Measures against aflatoxin B_1: a brief review about the use of flavonoids and clays

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Resumo

Medidas para o controle da aflatoxina B_1: uma breve revisão sobre o uso de flavonoides e argilas. A aflatoxina B_1 (AFB_1) é uma das micotoxinas mais abundantes e tóxicas produzidas por cepas toxigênicas. Esta substância afeta a viabilidade celular, sendo capaz de induzir a morte, tanto de células humanas, quanto de células animais. Medidas vêm sendo adotadas para minimizar os danos causados pela AFB_1, incluindo a utilização de flavonoides (compostos polifenólicos extraídos de plantas) e bentonitas (um tipo de argila). Nesta revisão, as características físico-químicas da AFB_1 e seus efeitos em diferentes tipos de células, *in vitro* e *in vivo*, foram abordados. Além disso, a capacidade de proteção celular a partir de substâncias e materiais naturais, tais como flavonoides e bentonita, foi brevemente descrita. Também relatamos os efeitos econômicos causados pelas micotoxinas e sugerimos alternativas (flavonoides e bentonita) para novas abordagens terapêuticas visando combater a toxicidade causada por estas substâncias (AFB_1).

Palavras-chave: Aflatoxina B_1; Argila; Flavonoides; Mecanismo de ação

Abstract

Aflatoxin B_1 (AFB_1) is one of the most abundant and toxic mycotoxins produced by toxigenic strains. This substance affects cell viability, inducing cell death in several human and animal cells. Measures have been taken to minimize damage caused by AFB_1, including the use of flavonoids (polyphenolic compounds extracted from plants) and bentonites (a type of clay). In this review, the physicochemical characteristics of AFB_1 and its effects on different cells are discussed. Furthermore, the cellular protective effects of flavonoids and bentonites are briefly reviewed. Finally, the emerging view of the economic effects caused by mycotoxins is briefly discussed, along with how flavonoids and bentonites might open new pathways to develop approaches to fight the toxic effects caused by aflatoxins.

Key words: Aflatoxin B_1; Flavonoids; Clays; Mechanism of action

Introduction

Aflatoxin B_1 (AFB_1) is a natural mycotoxin produced by *Aspergillus flavus* and *A. parasiticus*. This substance is sometimes present in food and feed and, consequently, causes human and animal diseases (ABBÈS et al., 2008; TIRMENSTEIN; MANGIPUDY, 2014; ZHANG et al., 2015). The main effects observed are severe biochemical and morphological cell changes, which can lead to liver, lung and kidney cancer, as well as harmful effects to heart tissues (ABDEL-WAHHAB; ALY, 2003; BEDARD; MASSEY, 2006; MULDER et al., 2014; TIRMENSTEIN; MANGIPUDY, 2014). Hemorrhagic enteritis and neurological symptoms have also been found in animals that ingested aflatoxins (TIRMENSTEIN; MANGIPUDY, 2014). AFB_1 was shown to have deleterious effects on the metabolism of eukaryotes and can affect the very early stages of mammalian embryonic development (NONES et al., 2013; 2015a; HARUTYUNYAN et al., 2015).

These diseases are caused mainly by the highly electrophilic AFB_1, which can react with nucleophilic sites of macromolecules, such as DNA, RNA and proteins, creating the basic mechanisms for cell death, mutagenesis, carcinogenesis and teratogenesis (AYUB; SACHAN, 1997; AGAR et al., 2013; HUUSKONEN et al., 2013).

Several strategies have been developed to reduce the risks of AFB_1 exposure (DIAO et al., 2013; WANG et al., 2015), including degradation, destruction, inactivation and removal of AFB_1 through chemical, physical, and biological methods (GIORDANO et al., 2012; AHLBERG et al., 2015; NONES et al., 2015a; 2015b; WANG et al., 2015). These methods are usually added to human foods or animal feeds; however, their use has some limitations, such as alteration in organoleptic characteristics and nutritional values of foods and feeds (MADRIGAL-SANTILLÁN et al., 2010).

Moreover, in order to reduce the risks of AFB_1, compounds and materials present in nature, such as flavonoids and bentonites, can modulate AFB_1 biotransformation and protect cells from AFB_1 damage (ABDEL-WAHHAB; ALY, 2003; NONES et al., 2013; 2015a; 2015b).

