The neurotoxic effect of deoxynivalenol in chronic intoxication of chickens

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Abstract

Deoxynivalenol (DON) is a mycotoxin produced by species of the Fusarium spp. that infects cereals such as corn, wheat, oats, barley, rice etc. stored in improper conditions. DON has a cytotoxic effect, affecting animal and human health, causing symptoms such as nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. Fourty ROS 308 broiler chikens were subchronic intoxicated with DON in order to observe histological changes in the cerebral cortex and cerebellum. Following the histological examination, the cerebral cortex and the cerebellum of the experimental lot showed a variable spongy appearance, abnormal aspect neurons, with highly acidophilic, granular, vacuolized cytoplasm and pycnotic nuclei. Also, in neurons from the cerebral cortex, loss of shape and loss of the layers arrangement was observed. These changes explain the altered clinical status of chickens from experimental group throughout the experiment.

Keywords: deoxynivalenol, chickens, chronic intoxication, neuronal degeneration

Introduction

Deoxynivalenol (DON or vomitoxin) is a trichothecenic mycotoxin causing loss of appetite, anorexia and weight loss (Pieters et al., 2002), immunosupression (Solcan et al., 2012). Laboratory studies have demonstrated that anorexia appears from the first day of DON oral administration (Amuzie et al., 2011, Hattori et al., 2011), or by intraperitoneal injection (Flannery et al., 2012). Acute toxicity studies have demonstrated the presence of toxin in the plasma, liver, spleen, brain only 5 minutes after inoculation (Pestka et al., 2008). In short-term and sub-chronic exposure, the decrease in feed consumption and body weight was observed (Pestka, 2007). A chronic toxicity study demonstrated reduced daily consumption, decreased of total weight, and increases in IgA serum levels (Iverson et al., 1995). In farm animals, the presence of DON has been reported in organs and tissues such as liver, kidneys, muscles, dorsal fat (Doll et al., 2008), eggs (Sypecka et al., 2004), milk (Keese et al., 2008). Considering these and the resistance of vomitoxin at high temperatures of 170°C to 350°C (Hughes et al., 1999), there is a risk of intoxication in humans by consumption of contaminated products.

Materials and methods

The effects of DON on 40 Ross 308 broiler chickens were studied. They were acclimatized for 6 days, then weighed and divided into 2 lots: a control group of 10 chickens and an experimental one of 30 chickens.The animals in the experimental lot were exposed to a dose of 50 μg / kg body weight of deoxynivalenol in sterilized sunflower oil suspension. Mycotoxin administration was performed by gavage for 36 days. Chickens from the control group received only sterilized sunflower oil. At 42 days of age they were weighed, slaughtered and sampled for blood, organs, respectively segments from the encephalus and cerebellum. The pieces were paraffin embeded, sectioned in slices of 5 μm using the Slee Meinz CUT 5062 microtome, then stained by HE, PAS and Gomori methods and examined with Olympus CX41 microscope.

Results and discussions

Ischemia accompanied by the presence of abnormal neurons and a reduction in the number of these neurons was observed in the cerebellum and cerebral cortex.

Neurons in the cerebral cortex showed changes in size, but also in the cytoplasmic staining affinity, appearing intense acidophilic, granular or vacuolar in PAS staining (fig1a). Betz neurons showed an atypical smaller aspect, loss of the characteristic layout of the layers (fig. 1b). The nuclei of the modified neurons were larger in size and had an eccentric position (Fig. 1c). Neuronal degeneration was indicated by free spaces without neurons (fig. 1d).

