into five groups of seven rats each as follows: group A (control) included healthy rats given distilled water orally for 7 days; group B had rats twice administered intraperitoneally on days 5 and 7 with aflatoxin B1 (2.5 mg/kg); group C rats were treated with melatonin for 7 days (10 mg/kg) along with aflatoxin B1 on the 5th and 7th days; Rats in groups D and E were treated with the flavonoid fraction from *C. odorata* at either 50 mg/kg (FCO1) or 100 mg/kg (FCO2), respectively, together with aflatoxin B1 on the 5th and 7th days. All animals were killed on the 8th day, about 24 h after the last treatment.

Determination of serum TNF-α and IL-1β

Blood samples were collected in plain sample bottles and later centrifuged at 3000×*g* for 10 min. Serum was collected as the resultant supernatant and was used in the assay of TNF-α and IL-1β by means of kits supplied by Abcam Biochemicals (Cambridge, UK) using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Histopathological analysis

The liver and small intestines were carefully dissected out, and small portions of different lobes of the liver were removed, while small portions of the proximal and distal areas of the duodenum and ileum were cut and opened along their lengths. The collected tissues were rinsed in normal saline to remove traces of blood and were immediately fixed by suspending in 10 % formal saline prior to histological processing. The fixed tissues were initially sectioned at a thickness of 4 mm, then embedded in paraffin wax and microtome sections at 4 μm were finally obtained. The sections were stained with hematoxylin and eosin (H&E) and examination was done by light microscopy by pathologists who were blind to the treatments in the experimental groups. The histological criteria for the assessment of lesions are as stated in Tables 1 and 2. The severity of lesions were graded as absent (−), mild (+), moderate (++), or severe (+++).

Results

Changes in serum cytokine levels after aflatoxin B1 administration and treatment with melatonin and flavonoid fractions of *C. odorata*

The serum levels of IL-1β and TNF-α are presented in Fig. 1. Animals exposed to AFB1 alone demonstrated a significant increase ($p < 0.05$) in IL-1β levels compared with the controls. Treatment with melatonin and the two doses of the flavonoid fractions from *C. odorata* produced significant reductions ($p < 0.05$) in IL-1β levels. On the other hand, a significant reduction ($p < 0.05$) in TNF-α levels was observed in rats exposed to AFB1 alone when compared with control. However, neither melatonin nor the flavonoid fractions were able to raise TNF-α levels to those in the control.

Table 1 Summary of severity of histopathological lesions induced by aflatoxin B1 in the liver

	Histopathological lesion	A	B	C	D	E
1	Hemorrhage	−	+++	−	−	−
2	Leukocyte infiltration	−	++	−	++	−
3	Sinusoidal dilatation	−	++	−	−	−
4	Portal tract congestion	−	++	−	−	−
5	Hepatocellular necrosis	−	−	−	−	−
6	Cytoplasmic vacuolation	−	−	−	−	−

A control, *B* AFB1 (2.5 mg/kg), *C* AFB1 (2.5 mg/kg) + melatonin (10 mg/kg), *D* AFB1 (2.5 mg/kg) + FCO (50 mg/kg), *E* AFB1 (2.5 mg/kg) + FCO (100 mg/kg), "−" absent (severity score), "+" mild (severity score), "++" moderate (severity score), "+++" severe (severity score)

Histopathology

Photomicrographs showing the histological appearance of the liver in the different groups are presented in Fig. 2. The

Table 2 Summary of severity of histopathological lesions induced by aflatoxin B1 in the duodenum and ileum

	Histopathological lesion		A	B	C	D	E
1	Hemorrhage	Duodenum	−	−	−	−	−
		Ileum	−	−	−	−	−
2	Sub-epithelial space	Duodenum	−	+++	+++	+	−
		Ileum	−	+++	+++	−	−
3	Villi degeneration	Duodenum	−	+	+	−	−
		Ileum	−	++	+	−	−
4	Loss of villi	Duodenum	−	−	−	−	−
		Ileum	−	−	−	−	−
5	Leucocyte infiltration	Duodenum	−	++	++	+	+
		Ileum	−	+	+	+	+
6	Lymphocyte infiltration	Duodenum	−	+	−	−	−
		Ileum	−	+	+	−	−
7	Hyperplasia of lamina propria	Duodenum	−	−	−	−	−
		Ileum	−	−	−	−	−