In this review, the physicochemical characteristics of AFB_1 and its effects on different types of cells are presented. Furthermore, the cellular protective effects of flavonoids and bentonites are briefly discussed.

## Effects of aflatoxin B_1 on different cell types

Although 20 aflatoxins have been identified, only four of them (i.e., aflatoxin B_1, B_2, G_1 and G_2 [AFB_1, AFB_2, AFG_1 and AFG_2]) occur naturally and are significant contaminants of a wide variety of foods and feeds (EL-NEKEETY et al., 2014). Among the aflatoxins, AFB_1 is the most abundant and toxic (AYUB; SACHAN, 1997).

AFB_1 is produced by the common molds *Aspergillus flavus* and *A. parasiticus*, which grow on grains, oilseeds and spices (ABDEL-WAHHAB et al., 1998; MULDER et al., 2014). The chemical structure of AFB_1 is similar to that of polysubstituted coumarins (Figure 1). Indeed, the bisfuran ring or 8,9-unsaturated carbon is considered the toxic structural component (ABDEL-WAHHAB et al., 1998; WU et al., 2015).

Exposure to AFB_1 induced chromosome aberrations consists mainly of gaps and breaks (ITO et al., 1989; ABDEL-WAHHAB et al., 1998) that can cause histological changes and abnormalities in human and animal cells (AUSTIN et al., 2012). AFB_1 affects cell membranes and viability, DNA, and induces apoptosis (EL-GIBALY et al., 2003; HARUTYUNYAN et al., 2015; NONES et al., 2015a; ZHANG et al., 2015). These changes may also affect the functionality of organs, mainly the liver and kidneys (AYUB; SACHAN, 1997).

Recently, we have shown that AFB_1 can influence the very early stages of mammalian embryonic development, affecting the survival and proliferation of neural crest stem cells, and, indirectly, the formation of neuronal cells (NONES et al., 2013; 2015a; 2015b). Moreover, AFB_1 affects placental steroid hormone synthesis, which may lead to anomalies in foetoplacental hormonal homeostasis (HUUSKONEN et al., 2013).

Abdel-Wahhab et al. (2015) showed that the *in vivo* application of AFB_1 (80 µg/kg b.w.) in male

FIGURE 1: 3D structure of aflatoxin B$_1$.
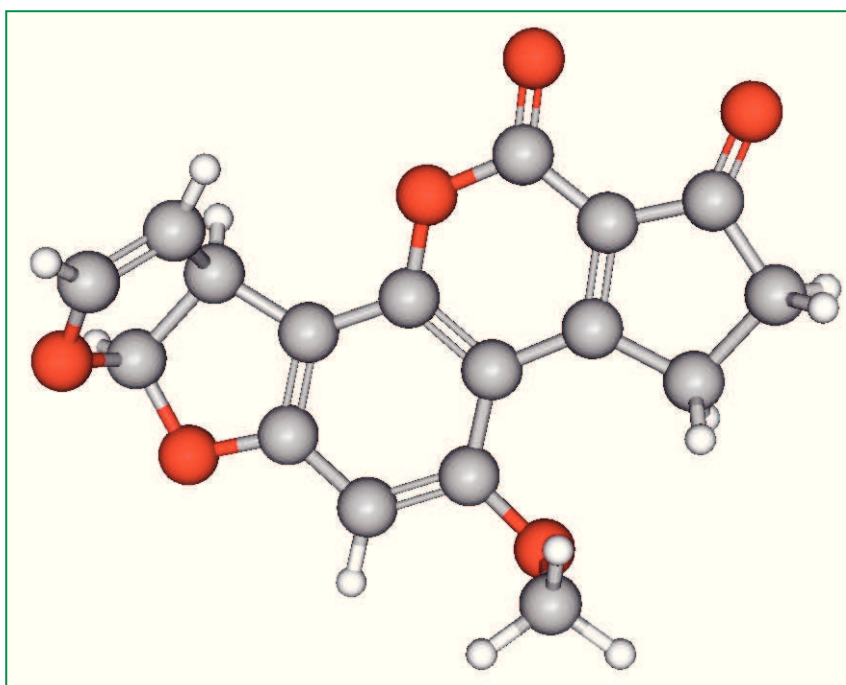
Sprague Dawley rats resulted in a significant increase in DNA fragmentation. Likewise, AFB$_1$ caused a marked decrease in cell viability and increased fragmented DNA levels in a concentration-dependent manner in monkey kidney cultures (GOLLI-BENNOUR et al., 2010) and neural crest stem cells (NONES et al., 2013). In fact, *in vitro* studies have shown that 5 μM of AFB$_1$ causes oxidative stress in a human hepatoma cell line (CHAN et al., 2003) and can react with components of cultured human blood cells, resulting in the formation of toxic intermediate compounds (TURKEZ; SISMAN, 2007). Furthermore, exposure of bovine peripheral blood mononuclear cells to AFB$_1$ (0, 5 and 20 μg/mL) increased intracellular reactive oxygen metabolites (BERNABUCCI et al., 2011).