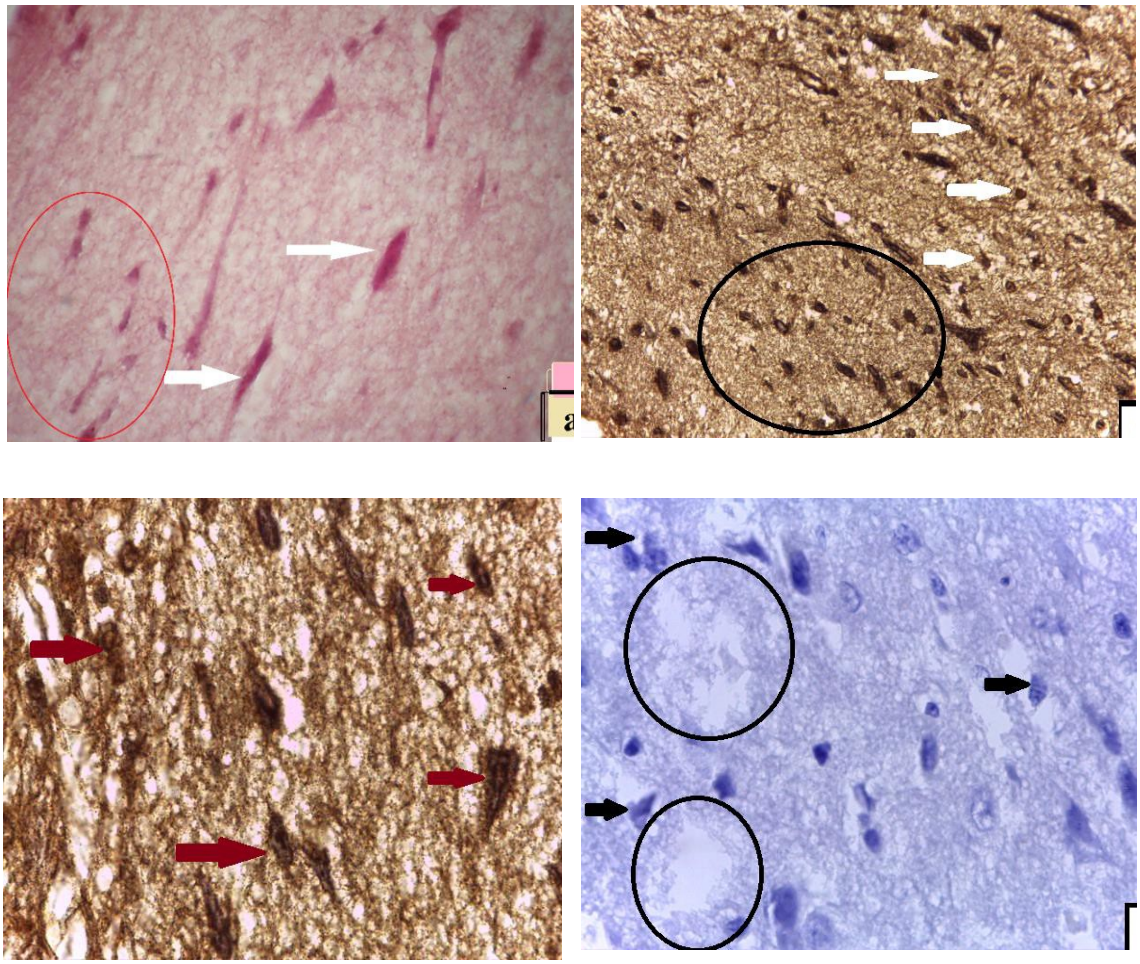

Fig 1. Cerebral cortex. A . Rare intense acidophilic neurons, smaller than normal (red cercle). PAS stain x400. B. A bigger number of astrocytes and small Betz neurons with abnormal smaller nuclei (black arrow) and neuronless regions, caused by neuronal degeneration (black circle) Gomori stain x400. C Betz neurons with abnormal bigger, eccentric nuclei (red arrow) Gomori stainx900. D. Abnormal smaller neurons (black arrow) and neuronless regions, caused by neuronal degeneration (black cercle). HE stain x900.

In cerebellar cortex were also observed lesions for suggestive for neurodegenerative effect of DON. So, Purkinje neurons were smaller, intensly acidophilic, containing granular intracytoplasmatic corpuscles

Any layer of cerebellum showed large regions where Purkinje neurons were absent (fig 1a).

An increased number of astrocytes around the Purkinje neurons was observed (Fig. 2b). Their role could be interpreted as a protection reaction from deoxynivalenol action. The degeneration and decrease of Purkinje neurons number was also observed (Fig. 2a, b,c). The nuclei of the modified neurons were larger in size and had an eccentric position (Fig. 2d).