A control, *B* AFB1 (2.5 mg/kg), *C* AFB1 (2.5 mg/kg) + melatonin (10 mg/kg), *D* AFB1 (2.5 mg/kg) + FCO (50 mg/kg), *E* AFB1 (2.5 mg/kg) + FCO (100 mg/kg), "−" absent (severity scores), "+" mild (severity scores), "++" moderate (severity scores), "+++" severe (severity scores)

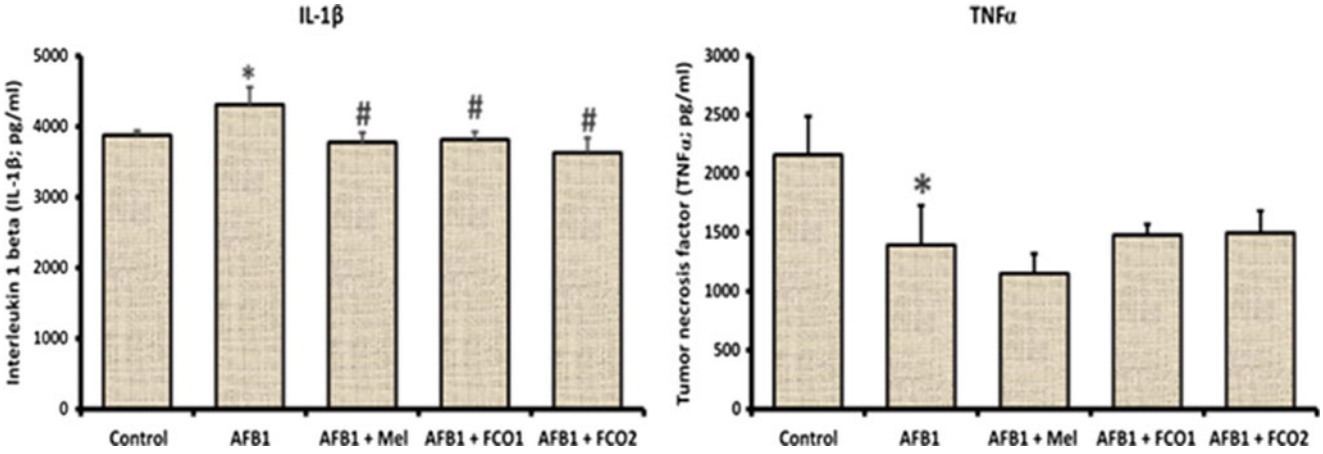


Fig. 1 Changes in serum tumor necrosis factor alpha (*TNF-α*) AND interleukin 1 beta (*IL-1β*) levels after aflatoxin B1 administration. Values are expressed as mean ± standard deviation. *Asterisk* indicates significant difference ($p < 0.05$) compared with control; *number sign* indicates significant differences compared with aflatoxin B1 group. *AFB1* aflatoxin B1, *Mel* melatonin, *FCO1* flavonoid fraction of *Chromolena odorata* (50 mg/kg), *FCO2* Flavonoid fraction of *C. odorata* (100 mg/kg)

severity of the lesions is as summarized in Table 1. Control rats had normal architecture of the liver with normal hepatocytes and clear sinusoids and portal tracts. Rats exposed to aflatoxin B1 alone showed hemorrhages with dilated sinusoids and inflammatory cellular infiltration. The portal tracts also exhibited some degrees of congestion. Considerable protection of haptic morphology was obtained in animals treated with melatonin and the higher dose of the flavonoid fraction of *C. odorata*. The lower dose of the latter, however, could not prevent inflammatory cell infiltration as was obtained with rats given aflatoxin B1 alone. In all the groups, the hepatocytes were not severely affected.