Oxidative damage is one of the potential mechanisms that makes AFB$_1$ able to induce cytotoxicity, carcinogenicity and mutagenicity in different cell and animal tissues (SHEN et al., 1995; CHAN et al., 2003; EL-GIBALY et al., 2003; LEE et al., 2005; ABBÈS et al., 2008; BERNABUCCI et al., 2011; AJIBOYE et al., 2013; MULDER et al., 2015). The oxidative process leads to the formation of the electrophilic AFB$_1$-8,9-epoxide (SMELA et al., 2001; CHAN et al., 2003;

HANIOKA et al., 2012), which reacts with guanine and other cellular macromolecules (i.e. RNA and proteins) (Figure 2). These genetic changes help cells along a pathway toward malignant transformation (SMELA et al., 2001; BEDARD; MASSEY, 2006).

Cell damage caused by AFB$_1$ is influenced by a number of human health factors, including age and the extent of aflatoxin exposure (ABDEL-WAHHAB; ALY, 2003). Furthermore, aflatoxin biotransformation may be influenced by diet, nutritional status and chemical treatments, which modulate various biological activities (AYUB; SACHAN, 1997; LEE et al., 2001; ABDEL-WAHHAB; ALY, 2003).

Therefore, aflatoxin contamination results in direct economic effects, such as product loss and healthcare damage, and indirect economic effects, such as animal death and food-borne disease surveillance costs (BHATNAGAR-MATHUR et al., 2015). In animals, AFB$_1$ has nutritional and immunologic consequences that may contribute to lower body weight gain and impaired reproductive efficiency (EWUOLA et al., 2014; BHATNAGAR-MATHUR et al., 2015; TREBAK et al., 2015). In addition, acute exposure to aflatoxins can cause aflatoxicosis, and in cases of severe hepatotoxicity

