THE DETOXIFICATION OF AFLATOXIN OXIDASE: THE MECHANISM AND APPLICATION STUDIES

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ABSTRACT

Aflatoxins, a group of mycotoxins, present with carcinogenic, teratogenic, immuno-suppressive and mutagenic toxicity. They can widely contaminate grains and food- and feed-stuffs, especially on the warm and wet area. Aflatoxin oxidase (AFO), isolated from edible Chinese fungus, previously named aflatoxin detoxify which is investigated due to its detoxification to aflatoxin B1. AFO has been cloned and expressed in an engineered yeast. Its oxidation-reduction catalyze action has been discovered by electrochemical research, and Protein Blast research indicate AFO is a new oxidase. Isothermal titration calorimetry determination shows AFO is a copper enzyme, and one molecular of AFO contains one copper ion. AFO acted on its substrate with oxygen dependence. AFO shows a high affinity to its substrate. AFO based biosensors have shown high sensitive, selective and a wide linear range to aflatoxin B1 and sterigmatocystin with immediate electric responses. To be used as additive of feed, the detoxification of AFO on aflatoxin B1 has been confirmed. AFO has performed the role of lessening the liver damages due to aflatoxin exposure, protecting animal’s liver and their health against the harmful aflatoxin contamination.

Key words: Aflatoxin, Microbial Enzyme, Biosensor, Feed

INTRODUCTION

Aflatoxins (AFs) are mycotoxins produced mainly by Aspergillus flavus (A. flavus) and A. parasiticus. They are a group of strong carcinogenic mutagens with great stability, among which, aflatoxin B1 was the most toxic. Aflatoxins may widely contaminate grains and commonly be found in foods and feeds. They present carcinogenic, teratogenic, immunosuppressive and mutagenic potential cause serious disadvantages to human health and high economic damage in the revenue of domestic animals (Enomoto, 1972; Hayes, 1981; Williams, 2004). The epidemiology investigations showed that the resulting implications include immuno-suppression, impaired growth, various cancers including hepatocellular carcinoma, and death depending on the type, period and amount of exposure. A synergistic effect between aflatoxins exposure and some important diseases such as kwashiorkor (severe malnutrition in children), HBV, HCV and HIV/AIDS have been suggested (Wagacha, 2008; Sherman, 2010; Awadelkarim, 2012). Mycotoxins can also reach the human food supply indirectly through animal products (e.g. milk) from livestock that have consumed contaminated feed, and lower doses that produce no clinical symptoms are more significant to public health due to the greater extent of this level of exposure. Ocean warming, climate change and extreme weather events such as floods and droughts may lead to contamination of soil, agricultural lands and affect infection of crops by toxigenic fungi, the growth of these fungi and the production of mycotoxins. Poor post-harvest management and droughts are all reported to stimulate aflatoxins accumulation.

The mineral absorbents used to remove mycotoxins are becoming arguable of the limited mineral resources, the disadvantages of losses of nutrients and the environmental ecologically recycle disorders. Aflatoxin may be degraded by physical and chemical (Nkana, 1987; Park, 1993). However, limitations such as losses of product nutritional and organoleptic qualities, undesirable health effects of such treatments and expensive equipment required
for other degradation techniques has encouraged recent emphasis on biological methods with features of gentle treatment and health and environment friendship (Samarajeewa, 1990; Philips, 1994). Biological transformation and detoxification is focused of attention. An enzyme isolated from *Armillariella tabescens* (E-20) has been confirmed having ability of detoxification towards aflatoxin B1 by Ames test, infrared spectrum and TLC determination (Liu, 1998). The enzyme previously was given a name of aflatoxin detoxifizyme (ADTZ). However, further investigations found this enzyme acts as an oxidase, so it is renamed aflatoxin oxidase (AFO) then. The following have reviewed the detoxification mechanism discoveries and application investigations.

