

## Enzyme-related aflatoxin production in vital organs of rats fed with *Aspergillus* species- inoculated rat chow

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### ABSTRACT

Wister strain Albino rats were fed with 40 mL distilled water and 20g of rat chow inoculated with *Aspergillus tamarii* Kita IMI 393765 and *Aspergillus flavus* Link IMI 393766 daily for 7 days. A progressive weight loss and reduced sluggishness accompanied very high activity at OD<sub>540</sub> of hepato-specific enzymes-Glutamate oxalate transaminase and Glutamate pyruvate transaminase in the heart and kidney of rats having continuous 7-day contact with *Aspergillus flavus*. Statistical analyses revealed significance at 0.05 % level of probability. A corresponding high aflatoxin level (above 20 ppb) was also determined in all the vital organs. Both enzyme and aflatoxin levels were comparatively lower in the liver and perforations were recorded in the gastrointestinal tract, leading to content leakage. Lower values, though higher than the control, were recorded in those fed on *Aspergillus tamarii*-inoculated rat chow, which also experienced no GI tract damage.

Key Words: Aflatoxin, *Aspergillus* spp., GOT, GPT, rat

### INTRODUCTION

The damaging effects of aflatoxin in animals (including man) tissues is on record (Reed and Kasali, 1987; Robens and Richard, 1992; Mclean and Dutton, 1995; Montesano et al., 1995; Verma and Mehta 1998). Studies on dietary aflatoxin exposure and incidence of liver cancer in China and the West African sub-region showed that the situation was alarming (Groopman and Wild, 2001).

Strains of *Aspergillus flavus* (*A. flavus*) and *Aspergillus parasiticus* (*A. parasiticus*) are typical producers of aflatoxin. However, with the first reported production of aflatoxins B1 and B2 by single spore cultures of *A. tamarii* in Japan in 1996 (Goto et al., 1996; Ito et al., 2001) the stage was set for intensive interest on this unique member of the *Aspergillus* section Flavi group. The interest became more attractive when it was realized that a year earlier, some strains of the group were screened and none of the *A. tamarii* isolates expressed aflatoxin gene. Klich et al. (1995) had then reported that they probably lacked at least one of the necessary genes for its biosynthesis.

Enzymes have been used as indices of mycotoxins production, in many forms including direct correlation between production and release (Hult and Gatenbeck, 1976; Han et al., 1987; Allameh et al., 2000; Atroshi et al., 2000), inverse relation in production between aflatoxin and enzymes involving, for example acetyl CoA carboxylase (Rao and Subramanyam, 2000). In 1988, there was a recorded link between enzymes and aflatoxin biosynthesis (Duton, 1988), with the microsomes playing a significant part in aflatoxin metabolism (Manson et al., 1997). The aim of this study was to observe aflatoxin levels in vital organs of rats using strain of rat that had diet laced with *A. flavus* and *A. tamarii* and relating this to enzyme production.

### MATERIALS AND METHODS

Six-week-old Wister strain albino rats were obtained from the Animal House, College of Medicine, University of Lagos. Glutamic oxalate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) markers were from Randox Laboratories, UK. Rat chow was purchased from Pfizer, Plc, Lagos, and Agri-screen aflatoxin 24-well test kit (Product number 8010, Lot number 11323) was manufactured by Neogen, USA. Confirmation of fungal isolates was done by CABI Bioscience, Surrey, UK. Fifty milliliter distilled water was added to 500g of sterile rat chow (1:10v/w). Each of this was prepared for inoculation with five, 6mm Potato Dextrose agar discs of *A. tamarii* Kita IMI 393765 and *A. flavus* Link IM393766 and incubated for 6 days after initiation of the feeding. Control had no inoculum. In replicates of 3 for each treatment, each rat was respectively given a daily meal of 40mL distilled water and 20g rat chow for 7 days. They were all fed on normal inoculated meal thereafter for another 7 days consecutively. This is to test the metabolite retention capacity (MRC) or the relative effectiveness in biotransformation. Rats were observed for physical