FIGURE 2:   Mechanism of action of AFB$_1$. The mycotoxin AFB$_1$ is an electrophilic molecule that is able to bind to the nucleophilic sites of macromolecules (DNA, RNA and proteins) and, consequently, causes cell damage.
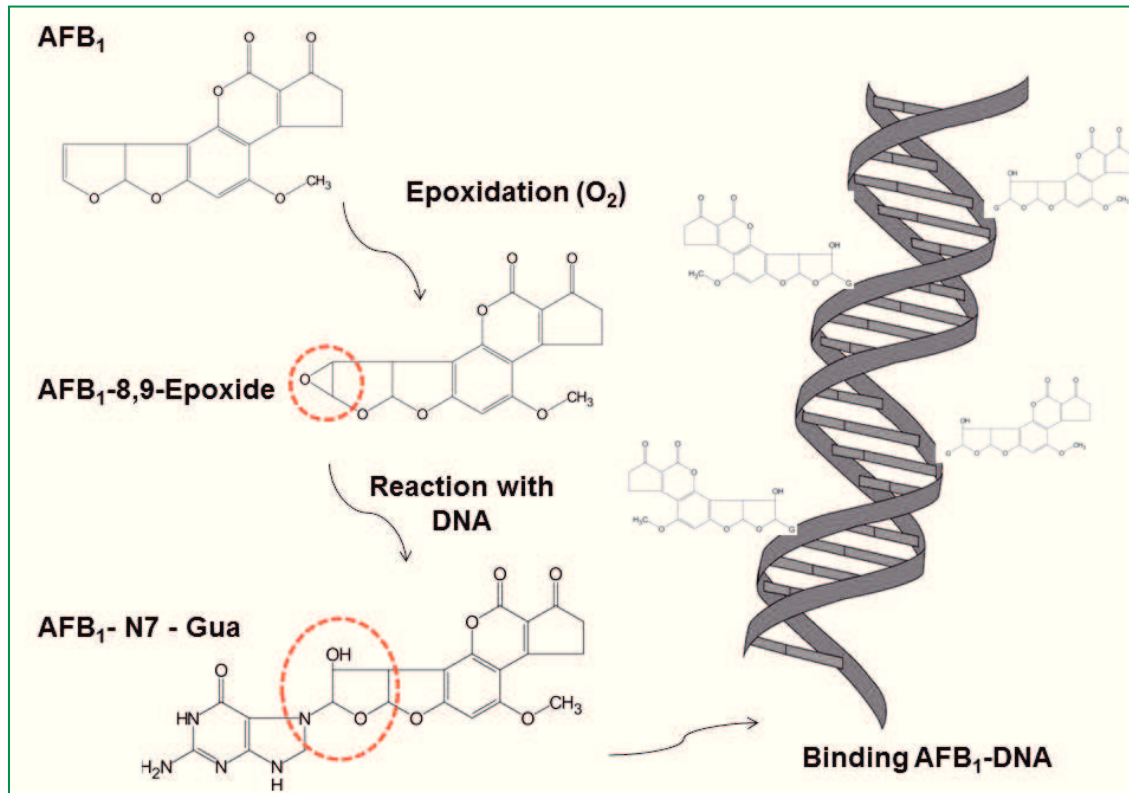
the mortality rate is approximately 25% (AHLBERG et al., 2015). AFB$_1$ may also affect early growth and some aspects of human immunity and nutrition (WILLIAMS et al., 2004).

During the past few years, a better understanding of cultured cells has suggested that flavonoids and bentonites can be used to control aflatoxin damage. Most of the physiological benefits of flavonoids and bentonites are generally thought to be due to their ability to indirectly reduce aflatoxin effects; however, emerging evidence has supported the hypothesis that their mechanism of action might go beyond these properties.

Protective effects of flavonoids and clays

Diet is an important factor that can influence favorable pathophysiological processes and may be very effective as a prevention strategy against various diseases (TURKEZ; SISMAN, 2012), including those caused by AFB$_1$. Likewise, chemoprevention is an attractive

strategy for protecting humans and animals from damage caused by exposure to this mycotoxin (COSTA et al., 2007; TURKEZ; SISMAN, 2012). Therefore, a measure adopted to combat the problems caused by this mycotoxin is associated with the addition of protective substances (i.e., flavonoids and bentonites) in human and animal diets or drugs.

Protective effects of flavonoids

Flavonoids are naturally occurring polyphenolic compounds of the human diet that are universally present as constituents of fruits and vegetables, as well as plant-derived foods and beverages, such as oil, tea and red wine (Figure 3) (BOUHLEL et al., 2010; NONES et al., 2012a; 2012b).

The biological activities of flavonoids cover a very broad spectrum, including anticancer, antioxidant, antiviral and antibacterial effects (SIESS et al., 2000; NONES et al., 2011; ALMEIDA et al., 2015), and several flavonoids are promising protectors when added to a