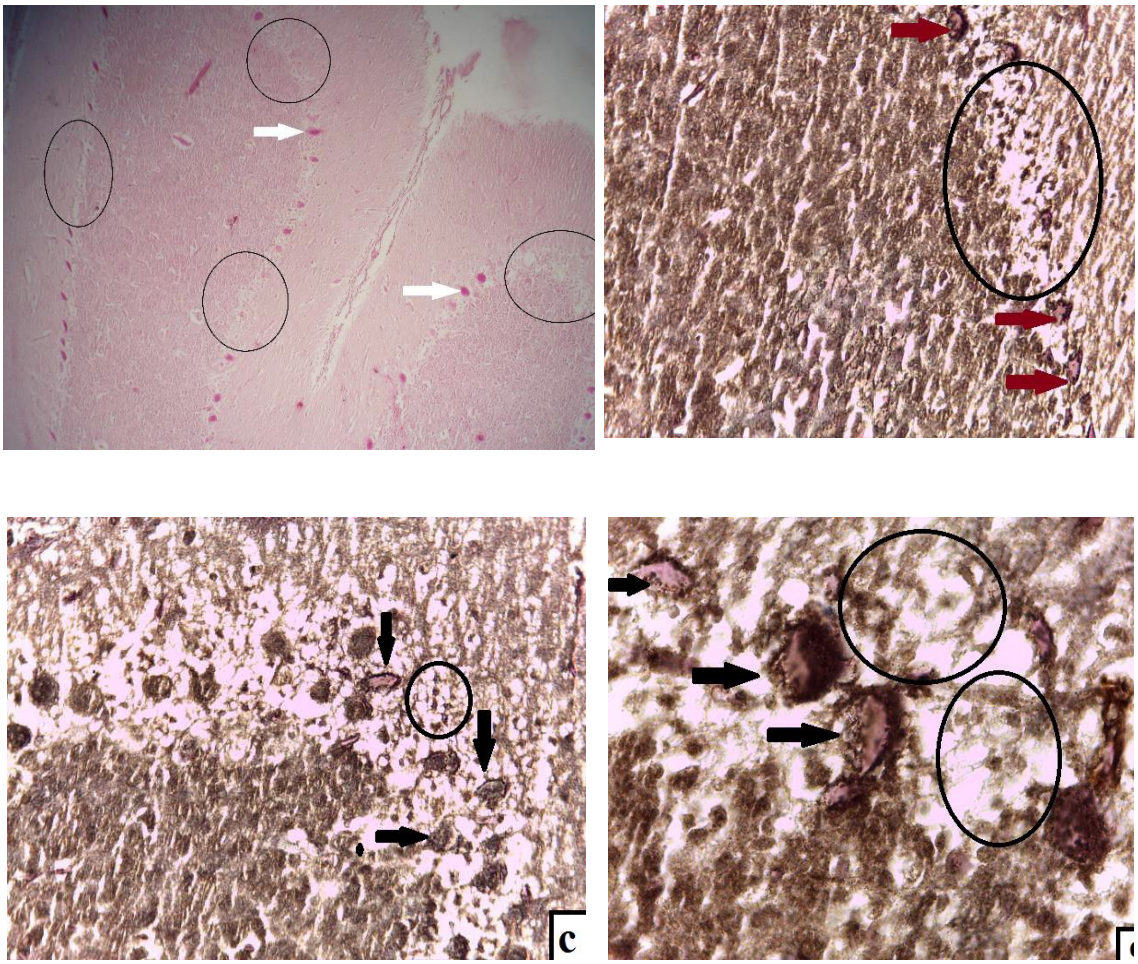

Fig. 2. Cerebellar cortex. A. Rare intense acidophilic neurons (white arrows) majority with smaller dimensions and neuronless regions (black cercle). PAS stain x200. B. Many astrocytes (black cercle) and smaller Purkinje neurons with bigger eccentric nucleus (red arrow). Gomori stain x400. C. Purkinje neurons with bigger degenerated eccentric nuclei. Gomori stain x400. D. Abnormal smaller neurons with big eccentric nuclei and many intracitoplasmatic granules (black arrow) and neuronless regions, delimitated by microglia and astrocytes. HE stainx900.

Vascular lesions were observed both in cerebellar cortex (fig 3a, b) and into the cerebellum (fig 3 c, d), consisting in intravascular blood clots (fig 3a), arteriolar degenerations (fig 3b), pericapillary edema (fig 3c) and zones of necrosis into molecular layerof the cerebellum (fig 3d).

No histological changes of the brain or cerebellum were seen in the control group subjects.

In our study we consider that neuronal degeneration characterized by changes in dimensions, vacuolization, the granules presence in the cytoplasm, changes in staining affinity, appearance and position of the nucclei occurred consecutively to a state of chronic hypoxia caused by DON intoxication.