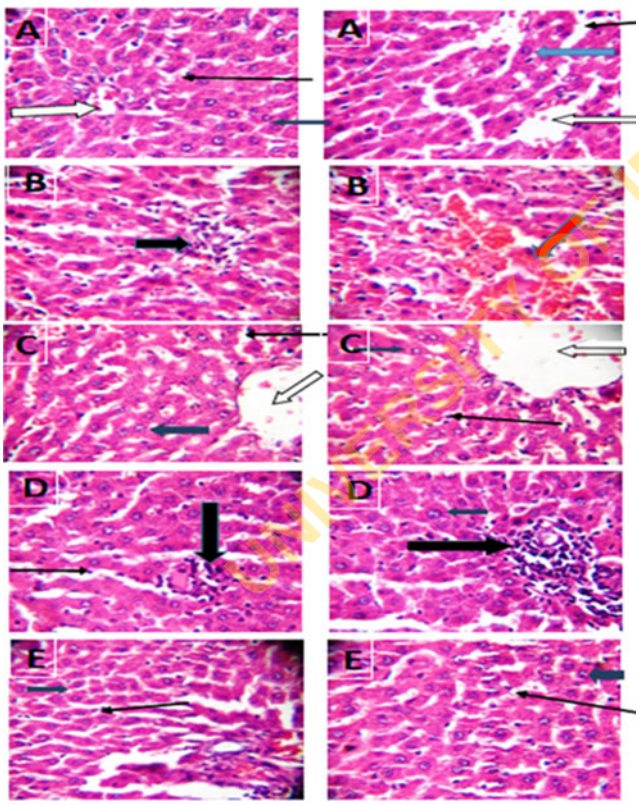


Fig. 2 Histological architecture of the liver (×400) following AFB1 administration. Photomicrographs show two different representative portions of the liver of each of **a** control rats, **b** rats given with AFB1 alone (2.5 mg/kg), **c** rats given AFB1 (2.5 mg/kg) and melatonin (10 mg/kg), **d** rats given AFB1 (2.5 mg/kg) with FCO1 (50 mg/kg), and **e** rats given AFB1 (2.5 mg/kg) with FCO2 (100 mg/kg). *Blue arrows* show normal hepatocytes; *thin black arrows* show normal sinusoids; *white arrows* show normal portal tracts; *black arrows* show inflammatory cell infiltration and red arrows show hemorrhages

In the duodenum and ileum (Figs. 3 and 4, respectively), the major lesions observed with aflatoxin B1 exposure included the development of sub-epithelial space, with mild to moderate degrees of villi degeneration. Moderate degrees of leucocyte and lymphocyte infiltration of the lamina propria were also observed. These lesions were also observed in the rats treated with melatonin. However, treatment with flavonoids from *C. odorata* produced significant amelioration of the aforementioned lesions compared with the rats in the aflatoxin B1 group.

Discussion

Aflatoxin B1 has been shown to induce immune-inflammatory responses in different conditions (Li et al. 2014; Qian et al. 2014) The immunosuppressive effects of aflatoxins, such as toxicity to T cell populations have also been well documented (Meisonnier et al. 2008). However, the effects of aflatoxin B1 on cytokine expression so far, still requires harmonization as some conflicting results have been obtained from in vitro, ex vivo and in vivo experiments (Meisonnier et al. 2008). Inflammation, immune response, and repair are major events in the early response to tissue injury. Measurement of serum cytokines have been considered valuable biomarkers in routine toxicity studies to detect drug-