**THE DISCOVERY OF AFO AND ITS FUNCTIONS**

**Purification, cloning and expressing of AFO**

*A. tabescens* (E20) is a Chinese edible and medicinal fungus, from where AFO has been separated and purified by a two-step procedure monitored with AFB1 conversion (Figure 1). (Cao, 2011). ESI-MS/MS analysis was carried out after a trypsin digested procedure (Figure 2). From the sequenced fragments of AFO, full length of AFO has been cloned, expressed and sequenced (Figure 3). Database searching (Protein Blast on NCBI) results do not show any homologous oxidase protein, which implied that AFO was mostly a new oxidase differing from other reported aflatoxin-converting enzymes such as fungal laccase and horse radish peroxidase.

**Electrochemical studies**

To carefully inspect the contradictory bioinformatics result, we have performed electrochemical studies on AFO. AFO acted on substrate with oxygen dependence (Figure 4). Electrochemistry investigations display that the enzyme catalyze oxidation-reduction reaction and exchanges one electron with one proton during the reaction occur. And subsequently, the producing of hydrogen peroxide has been confirmed by HRP (horseradish peroxidase) modified electrode (Figure 5).

And is has also been found the post-reacted mixture of AFO can converse RBG (recessive brilliant green) to BG (brilliant green) with HRP existing, which ensure liberation of hydrogen peroxide. But these results are somewhat confused to us since AFO is neither a NADH or NADPH required enzyme nor a heme protein. Therefore, AFO has supposed to be a divalent metal enzyme. And the following investigations of AFO focus on the binding ability to metal ion

**Metal ion binding ability and substrate affinity determinations**

To explain the function of oxidation-reduction catalysis, effective metal ion bindings are determinate by using isothermal titration calorimetry (ITC), and copper ion is found play an important role in AFO. Each AFO molecule combined with one copper ion (Figure 6 A and B). In the presence of copper ions, AFO demonstrated the ability of

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**Figure 1.** Purification of AFO on column of Phenyl Sepharose Fast Flow, and then a Cu⁺⁺ chelating Sepharose
Figure 2. Analysis of purified AFO by ESI-MS and ESI-Q-TOF. (A) ESI-MS spectra of AFO digested by trypsin; (B) one typical example of ESI-Q-TOF spectra of the trypsin digested fragments of AFO.

Figure 3. SDS-PAGE of AFO purified by IMAC from engineered \textit{E.coli}. M: low molecular weight protein marker; 1, 2, bovine serum albumin; 3, 40 – 70\% saturation ammonium sulfate precipitation of crude extract; 4, Cu\textsuperscript{2+} chelating Sepharose purification of AFO.

Figure 4. Electrochemical response of AFO modified electrode towards AFB1 by CV (left) and DPV (right), which shows the reaction is O\textsubscript{2} dependent. A: control, B: N\textsubscript{2} blow, and C: O\textsubscript{2} blow.
oxygen binding (Figure 6 C). These results may give an explanation to the previous findings that AFO can exchange an electron between oxidation and reduction state and the reaction is oxygen dependent.

Since in the real case, the pollution of AFs are mostly in an extreme as low as ppb or even ppt levels. To estimate the possible value of AFO in practical using, the enzymatic dynamics of AFO towards AFB1 and sterigmatocystin (ST) have been under investigated. The results show by fitting ITC titration curves (Figure 7): KmAFB1 = 0.334 μmol/l, KmST = 0.106 μmol/l; KcatAFB1 = 2.70 min⁻¹, KcatST = 1.74 min⁻¹; ΔHAFB1 = -4.027 × 10⁶ cal/mol, ΔHST = -2.172 × 10⁶ cal/mol, which suggests a high affinity of AFO to bind with AFB1 and ST and implies potential valuable for practical applications.

RESEARCHES OF AFO APPLICATIONS

To be a recognizer used in biosensor

Biosensors which provide selective, sensitive, and accurate measurements as well as fast analysis are attractive in mycotoxins determination (Prieto-Simón, et al., 2007). Available mycotoxins biosensors are mostly based on an immune principle and have one
significant limitation, in that it is necessary to label the molecule such as the enzyme and fluorophore/luminophore to the biospecifically interacting molecules (Melikhova et al., 2006; Tan et al., 2009; Jin et al., 2009), or a cross-reactivity may occur with the secondary antibody, resulting in non-specific signal (Turner et al. 2009). The development of convenient alternative label-free detection techniques is under requirement. Beside of selective and sensitive, enzymatic amperometric biosensors have wider linear range with minor cross or non-specific response due to their definite substrate with definite catalysis reaction.