FIGURE 3: 3D structures of main flavonoid groups.
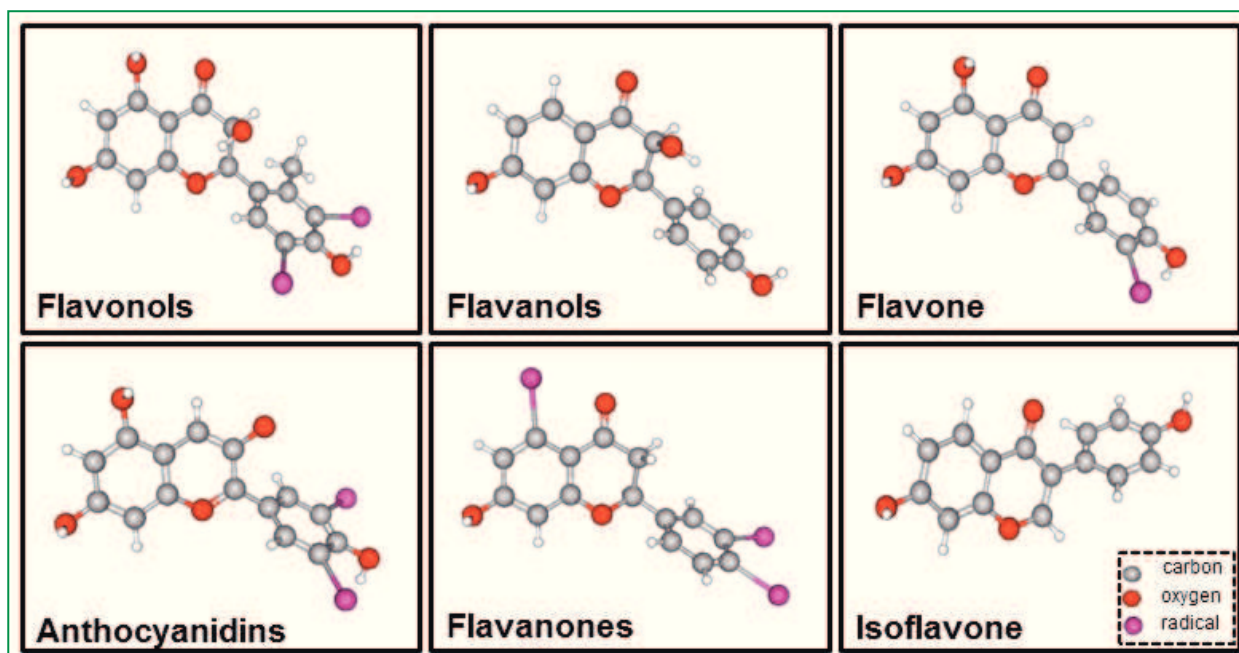
diet (NONES et al., 2013; SIESS et al., 2000). Studies have demonstrated that different natural compounds, such as flavonoids (NONES et al., 2013), carotenoids (WANG et al., 1991) and anthocyanins (AJIBOYE et al., 2013), have several positive biological actions and protect cells against AFB$_1$ damage (CHOI et al., 2011; LANGESWARAN et al., 2012; NONES et al., 2013).

These compounds can enhance the detoxification of AFB$_1$, possibly by improving the activities of reactive oxygen species detoxifying enzymes that prevents the oxidation and fragmentation of cellular macromolecules, such as DNA, lipids and proteins, as well as AFB$_1$ induced redox imbalance (AJIBOYE et al., 2013; 2014).

Flavonoids are a particularly attractive class of polyphenols with interesting therapeutic properties, which contain strong nucleophilic centers that can react with electrophilic compounds, protecting cells from damage (Figure 4) (MARNEWICK et al., 2000; NONES et al., 2013; PUNVITTAYAGUL et al., 2014).

Recently, by using an *in vitro* culture system of quail neural crest cells, we demonstrated for the first time that the flavonoids hesperidin and rutin modulate neural crest cell death by a mechanism dependent on the ERK2 and PI3 kinase pathways (NONES et al.,

2012a). We have also shown that AFB$_1$ affects neural crest cell development *in vitro* and the co-administration of hesperidin and rutin partially prevents cell death caused by this mycotoxin (NONES et al., 2013; 2015d). Similar results were obtained using human skin-derived-multipotent mesenchymal stromal cells, where rutin and quercetin increased cell viability and proliferation in a concentration-dependent manner (ALMEIDA et al., 2015).

Corroborating our results, Barcelos et al. (2011) demonstrated the antigenotoxic activity of quercetin and rutin against DNA damage induced by AFB$_1$ in human hepatoma cells. Indeed, quercetin prevented AFB$_1$-induced genotoxicity in rat liver microsomes (KOHLI et al., 2002) and also showed a protective effect against the cytogenetic outcomes in rat livers (ABDEL-WAHHAB et al., 2015). Similar results were also reported by El-Nekeety et al. (2014), where quercetin demonstrated potential antioxidant activity, a protective action, and was able to regulate gene expression alterations induced by aflatoxins.