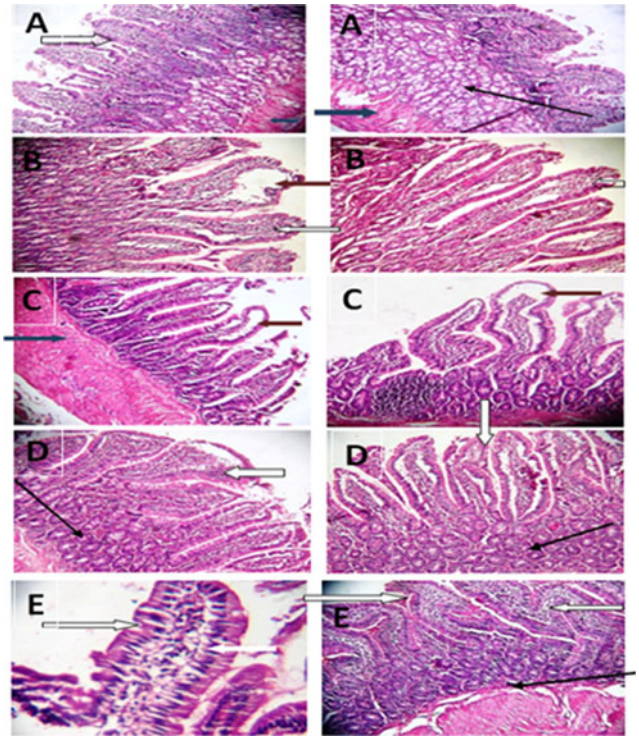


Fig. 3 Histological architecture of the duodenum (×100) following aflatoxin B1 administration. Photomicrographs show proximal and distal portions of the duodenum of each of **a** control rats, **b** rats given with AFB1 alone (2.5 mg/kg), **c** rats given AFB1 (2.5 mg/kg) and melatonin (1 0mg/kg), **d** rats given AFB1 (2.5 mg/kg) with FCO1 (50 mg/kg), and **e** rats given AFB1 (2.5 mg/kg) with FCO2 (100 mg/kg). *White arrows* indicate normal villi; *thin black arrows* show intestinal glands; *blue arrows* indicate normal muscularis mucosa; *red arrows* show development of sub-epithelial space

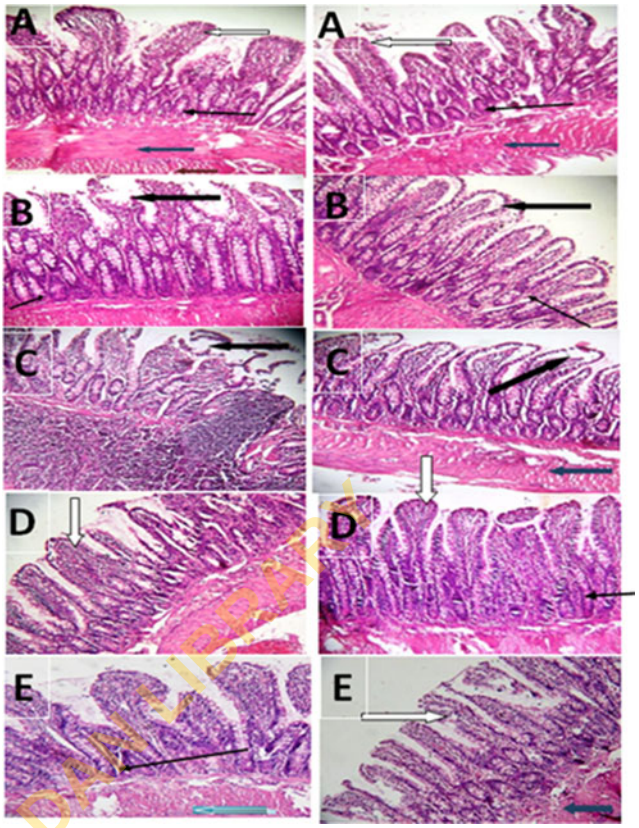


Fig. 4 Histological architecture of the ileum (×100) following aflatoxin B1 administration. Photomicrographs show proximal and distal portions of the duodenum of each of **a** control rats, **b** rats given with AFB1 alone (2.5 mg/kg), **c** rats given AFB1 (2.5 mg/kg), and melatonin (10 mg/kg), **d** rats given AFB1 (2.5 mg/kg) with FCO1 (50 mg/kg), and **e** rats given AFB1 (2.5 mg/kg) with FCO2 (100 mg/kg). *White arrows* indicate normal villi; *thin black arrows* show intestinal glands; *blue arrows* indicate normal muscularis mucosa; *thick black arrows* show development of sub-epithelial space

induced toxicities to liver and vascular tissues (Lacour et al. 2005; Kerns et al. 2005). The accessibility to blood for serial quantitative monitoring of these cytokines in a patient with availability of assay platforms makes the utilization of cytokines as markers of inflammation highly useful in clinical medicine (Tarrant 2010).

In this study, we aimed to investigate whether changes in the morphology of the liver and intestines induced by aflatoxin B1 were accompanied by any significant alterations in serum cytokine levels. The reported anti-inflammatory potentials of melatonin and *C. odorata* were also investigated for their roles against AFB1-induced acute inflammation. We observed a significant increase in interleukin 1 beta levels in rats given AFB1 alone, while treatments with melatonin and the flavonoid fractions of *C. odorata* reversed this trend and returned IL-1β levels to those of control rats. Elevation of this pro-inflammatory cytokine clearly indicates the occurrence of some degree of systemic inflammation (Safieh-Garabedian et al. 1995). Inflammation is basically a component of the body's response to many chemical insults that normally serves to protect tissue integrity, although excessive inflammatory responses can eventually lead to damage to normal tissues (Rostasy 2005).