**Enzymatic amperometric biosensor of AFB1**

Previously we have reported an amperometric aflatoxin biosensor (Li et al., 2011). The sensor was developed by AFO, embedded in sol-gel, linked to multiwalled carbon nanotubes (MWCNTs)-modified Pt electrode. The covalent linkage between AFO and MWCNTs retained enzyme activity and response to the oxidation of AFB1 (Figure 8).

![Figure 7. Enzymatic kinetic analysis of AFO catalysis the reaction with AFB1 (left) and ST (right) by ITC](image1)

![Figure 8. The chronamperometry determination of AFB1 at various potentials by AFO-electrode (a); and the dynamic response of the resulting biosensor upon successive addition of AFB1 (from 1ng/ml to 260 ng/ml) (b); Inset of Fig. 8 (b) shows calibration plots of current versus AFB1 conc. (c); Determination of the apparent Km value: the dynamic amperometric response of the successive additions of aflatoxin B1 (from 16 nmol·L⁻¹ to 224 nmol·L⁻¹), at a working potential of 400 mV. The upper inset shows a calibration plot of the reciprocals of steady-state current versus AFB1 conc. The lower inset shows a calibration plot of steady-state current versus AFB1 conc.](image2)
The apparent Michaelis-Menten constant of the immobilized AFO has been found to be 7.03 μmol·L$^{-1}$, showing a good affinity (Figure 8c). The sensor exhibited a linear range from 3.2 nmol·L$^{-1}$ to 721 nmol·L$^{-1}$ (1 ng/ml to 225 ng/ml) with limits of detection of 1.6 nmol·L$^{-1}$ (0.5 ng/ml, signal-to-noise ratio=3), an average response time of 44 s (less than 30 s when AFB1 Concentration is greater than 45 ng/ml), and a high sensitivity of 0.33 A·mol$^{-1}$·L·cm$^{-2}$ (Figure 8a and b). The active energy was 18.8 kJ mol$^{-1}$, demonstrating the significant catalysis of AFO on oxidation of AFB1 in this biosensor.

**Enzymatic amperometric biosensor for sterigmatocystin (ST)**

Sterigmatocystin (ST), a mycotoxin with the toxicity second to aflatoxins produced by fungi of many Aspergillus species including A. versicolor, A. nidulans, A. chevalieri, A. ruber, A. amstelodami, A. aureolatus, and A. sydowi. Other species such as Bipolaris spp, Emericella venezuelensis are also reported to produce ST (Sivakumar, 2001; Frisvad, 2004; Nielsen, 1999; Atalla, 2003; Abdell-Mallek, 1993; Rabie, 1997; Frisvad, 2005; Schroeder, 1975). ST is a biogenic precursor of aflatoxin B1 (Sreemannarayana, 1987) in aflatoxins biosynthesis; bears a close structural relationship to aflatoxin B1 (Rabie, 1977). The carcinogenic of ST to humans and has been classified as group 2B by the International Agency for Research on Cancer (IARC). The toxicity of ST is primarily confined to the liver and kidney and closely correlated to the occurrence of hepatocellular carcinoma, gastric carcinoma and esophagus carcinoma (Sreemannarayana, 1987; Sivakumar, 2001; Reijula, 2003). ST is found to be one of the predominant contaminating mycotoxins in food and grains (Abraham, 1983; Vesonder, 1985) and relative with the high-incidence areas of malignant tumors in China (Lou, 1995; Wang, 2002; Tian, 2004).