AFB$_1$ mediated hepatic damage is related to the production of AFB$_1$-8,9-epoxide and reactive oxygen species; in this way bioactive compounds that have antioxidant activity are potentially capable of reducing

FIGURE 4: Possible flavonoid effects on cells previously treated with AFB_1. The protective effects are probably related to a decrease of covalent binding of macromolecules (DNA, RNA, proteins) when AFB_1 is added. This action probably reduces AFB_1 toxicity and ROS formation, maintaining cell integrity and, consequently, increasing the total number of cells.
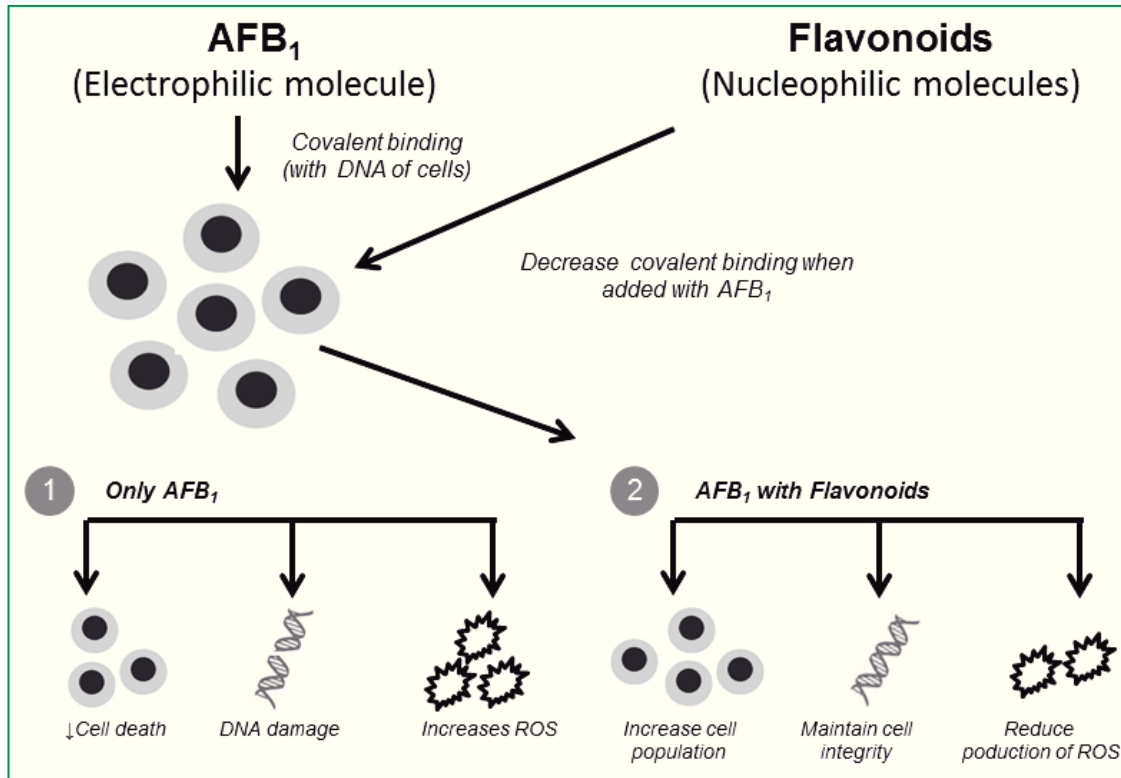
AFB_1-induced toxicity (CHOI et al., 2011). Langeswaran et al. (2012) studied the anticancer action of the flavonoid kaempferol against AFB_1 induced hepatocarcinogenesis. They found that this mycotoxin (2 mg/kg body weight i.p) induced hepatocellular carcinoma in experimental animals, and when the animals were treated with kaempferol (100 mg/kg body weight p.o) the nucleic acids levels were brought back to normal, as well as the altered levels of biological enzymes, such as membrane bound ATPase, carbohydrate metabolizing enzymes and mitochondrial TCA cycle enzymes. Likewise, Lee et al. (2001) showed that flavones, coumarins, and quinones had a strong inhibitory effects on AFB_1 transformation to AFB_1-8,9-epoxide using cell culture models. On the other hand, isoflavones, flavanones, flavone glycosides, monoter-penes, curcuminoids, limonoids, and alkaloids were less active (LEE et al., 2001).