Contrary to the response obtained for IL-1β, significant reduction in TNF-α was obtained in all the groups exposed to AFB1, compared with control rats, irrespective of treatments with either melatonin or *C. odorata*. AFB1 has been reported to inhibit the production of TNF-α and other cytokines in mice in vivo (Moon et al. 1999a) and in murine macrophages (Moon et al. 1999b) and lipopolysaccharide-activated human macrophages (Rossano et al. 1999) in vitro. The reduction in cytokine expression induced by aflatoxin B1 may be attributed to impairment in immune functions usually seen with aflatoxins (Bruneau et al. 2009). The lowering of TNF-α in all the groups exposed to AFB1, including the treated groups, may suggest a more selective effect towards TNF-α, rather than IL-1β. Previous studies have also attributed the lowering of TNF-α production to a reduction in secretory molecules, such as CD14, which stimulate the secretion of TNF-α by macrophages (Takai et al. 1997). Melatonin has been previously reported to reduce the expression of TNF-α (Wu et al. 2001). The profound reduction in TNF-α obtained in the group of rats treated with melatonin in this

study may indicate a combination of the immunosuppressive effects of AFB1 and the anti-inflammatory effects of melatonin. The same reasoning could be responsible for the similar response obtained with *C. odorata*.

The liver has been identified as a major target for the toxicity of aflatoxin (Kumagai et al. 1998). There are intricate links between the liver and the intestines with regard to vascular supply and the composition of xenobiotic-metabolizing enzymes, which could be relevant in activation of AFB1 into the toxic metabolites (Sergent et al. 2008). The rise in serum pro-inflammatory IL-1β seemed to be consistent with histologic evidence of inflammation observed in the liver and small intestines. The ability of other types of toxins administered intraperitoneally to cause toxicity in the gastrointestinal tract has also been reported elsewhere (Botta et al. 2004). Our study revealed histologic evidences of inflammation including hemorrhages, congestion and leucocytic infiltration around sinusoids and portal tracts in the liver, as well as leucocytic and lymphocytic infiltration of the lamina propria in the intestinal mucosa, in rats given aflatoxin B1. Histo-morphological examination is a recognized tool for assessing inflammatory-related changes in many animal models (Erben et al. 2014). Previously, Kumagai et al. (1998) reported similar findings of perivascular infiltration of leucocytes in hepatic connective tissues, when 2 mg/kg of AFB1 was administered orally to rats. Hemorrhages are consequences of alterations in hemodynamic processes which characterize inflammation (Palipoch et al. 2013). The occurrence of histological lesions of hemorrhages (Baker and Green 1987; Dafalla et al. 1987) and dilated sinusoids (Salim et al. 2011) has previously been reported in aflatoxicosis. The recruitment of leucocytes to inflammatory sites is mediated by chemokines secreted by monocytes and macrophages, as well as products of arachidonic acid metabolism (e.g., leukotriene B_4) derived from the lipo-oxygenase pathway (Kanaoka and Boyce 2004). These mediators normally stimulate the production of pro-inflammatory cytokines, including IL-1β and TNF-α. Further histological lesions observed in this study included development of subepithelial space and some evidence of villi degeneration.

The modulation of cytokine production is among new approaches to the treatment of various disease conditions and herbal extracts are currently being investigated as potent immunomodulators to regulate cytokine expression (Spelman et al. 2006). The pathology of many inflammatory conditions involves excessive generation of reactive oxygen species (ROS) by polymorphonuclear leucocytes (Babior 2000). Naturally occurring compounds or those isolated from natural sources can be useful to modulate the oxidative and/or inflammatory damage associated with toxic conditions. Oral administration of flavonoids from *C. odorata* prevented the AFB1-induced elevation of IL-1β and histologic changes in the duodenum and ileum, while only the higher dose was effective in the liver. Immuno-modulatory effects of methoxylated flavonoids from *C. odorata* have been associated with inhibition of oxidative stress, and this was found to be related to the number and positions of methoxy and hydroxyl groups on the flavonoid rings (Kanashiro et al. 2004; Taleb-Contini et al. 2006).

