SUBCELLULAR ALTERATIONS IN THE HEPATOPANCREAS OF *ARCHACHATINA MARGINATA* EXPOSED TO AFLATOXIN CONTAMINATION

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ABSTRACT

Aflatoxin (AFT) is a major concern to public health and to toxicologists. Sixty (60) fresh Archachatina marginata (50.00±0.5g) were reared in plastic snaileries in the laboratory for 3 weeks, with 5 snails randomly allocated to each of 4 dietary contaminations of 0 (control), 2000, 6000, and 10,000 AFT ppb replicated 3 times. Responses were recorded as weekly hepatosomatic index (HSI) on the 21st day hepatopancreas photomicrographic sections (PMS) at 400x magnification. Results revealed that HSI significantly decreased with increasing AFT dietary contamination doses for non-control snails; while PMS observations showed cellular degenerations with histopathological lesions of increasing varying severity respectively. Exposure of snail to dietary AFT contamination leads to severe histopathological alternations.

Key words: Snail, Aflatoxin, Dietary contamination, Cell damage, Hepatopancreas

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INTRODUCTION

The mid-gut gland or digestive gland or hepatopancreas (HPS) in snail is similar in its function to the pancreas and liver for humans (Boucenna *et al.,* 2015). The HPS of mollusc is a large digestive gland which is involved in several functions including the extracellular and intracellular digestion of food, storage of lipids, glycogen and minerals. The HPS is also the main site of nutrient absorption and plays a major role in detoxification (Bebby and Richmond, 1988). The digestive gland (or HPS) was chosen as a target organ in toxicity evaluations due to its ability to uptake and to concentrate the contaminants by 5-10 folds than other organs (Bebby and Richmond, 2003). The HPS shows critical ultra-structural variations at very early stages of exposure, even before morphological manifestation (Gopinath *et al.,* 2011).

Human foods can be contaminated by mycotoxins from the genera *Aspergillius* (aflatoxins) and *Penicillium* (ochratoxins) at various stages in the food chain, (Ebenso *et al.,* 2013). Human consuming aflatoxin (AFT) above the limit of 20µg/kg (FAO, 1997) and above African maximum permissible limit of 5mg/kg (Oyero and Oyefolu, 2011) will be at risk of additional chance of developing adverse health conditions.

Cellular assay as an important biological index provides insight into cellular injuries (Moore, 1990). The present study was aimed at studying microscopic HPS subcellular structures of *Archachatina marginata* exposed to dietary contaminations of AFT in the laboratory.

MATERIALS AND METHODS

Experimental animal: Fresh samples of *A. marginata* were sourced locally and

transported in sterile poly bags to the laboratory. The laboratory had an environmental condition of 24±2^{o}C, with relative humidity of 90% and photoperiod of 12h light and 12h darkness. A total of 60 *A. marginata* (50.00±0.5g) with 5 snails randomly allocated to each of 4 dietary treatments and replicated 3 times, in a completely randomized design. The microcosm was plastic snaileries of 0.25 x 0.25 x 0.25m^3 dimensions with mosquito netting on the lid to allow for light, air and protect snails against predators. Floor of each snailery had up to 6cm loamy soil. The *A. marginata* were starved for 7 days before commencement of the study in plastic snaileries to acclimatize to laboratory conditions.

Experimental fungus: The fungus AFT was released from *Aspergillus paraciticus* isolated by cultivation of 1g contaminated soil sample using several dilutions. A 1ml of the inoculum was transferred onto molten potato dextrose agar (PDA) sterilized at 121^{o}C for 15 minutes and incubated at 34ºC for 7 days. A loopful of the colonies for sub-culturing was transferred onto prepared sabouraud dextrose agar (SDA) thereafter into tryptone dextrose brooth (TDB) for incubation at 34ºC for 7 days respectively. The AFT filtrate for dietary treatments was obtained using millipore filtration system. The above procedures were according to methods of Prescott *et al.* (1993); Willey *et al.* (2011); Carlos and Joseph (2012); Abubakar *et al.* (2013).