Flavonoids probably inhibit AFB_1 effects by decreasing the covalent binding of AFB_1 to DNA, which results in the production of less toxic metabolites of AFB_1 via phase 2 enzyme induction (mostly GST)

(SIESS et al., 2000). The action of flavonoids is possibly mediated through interaction with microsomal activating enzymes (FRANCIS et al., 1989).

The protective effect depends on the flavonoid structure (SIESS et al., 2000). A nonpolar flavonoid, like apigenin, is more capable of chemoprevention of AFB_1-mediated immunotoxicity than a polyhydroxylated flavonoid, such as quercetin, fisetin, and myricetin (CHOI et al., 2010). Nonpolar flavonoids without free hydroxyl groups show a protective effect, which suggests that intestinal absorption and bioavailability of flavonoids are probably different depending on whether they have hydroxyl groups or not (SIESS et al., 2000).

Protective effects of clay

Clays are ubiquitous, especially in geologic deposits, weathering terrestrial rocks and marine sediments (TURKEZ; SISMAN, 2007). They have numerous applications due to their structural characteristics, their abundance in nature, low cost and availability (NONES

et al., 2015c). Among clays, bentonites are commonly used by the pharmaceutical industry as excipients, active substances or dispersal agents that fulfill technological functions (MURRAY, 2006; RODRIGUES et al., 2013).

Our recent work suggests that bentonites may be excellent cell protectors and can reduce some of the side effects of drugs, such as those used for cancer treatments (NONES et al., 2015c). Once inside human and animal bodies, bentonite particles are efficient at promoting growth (acting as adsorbents of toxins), improving health (reducing the harmful effects of drugs) and promoting well being (active principles in cosmetics and pharmaceuticals) (NONES et al., 2015c).

Recently, by using an *in vitro* culture system of quail neural crest cells, we investigated the effects of bentonite on neural crest differentiation and survival when exposed to AFB$_1$. We demonstrated for the first time that bentonite reduces the toxicity of AFB$_1$, which is possibly due to its ability to adsorb AFB$_1$ in the culture medium (NONES et al., 2015a). We believe that AFB$_1$ participates in a specific bonding mechanism with the interlayer space of montmorillonite (a clay mineral which is the main component of bentonite), probably forming complexes with metals that lead to a reduction in

AFB$_1$ availability and, consequently, cell damage caused by the mycotoxin (NONES et al., 2015a).

AFB$_1$-bentonite binding depends on the physio-chemical characteristics of clays (i.e., surface area, pore size, particle distribution and charge) (HUWIG et al., 2001), which can be affected by different treatment types (Figure 5). Previous studies have demonstrated that metallic ions, organic and thermal treatments may affect the AFB$_1$ adsorption capacity of clays (DAKOVIĆ et al., 2008; JAYNES; ZARTMAN, 2011; NONES et al., 2015b; 2016). We have shown that bentonite, even after a thermal treatment (125 to 1000ºC), can maintain the viability of neural crest stem cells previously treated with AFB$_1$ (NONES et al., 2015b).

Abbès et al. (2008) reported that the addition of montmorillonite (the main compound of bentonite) reduced AFB$_1$ related death in a colon-cancer cell line *in vitro*. Moreover, Anadón et al. (2014) demonstrated that the co-incubation of AFB$_1$ and clays (green and green montmorillonite) was effective at reducing the toxicity induced by mycotoxins in Caco-2 cells (after a 24 h treatment). Protective effects against AFB$_1$ were also reported by Turkez and Sisman (2007), where hydrated sodium calcium aluminosilicate prevented the adverse effects induced by AFB$_1$ in human lymphocytes.