Although melatonin is naturally secreted in the pineal gland and many other tissues including the bone marrow, testes, retina, lymphocytes, and the gastrointestinal tract (Tijmes et al. 1996; Conti et al. 2000; Bubenik 2002), exogenous administration of this anti-oxidant and anti-inflammatory compound has also been found to be beneficial in many models of organ damage (Mathes 2010). Its role in the suppression of inflammatory response related to increased formation of pro-inflammatory cytokines in models of carbon-tetrachloride and dimethyl nitrosamine toxicities have been documented (Carrillo-Vico et al. 2005; Wang et al. 2005; Jung et al. 2009). In the present study, melatonin clearly ameliorated cytokine-related changes. This effect was positively correlated with its protective effects in the liver tissues, but not in the intestines. It appears, therefore, that while extracts of *C. odorata* produced amelioration of tissue injury in both liver and intestines, melatonin was effective predominantly in the liver. The differences observed in the effectiveness of these compounds could be related to their availability in the tissues examined, due to the differences in their routes of administration.

Taken together, aflatoxin B1 produced alterations in pro-inflammatory cytokines as part of an inflammatory and/or immuno-modulatory process that also included associated histologic changes in the liver and small intestines. Modulation of cytokine activity may partially play a role in protection of tissue integrity observed with treatment with melatonin and/or flavonoids from *C. odorata*, and this may, in part, justify their potential incorporation in diet against aflatoxicosis.

Acknowledgments The authors are grateful to Dr. Joseph Atehnkeng of the Aflasafe Laboratory at the International Institute of Tropical Agriculture, Ibadan, Nigeria for provision of aflatoxin B1 used in this study.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest whatsoever.

References

Abdulmajeed NA (2011) Therapeutic ability of some plant extracts on aflatoxin-B1 induced renal and cardiac damage. Arabian J Chem 4: 1–10

Akinrinmade JF, Akinrinde AS, Odejobi A, Oyagbemi AA (2015) Evidence of attenuation of intestinal ischemia-reperfusion injury following pre-treatment with methanolic extracts of Chromolena odorata in rats. J Complem Integr Med 12(1):23–32

Babior BM (2000) Phagocytes and oxidative stress. Am J Med 109:33–44

Baker DC, Green RA (1987) Coagulation defects of aflatoxins in toxicated rabbits. Vet Pathol 24:62–70

Bedard LL, Massey TE (2006) Aflatoxin B1-induced damage and its repair. Cancer Lett 241:174–183

Botta N, van de Venter M, Downing TG, Shephard EG, Gehringer MM (2004) The effect of intraperitoneally administered microcystin-LR on the gastrointestinal tract of BALB/c mice. Toxin 43:251–254

Bruneau J, Loscher C, O'Kennedy R (2009) Investigation of the immunemodulatory effects of aflatoxin B1, B2 and G1. Epidemiology 20(6): S42–S43

Bubenik GA (2002) Gastrointestinal melatonin: localization, function, and clinical relevance. Dig Dis Sci 47:2336–2348

Carrillo-Vico A, Lardone PJ, Naji L, Fernández-Santos JM, Martín-Lacave I, Guerrero JM, Calvo JR (2005) Beneficial pleiotropic actions of melatonin in an experimental model of septic shock in mice: regulation of pro-/anti-inflammatory cytokine network, protection against oxidative damage and anti-apoptotic effects. J Pineal Res 39:400–408

Cometa MF, Lorenzini P, Fortuna S, Volpe MT, Menequz A, Palmery M (2005) In vitro inhibitory effect of aflatoxin B1 on acetylcholinesterase activity in mouse brain. Toxicology 206(1):125–135

Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni JM (2000) Evidence for melatonin synthesis in mouse and human bone marrow cells. J Pineal Res 28:193–202

Dafalla R, Yagi A, Adam S (1987) Experimental aflatoxicosis in hybrotype chicks: sequential changes in growth and serum constituents and histopathological changes. Vet Hum Toxicol 29:222–225

Eaton D, Ramsdell H, Neal G (1994) Biotransformation of aflatoxins. In: Eaton L, Groopman J (eds) The toxicology of aflatoxins, human health, veterinary agricultural significance. Academic Press, New York, NY

Erben U, Loddenkemper C, Doerfel K, Speickermann S, Haller D, Heimesaat MM, Zeitz M, Siegmund B, Kuhl AA (2014) A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Cli Exp Pathol 7(8):4557–4576

Grenier B, Applegate TJ (2013) Invited review—modulation of intestinal functions upon mycotoxin ingestion: meta-analysis of published experiments in animals. Toxins 5:396–430

Grenier B, Bracarense AP, Schwartz HE, Trumel C, Cossalter AM, Schatzmayr G, Kolf-Clauw M, Moll WD, Oswald IP (2012) The low intestinal and hepatic toxicity of hydrolyzed fumonisin B 1 correlates with its inability to alter the metabolism of sphingolipids. Biochem Pharmacol 83:1465–1473