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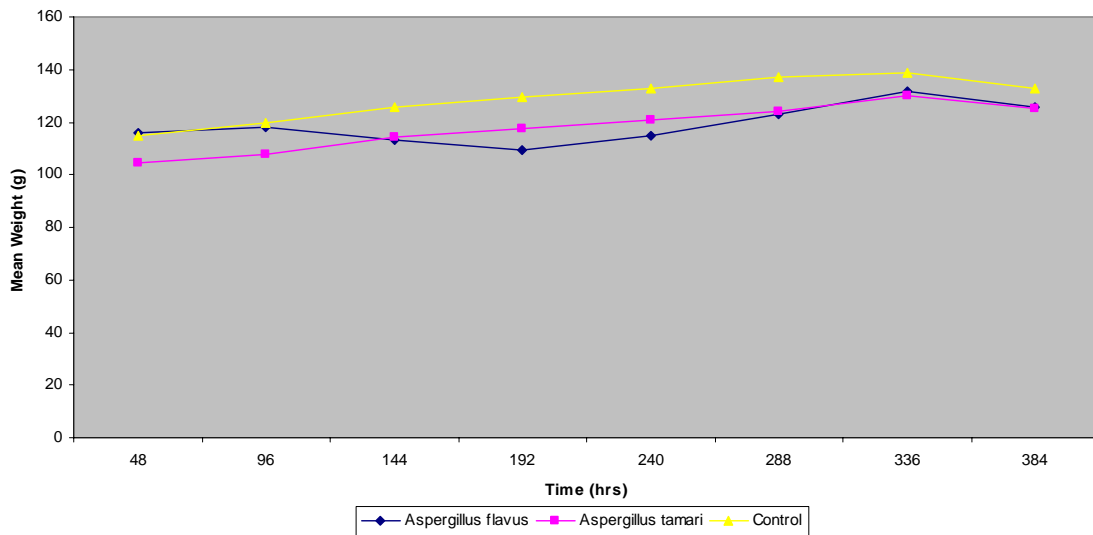
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activity and appetite, and body weight were taken at 2-day intervals. After sacrifice, the GOT, GPT and total aflatoxin levels in the heart, liver and kidney were measured as instructed by the kits.

Also, the ELISA procedure was further carried for confirmation. Serum analyses for the heart, kidney and liver were done using the AgraQuant total aflatoxin assay 4/40 procedure with the aid of a microwell reader at an absorbance filter of 450 nm and a differential filter of 630 nm. The OD of samples were compared with the OD of the standards to give the interpretative result.

## RESULTS AND DISCUSSION

The growth of *A. tamarii* on rat chow was not profuse, when a visual observation was conducted on the sample before feeding the rats. Although the average weight increased throughout the period of observation (Figure 1), the animals expressed slight weakness and sluggishness. In humans, stunted growth had been recorded after ingestion of aflatoxigenic strains along with meal (Gong et al., 2002). *In vivo*, the fungal growth did not incite visible destruction to the gastro intestinal (GI) tract and the vital organs -liver, kidney and heart - tested positive for aflatoxin (Table 1). This directly correlated with a relatively abnormal release of GOT and GPT compared to control.



**Figure 1.** Body weight responses of Albino rats fed with *Aspergillus* spp- inoculated chow

**Table 1.** Total Aflatoxin profile of vital organs of rats after exposure to fungi (Agri-screen)

	A. flavus	A. tamarii	Control
Kidney	red (very high*)	faint red (high**)	blue***
Liver	red (very high)	light blue (low)	blue
Heart	red (very high*)	faint red (high)	blue