FIGURE 5: Treatments of bentonites affect their chemical structure. Changes depend on the mineralogical composition of the bentonite, as well as the concentration, time and temperature at which samples are exposed.
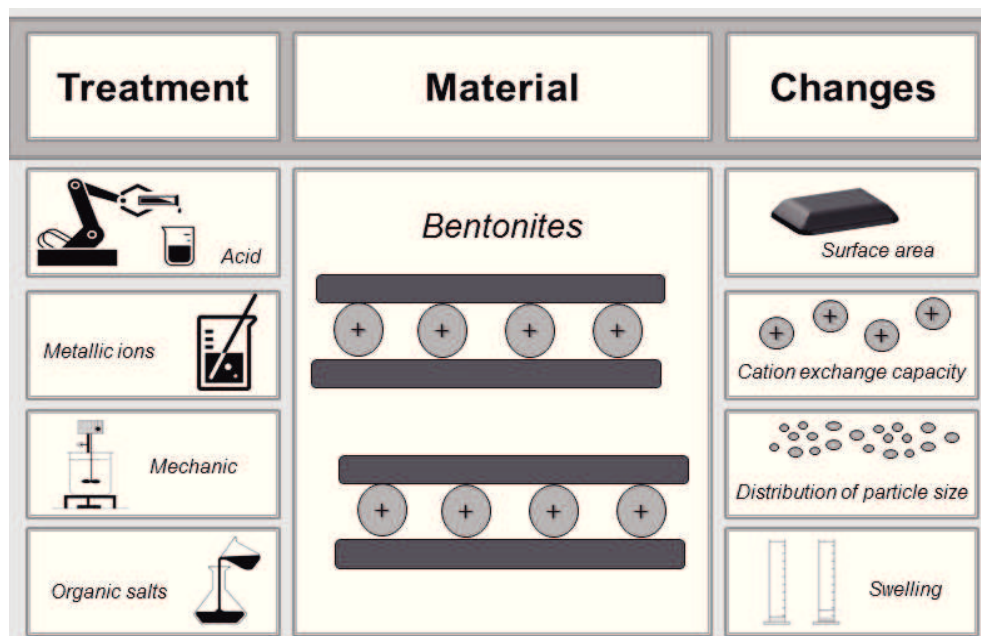
A hematological study performed with female albino rats revealed that the treatment with aflatoxins (2.5 mg kg^{-1} diet) caused a significant decrease in haemoglobin, packed cell volume, total red and white blood cells counts, and neutrophilsand basophils (ABDEL-WAHHAB et al., 2002). On the other hand, animals treated with aflatoxins plus montmorillonite, or hydrated sodium calcium aluminosilicate, exhibited improved hematologic parameters (ABDEL-WAHHAB et al., 2002).

Although bentonites can prevent the harmful effects of AFB$_1$ when present in diets or drugs, the effects of this material may differ according to mineralogical composition and structure.

Concluding remarks

Mycotoxins, including AFB$_1$, account for millions of dollars spent every year worldwide on human and animal health, and condemned agricultural products (MADRIGAL-SANTILLÁN et al., 2010). In relation to human health, for example, of the 550,000-600,000 new hepatocellular carcinoma cases worldwide each year, about 25,200-155,000 may be associated with aflatoxin exposure (LIU; WU, 2010). These data are a concern. Thus, actions to fight the harmful effects of aflatoxins are needed.

In this context, culture cells can be a good experimental model to help explain the main AFB$_1$ toxicological effects and the mechanism of actions of different protective substances and materials, such as flavonoids and clays.

This review shows that flavonoids and bentonites can provide great benefits when properly employed, because they modulate cell viability and reduce cell damage caused by AFB$_1$. Nevertheless, additional research is needed to more precisely assess the preventive effects of diets and drugs using these substances and materials against AFB$_1$. In fact, concentration-response studies and experimental culture models using different cell types are also useful in evaluating inhibitory effects (SIESS et al., 2000) and can ensure safety applications. These studies could extend our understanding of how

cell cultures could be used for monitoring developmental toxicity and its relevance in relation to its differentiation progress. In addition, perhaps flavonoids and bentonites could be used as a model system for screening for the developmental toxicity of various other chemicals.

Acknowledgments

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