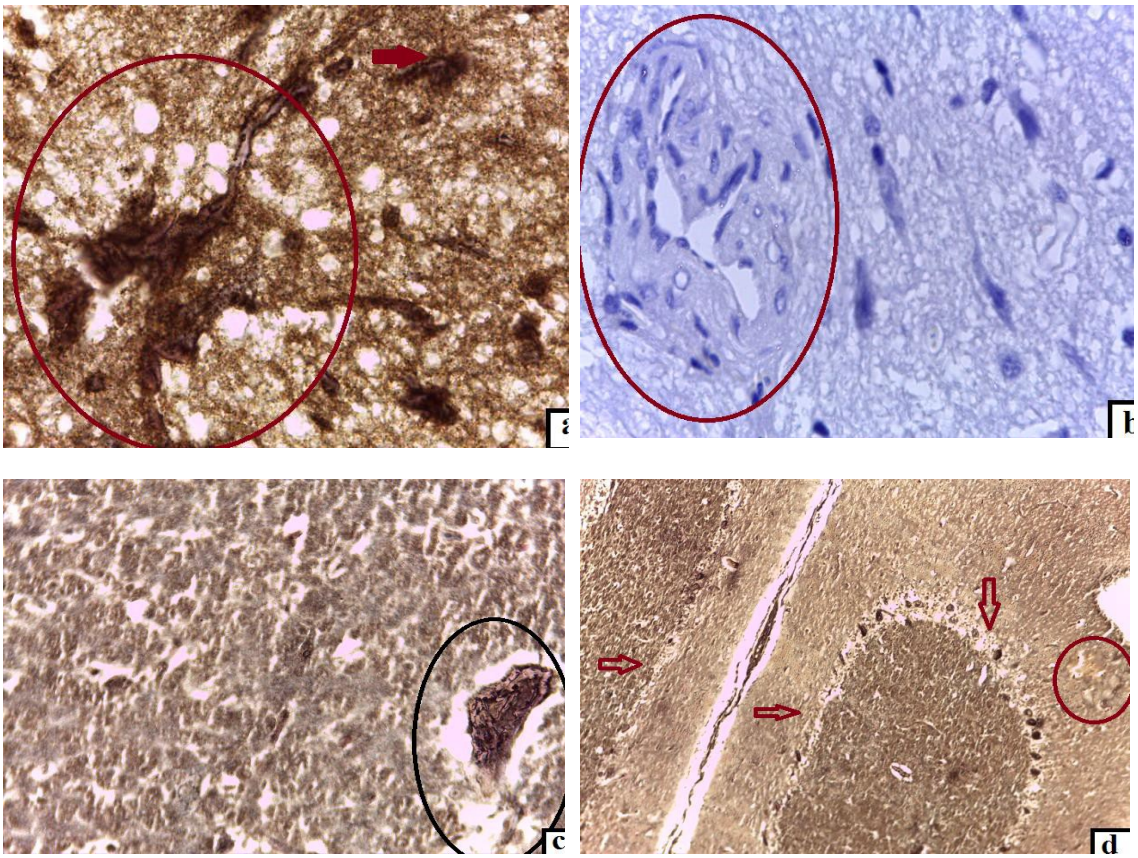

Fig 3. A. Cerebral cortex. Intracapillary blood clots (red cercle) and smaller neurons (red arrow). Gomori stain x900. B. Degenerated arteriola (red cercle) and smaller Betz neurons. HE stain x900. C. Cerebellum granular layer. Blood clot in a capillary vessel and edema arround it. Gomori stain x900. D. Cerebellar blade showing absence of Purkinje neurons (degenerated) (red arrow) and coagulation necrosis in molecular layer (red cercle). Gomori stain x1oo.

DON has the ability to cross the blood-brain barrier and to affect the central nervous system (Girardet et al., 2011a, Maresca M., 2013) increasing the level of proinflammatory cytokines such as interleukin 1β, interleukin 6, necrosis factor α (Bonnet et al., 2012, Girardet et al., 2011b, Tominaga et al., 2016). DON has been shown to have the ability to induce cellular apoptosis (Wang et al., 2016, Deng et al., 2016). The purpose of this study was to observe the histological aspects of cerebral cortex and cerebellum caused by chronic DON intoxication in chickens.

Long-term administration of this mycotoxin has progressively disrupted the neuronal metabolism, the membrane transport, altered intracellular organelles and triggered the release of lysosomal proteinases - aspects described in the literature (Pestka, 2010, Maresca, 2013).
Modifications have completely destroyed the neuron, causing its disappearance and the spongy aspect of some areas in the microscopic field. Thus, administration of DON determines the same reaction from neurons as in the case of inflammatory factors, especially interleukin β1 (Girardet et al., 2011a, Bonnet et al., 2012, Langhans, 2000, Clark et al., 2015).
Histopathological aspects showed significant changes in both neuronal hyaloplasma and cellular organelles. The role of mitochondria in cell death in DON intoxication was suggested by highlighting mitochondrial factors such as the pro-apoptotic Bcl-2 proteins and cytochrome C (Wang et al., 2016; Deng et al., 2016; Ren et al., 2015).

The most sensitive to hypoxia appear to be neurons from the surface of the gray matter where associative processes take place (Girardet et al., 2011a, Flannery et al., 2012).

Conclusions

Oral administration of 50 micrograms/kg b.w DON in chickens for 36 days caused histological changes in the central nervous system correlated with changes in the food behavior. Microscopic aspects were: atypical neurons, variations in size, cytoplasmic granules, nucleus changes, and apoptosis in both the cerebral cortex and the cerebellum.

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