Gross-Steinmeyer K, Eaton DL (2012) Dietary modulation of the biotransformation and genotoxicity of aflatoxin B1. Toxicology 299: 69–79

Gupta RC (2011) Aflatoxins, ochratoxins and citrinin. In: Gupta RC (ed) Reproductive and developmental toxicology. Academic Press, San Diego, CA

Hanh TTH, Hang DTT, Minh CV, Dat NT (2011). Anti-inflammatory effects of fatty acids isolated from Chromolena odorata. Asian Pacific J Trop Med 760–763.

Hsieh D, Wong JJ (1994) Pharmacokinetics and excretion of aflatoxins. In: Eaton DL, Groopman J (eds) The toxicology of aflatoxins: human health, veterinary and agricultural significance. Academic Press, New York, NY

Ito E, Kondo F, Harada K (2001) Intratracheal administration of microcystin-LR and its distribution. Toxicon 39:265–271

Joseph GS, Jayaprakasha GK, Selvi AT, Jena BS, Sakariah KK (2005) Antiaflatoxigenic and antioxidant activities of Garcinia extracts. Int J Food Microbiol 101:153–160

Jung KH, Hong SW, Zheng HM, Lee DH, Hong SS (2009) Melatonin downregulates nuclear erythroid 2-related factor 2 and nuclear factor-kappa B during prevention of oxidative liver injury in a dimethylnitrosamine model. J Pineal Res 47:173–183

Kanaoka Y, Boyce JA (2004) Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. J. Immunol. 173:1503–1510

Kanashiro A, Kabeya LM, Polizello ACM, Lopes NP, Lopes JLC, Lucisano-Valim YM (2004) Inhibitory activity of flavonoids from Lychnophora sp. on generation of reactive oxygen species by neutrophils upon stimulation by immune complexes. Phytother Res 18: 61–65

Kerns W, Swhartz L, Blanchard K, Burchiel S, Essayan D, Fung E, Johnson R, Lawton M, Louden C, MacGregor T et al (2005) Drug-induced vascular injury—a quest for biomarkers. Toxicol Appl Pharmacol 203:62–87, 2005

Kumagai S, Sugita-Konishi Y, Hara-Kudo Y, Ito Y, Noguchi Y, Yamamoto Y, Ogura A (1998) The fate and acute toxicity of aflatoxin B1 in the mastomys and rat. Toxicon 36(1):179–188

Kumar R, Mishra AK, Dubey NK, Tripathi YB (2007) Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int J Food Microbiol 115:159

Lacour S, Gautier JC, Pallardy M, Roberts R (2005) Cytokines as potential biomarkers of liver toxicity. Cancer Biomark 1:29–39

Li Y, Ma QG, Zhao LH, Wei H, Duan GX, Zhang JY, Ji C (2014) Effects of lipoic acid on immune function, the antioxidant defense system and inflammation-related gene expression of broiler chickens fed aflatoxin-contaminated diets. Int J Mol Sci 15(4):5469–62

Ling SK, Mazura MD, Salbiah M (2007) Platelet-activating factor (PAF) receptor binding antagonist activity of the methanol extracts and isolated flavonoids from *Chromolaena odorata* (L.) King and Robinson. Biol Pharm Bull 30:1150–1152

Mathes AM (2010) Hepatoprotective actions of melatonin: possible mediation by melatonin receptors. World J Gastroenterol 16(48): 6087–6097

Meisonnier GM, Pinton P, Laffitte J, Cossalter A, 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

Moon E, Rhee D, Pyo S (1999a) In vitro suppressive effect of aflatoxin B1 on murine peritoneal macrophage functions. Toxicology 133: 171–179

Moon E, Rhee D, Pyo S (1999b) Inhibition of various functions in murine peritoneal macrophages by aflatoxin B1 exposure in vivo. Int J Immunopharmacology 21:47–58

Nur Jannah MH, Mahmood AA, Sidik K, Salmah I (2006) Cytoprotective effects of honey in combination with aqueous and ethanol extracts from *Chromolaena odorata* L (Eupatorium odoratum) in rats. JUMMEC 9:7–13

Omokhua AG, et al. (2015) *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. J Ethnopharmacol http://dx.doi.org/10.1016/j.jep.2015.04.057i.