Experimental diets: The AFT dietary contaminant was presented orally for 3 weeks to *A. marginata* as cassava flour (CF) as control; and blended mixture (v/w ratio of 1ml AFT: 50g CF) at 3 concentration treatments of 2000, 6000 and 10000 AFT ppb (10^6 AFT spores/ml = 10^{-6} µg/L = 1ppm = 1000 ppb) respectively. Spore concentrations were according to methods of Madhyastha and Bhat, (1984). The *A. marginata* were fed on alternate days *ad libitum.*

Experimental microscopy: To obtain weekly hepatosomatic index [HSI% = (weight of HPS ÷ weight of whole snail) x 100], fresh wet specimens of HPS from exposed and control *A. marginata* (randomly sampled weekly from each of 2 snails/treatment) were washed in phosphate buffer solution and weighed using digital balance (ESA-1200 Olympic, USA). On the 21st day, HPS were dissected and immediately fixed in 10% neutral buffered formalin for 24hrs, thereafter the preserved HPS specimens were dehydrated through graded series of ethanol and embedded in paraffin wax. The paraffin sections were cut into 4mm semi-thin slices using rotary microtome (Leica RM2235, Germany) and stained with hematoxylin and eosin for microscopy. The sections were viewed and examined with light microscope (Leica DM 2500 Olympic, USA). Experimental microcopy was according to methods of Boucenna *et al.,* (2015).

Statistical analysis: Data obtained were analysed using one way Analysis of Variance (ANOVA) and the means compared by Duncan Multiple Range Test (DMRT) according to methods of SAS (1999), and presented as descriptive statistics.

RESULTS AND DISCUSSION

Results of the present study (Fig. 1) in descriptive statistics reveal that *A. marginata* recorded significant ($p<0.05$) decreasing HSI values of 1.2, 1.0, 0.8; 1.1, 0.8, 0.5; and 1.0, 0.7, 0.3 % at increasing dietary contaminations doses of 200; 600 and 10000 AFT ppb for non-control snails respectively. Sellers *et al.* (2007) reported that although statistics are commonly utilized in evaluation of organ weights in general toxicology studies, organ weights may be reliably interpreted with only descriptive statistics (individual animal

death, number of animals, evaluated means and standard deviation) in consideration with other study data.

The decrease in HSI data as observed in Fig. 1 is comparable to the report of the study carried out by Huang *et al.* (2014) that HSI in groups fed AFT B1 were significantly lower than in the control group. In previous study, the observation of higher HSI in highest dose signifies toxicity (Knuckles *et al.,* 2001); and hepatic response to injury (Sellers *et al.,* 2007). According to Hou *et al.* (2013) dietary mycotoxins led to not only hyperemia and swelling, but also the increased organ index of liver, kidney and spleen. Mikaelian *et al.* (2002) reported that somatic index was not altered by the presence of hepatic lesions or other lesions. Wooley (2003) noted that the use of organ-to-body weight ratios is often helpful for clarifying treatment related organ changes, particularly in non-rodents in which there can be notable variations in organ and body weights. Adams and Molean (1985) while estimating liver somatic indices concluded that individual indices are relatively easy to measure and can reflect adverse effects at the organism level. Sellers *et al.* (2007) reports that although statistics are commonly utilized in evaluation of organ weights in general toxicology studies, organ weights may be reliably interpreted with only descriptive statistics (individual animal death, number of animals, evaluated means and standard deviation) in consideration with other study data.

The histopathological examination of HPS showed (Fig. 2a) intact cellular junctions in the control group. According to Zhu *et al.* (2001) at the beginning of exposure, all cell organelles could still protect the snails from the environmental toxins. Studies of Vogt *et al.,* (1985); Gopinath *et al.* (2011) reported normal structure of different cells and cell organelles in the control group. Encarnacao, (2008) previously explained that even when animals consumed low or moderate contaminated products, as such its effects pass unnoticed and the economic losses are normally just associated with the diseases outbreak that caused the damage.

In Fig. 2b, dilation of intercellular spaces, tubule lumen and lesions was observed. Thomson (1984) reported that breakage of the cell membrane would affect normal cell function and structure. Domonhtsidou and Dimitriadis (2001) inferred that morphological and functional changes caused by pollutants may be a valuable bio-indicator of environmental pollution.