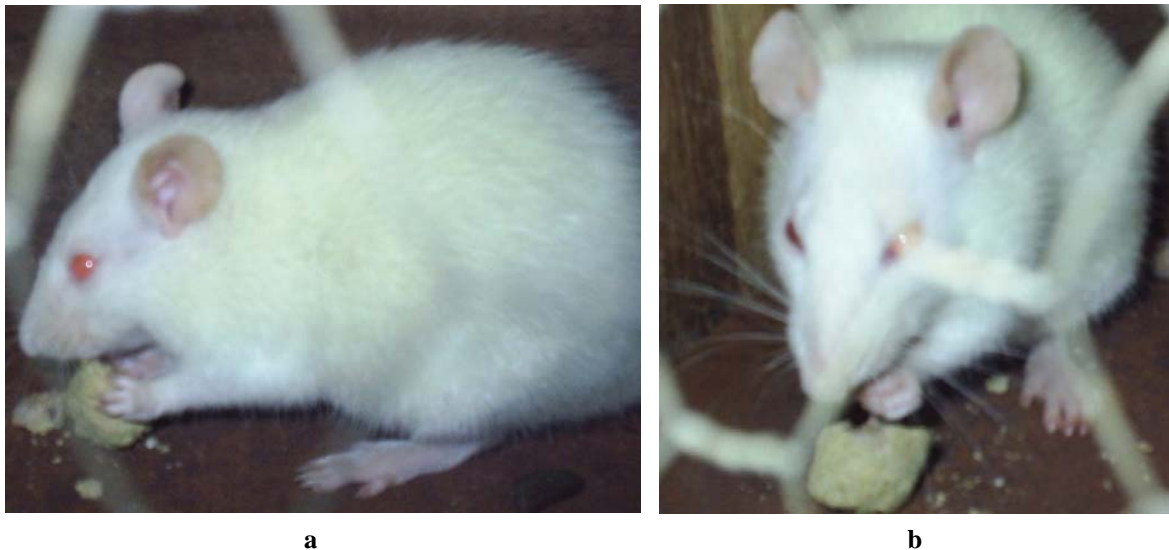
\* in excess of 20ppb

\*\* ≤ 20ppb

\*\*\* no aflatoxin detected

*A. flavus* had a profuse growth on the pellets just as the weight of rats increased up to 48hrs after which it started declining until the end of the feeding time towards day 6, when the animals rejected food. The consumption of *A. flavus*-contaminated grains had earlier been reported to significantly reduce growth rates of broiler chickens (Smith et al., 2006). No recorded effect on the impact of *A. tamarii*-infested meal on the growth of rats or chicken has been noted in literature. When this is related to an earlier strong appetite for food (Fig 2 a,b) up to 48 hrs, it can be deduced that the rapid growth of *A. flavus* altered the taste of the chow and its accumulation adversely affected the body and subsequent reduced growth physiology (Gong et al., 2002). All control subjects showed increase in body weight and no adverse physical and metabolic findings

were detected. Along the length of GI tract, there were openings that gave rise to content leakage (data not shown). Since this did not occur in both the control and the rats fed with meal contaminated with *A. tamaritii*, it was only the competitive growth and metabolites produced by *A. flavus* which equally had the capacity for producing cyclopiazonic acid even when not aflatoxigenic (Purchase, 1971), that could have accounted for the collapse of the tract. Generally and in the 3 organs, the GOT and GPT release was higher than in *A. tamaritii* (Table 2). Using the GLM procedure of the SAS (1999) V8 package, the Duncan's multiple range test for effects in all the vital organs, the GOT and GPT levels with respect to *A. flavus* were significantly higher just as in aflatoxin production than *A. tamaritii* and the 2 species recorded significant difference from the control, all at 5% level of probability. Resting on the principle of reasonable estimates in mycotoxins detection (Whitaker, 2006), there was a direct correlation between enzyme production and aflatoxin profile in all. However, there was a reduction in the level of aflatoxin in the liver. Manson et al. (1997) had explained that this could be related to the activity of microsomes that can metabolize aflatoxin B1 and cytosol that can readily conjugate aflatoxin B1 epoxide to AFB1-dialcohol, and the conversion to metabolic products like aflatoxin Q1 or P1 or M1, and other detoxification residues. However this type of bioconversion to chemoprotective and detoxifying agents depends largely on the genetic make up and other nutritional factors of the rat (Howard and Eaton, 1990; Eaton and Gallagher, 1994; Maxwell et al., 1998).



**Figure 2 (a, b).** Albino rat showing strong appetite for *Aspergillus*-infected rat chow

**Table 2.** Enzyme activity in vital organs at OD<sub>540</sub>

GOT (U/L)	<i>A. flavus</i>	<i>A. tamaritii</i>	Control
Kidney	89 ± 0.00 <sup>a</sup>	53 ± 1.15 <sup>b</sup>	18 ± 0.58 <sup>c</sup>
Liver	89 ± 0.00 <sup>a</sup>	75 ± 0.00 <sup>b</sup>	12 ± 1.73 <sup>c</sup>
Heart	89 ± 0.00 <sup>a</sup>	55 ± 0.58 <sup>b</sup>	20 ± 1.15 <sup>c</sup>
GPT (U/L)	<i>A. flavus</i>	<i>A. tamaritii</i>	Control
Kidney	94 ± 0.00 <sup>a</sup>	65 ± 0.00 <sup>b</sup>	23 ± 0.00 <sup>c</sup>
Liver	94 ± 0.00 <sup>a</sup>	80 ± 0.58 <sup>b</sup>	15 ± 0.00 <sup>c</sup>
Heart	94 ± 0.00 <sup>a</sup>	50 ± 0.58 <sup>b</sup>	24.50 ± 0.50 <sup>c</sup>

Normal values:

GOT: 5-40 U/L

GPT: 8-40 U/L

NOTE: Mean GOT and GPT values for treatments per tested vital organs having the same Duncan's Multiple Range letter are not significantly different at  $P \geq 0.05$ .

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