Owoyele VB, Adediji JO, Soladoye AO (2005) Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata*. Inflammopharmacology 13(5–6):479–484

Palipoch S, Punsawad C, Chinnapun D, Suwannalert P (2013) Histopathology of the small intestine induced by cisplastin in male Wistar rats. Walailak J Sci Tech 10(6):657–663

Peraica M, Radic B, Lucic A, Pavlovic M (1999) Toxic effects of mycotoxins in humans. Bull World Health Organ 77:754–766

Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST (2001) Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol Pharm Bull 24:1373–1379

Qian G, Tang L, Guo X, Wang F, Massey ME, Su J, Guo TL, Williams JH, Phillips TD, Wang JS (2014) Aflatoxin B1 modulates the expression of phenotypic markers and cytokines by splenic lymphocytes of male F344 rats. J Appl Toxicol 34(3):241–249

Ravinayagam V, Jaganathan R, Panchanadham S, Palanivelu S (2012) Potential antioxidant role of tridham in managing oxidative stress against aflatoxin B1-induced experimental hepatocellular carcinoma. Int J Hepatol. doi:10.1155/2012/428373

Rossano F, Ortega D, Buommino E, Cusumano V, Losi E, Catania MR (1999) Secondary metabolites of Aspergillus exert immunobiological effects on human monocytes. Res Microbiol 150:13–19

Rostasy KM (2005) Inflammation and neuroaxonal injury in multiple sclerosis and AIDS dementia complex: Implications for neuroprotective treatment. Neuropediatrics 36:230–239

Safieh-Garabedian B, Poole S, Allchorne A, Winter J, Woolf CJ (1995) Contribution of interleukin 1beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. Bri J Pharmacol 115:1265–1275

Salim AB, Zohair A, Hegazy AES et al (2011) Effect of some strains of probiotic bacteria against toxicity induced by aflatoxicosis in vivo. J Am Sci 7(1):1–12

Sergent T, Ribonnet L, Kolosova A, Garsou S, Schant A, De Saege S, Van Peteghem C, Larondelle Y, Pussemier L, Schneider YJ (2008) Molecular and cellular effects of food contaminants and secondary plant components and their plausible interactions at the intestinal level. Food Chem Toxicol 46:813–841

Shibamoto T, Bjeldanes LF (2009) Introduction to Food toxicology. Academic Press.

Singh KB, Maurya BK, Trigun SK (2015) Activation of oxidative stress and inflammatory factors could account for histopathological progression of aflatoxin B1 induced hepatocarcinogenesis in rat. Mol Cell Biochem 401(1–2):185–196

Spelman K, Burns JJ, Nichols D, Winters N, Ottersberg S, Tenborg M (2006) Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. Alt Med Rev 11(2):128–150

Takai N, Kataoka M, Higochi Y, Matsuura K, Yamamoto S (1997) Primary structure of rat CD14 and characteristics of rat CD14, cytokine and NO synthase mRNA expression in mononuclear phagocyte system cells in response to LPS. J Leukocyte Biol 61:736–744

Taleb-Contini SH, Kanashiro A, Kabeya LM, Polizello ACM, Lucisano-Valim YM, Oliveira DCR (2006) Immunomodulatory effects of methoxylated flavonoids from two Chromolena species: structure activity relationships. Phytother Res 20:573–575

Tarrant JM (2010) Blood cytokines as biomarkers of in vivo toxicity in pre-clinical safety assessment: considerations for their use. Toxicol Sci 117(1):4–16

Tijmes M, Pedraza R, Valladares L (1996) Melatonin in the rat testis: evidence for local synthesis. Steroids 61:65–68

Wang H, Wei W, Wang NP, Gui SY, Wu L, Sun WY, Xu XY (2005) Melatonin ameliorates carbon tetrachloride-induced hepatic fibrogenesis in rats via inhibition of oxidative stress. Life Sci 77: 1902–1915

Wu CC, Chiao CW, Hsiao G, Chen A, Yen MH (2001) Melatonin prevents endotoxin-induced circulatory failure in rats. J Pineal Res 30: 147–156

Zhu H, Wang Y, Liu Y, Xia Y, Tang T (2010) Analysis of flavonoids in *Portulaca oleracea* L. by UV–vis spectrophotometry with comparative study on different extraction technologies. Food Anal Methods 3(2):90–97