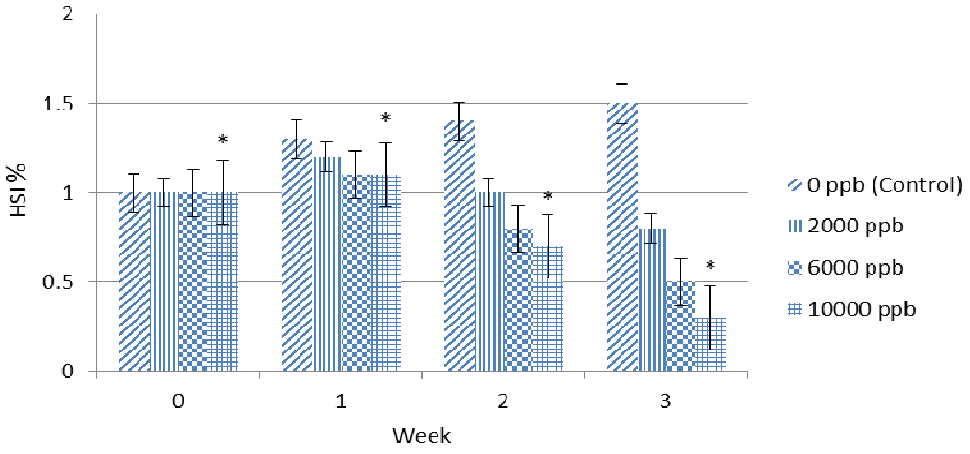

Fig. 1: Hepatosomatic index (HSI) % of *A. marginata* fed AFT

However, Zhu *et al.* (2011) stated that this lysosome activation could be an adaptive mechanism to eliminate or reduce cell damage. Boucenna *et al.* (2015) reported that intercellular exchange and fluidity are disrupted.

The PMS (Fig. 2c) indicates degradation of epithelial cells. This agrees with reports of Gust *et al.* (2011) that lesions of the digestive gland were observed with hypertrophy of calcium cells and vascularization of digestive cells. Biochemically, AFT affects cells metabolism (Ellis *et al.,* 1991).

In *A. marginata* fed the highest dietary AFT dose in this study (Fig. 2d) elucidated sever necrosis and loss of cellular organelles. Gopinath *et al.* (2011) reported AFTs may be considered as biosynthetic inhibitors with large doses causing total inhibition of biochemical system and lower doses affecting different metabolic systems. According to Boran *et al.* (2012) histopathological alterations in the liver are common and these structural changes may cause obstruction of circulation and digestive system. Triebskorn (1989) reported that cell damages by toxins did not necessarily cause body death, but could result in cell death. .

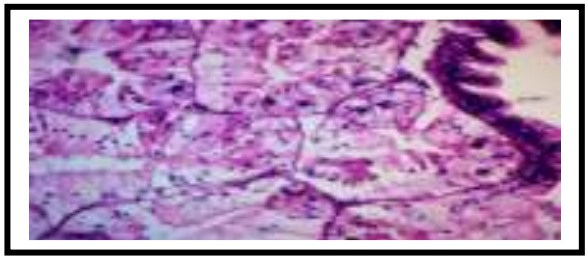

Figure 2a

Figure 2b.

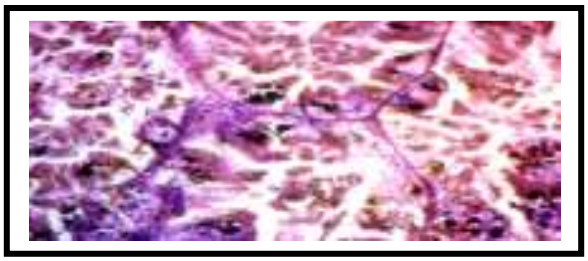

Figure 2c.

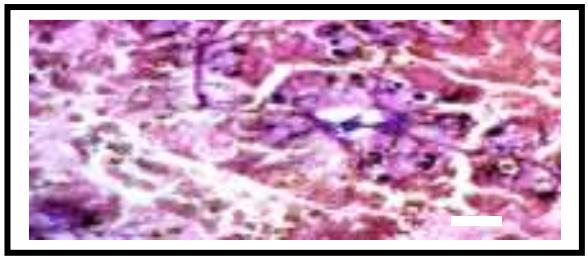

Figure 2d.

Fig. 2: 21st day PMS of *A. marginata* fed AFT-contaminated diets showing alterations at 400x magnification.
2a: 0 ppb (control): Cellular junctions are intact.
2b: 2000 ppb: Dilation of intercellular spaces.
2c: 6000 ppb: Degeneration of epithelial cells, with hypertrophy and cell separation.
2d: 10000 ppb: Excessive vacuolation, necrosis and autophagy.

CONCLUSIONS

- The presence of AFT in this study caused histopathological damages in snail.
- Since the Nigerian weather is favourable for fungal growth and AFT production and with high consumption of snail by rural southern consumers; the public

health consequence of developing adverse conditions merits more investigation

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