A sensitive electrochemical biosensor based on AFO was developed for detection of ST (Liu, 2011). The enzyme was immobilized on chitosan-single-walled carbon nanotubes (CS-SWCNTs) hybrid film, which attached to the poly-o-phenylenediamine (POPD)-modified Au electrode. The enzymatic electrode exhibited excellent electrocatalytic response to ST (Figure 9 A) with the interferences existing (Figure 9 B). The linear range of ST determination was from 10 ng·mL$^{-1}$ to 310 ng·mL$^{-1}$ with correlation coefficient of 0.997, the detection limit was 3 ng·mL$^{-1}$ (S/N=3), and the response time was less than 10 seconds. The apparent Michaelis-Menten constant (Km$^{app}$) was estimated to be 7.13 μmol·L$^{-1}$. The biosensor had the advantages of good repeatability and stability, remaining 85.6% of its original current value after storage at 4°C for a month, and the RSD for 11 replicate determination of 20 ng·mL$^{-1}$ ST was 3.9%. This AFO-modified electrode showed high selectivity and sensitivity in real sample analysis, giving values of recovery in the range of 91.9 to 100.3% for ST concentration levels of 20, 100, 200, and 500 ng·mL$^{-1}$ in model samples. The data indicate that the developed biosensor is promising for the rapid detection of ST in real samples.

![Figure 9](image-url)  
*(A) The cyclic voltammograms of AFO-modified electrode under various conc. of ST. (B) Amperometric current-time curve illustrating the interferences free sensing of ST at the proposed biosensor in ST (20 ng/ml) and the potential interfering substances (4 g/ml) were added at regular intervals as indicated by the arrows.*
Figure 10. (A) Typical amperometric current-time curve of AFO-modified electrode to successive addition of ST. (B) The corresponding calibration curve of the electrode.

of 82.0 – 115.0% (results not shown here). The proposed method can be applied to the determination of ST in real samples with satisfactory results.

**AFO to be a prepared enzyme agent used in feeds additives**

To determine the effects of ADTZ (prepared agent of AFO) on chicken growth, 840 chickens (Lingnan yellow broilers) in 1-day-old are randomly divided into normal group (basal diet, marked as A), toxin control group (basal diet added with three levels of AFB1, 20 µg/kg, 50 µg/kg and 100 µg/kg marked as B, C and H, respectively), and treatment test group (basal diet added with three levels of AFB1 and five levels of ADTZ at 0.1, 0.2, 0.3, 0.4 and 0.5%, separately, shown as Table 1) (Cao, 2010; Yin, 2010). The experiment lasted for 42 days.

The results showed that: (1) growth performance of broiler fed 20 µg/kg and 50 µg/kg AFB1 were affected: final weight and ADFI were significantly decreased, while F/G was increased, the contents of total protein and albumin in serum were significantly decreased with activities of GOT (glutamic oxalacetic transaminase) and GPT (glutamic pyruvic transaminase) significantly increased. The generalized necrosis of liver and abnormal proliferation of bile duct found through liver tissue sections were found in 100 µg/kg AFB1 toxin group and (2) 20 µg/kg, 50 µg/kg and 100 µg/kg AFB1 added with ADTZ improved growth performance, downgrade residual AFB1 in blood and liver and related parameters to normal levels. These results may lead a conclusion that ADTZ may reduce or almost eliminate the negative effects of AFB1 on growth performance and organs of Lingnan yellow broiler and the appropriate amounts of ADTZ added is suggested to be 0.3%.

As Table 2 shows, the reduced of average slaughtering weight of group B indicate that as low as 20 ppb (*may be higher than this level) contamination of feed may influence the growth. The effective of ADTZ has shown when compare group D and E with group B, the average slaughtering weight enhanced by 1.45 and 2.42%, respectively. Compare group J, K, L and M with group H the average slaughtering weight raised by 1.40, 1.58, 4.62 and 2.10%, respectively, while the average slaughtering weight of group O was reduced.

**Table 1. The groups of AFB1 levels and the amount of ADTZ added in basal diets**

<table>
<thead>
<tr>
<th>Basel diet added with AFB1</th>
<th>0% AFB1</th>
<th>0.1% ADTZ</th>
<th>0.2% ADTZ</th>
<th>0.3% ADTZ</th>
<th>0.4% ADTZ</th>
<th>0.5% ADTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg/kg</td>
<td>A</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>20 µg/kg AFB1</td>
<td>B</td>
<td>D</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>50 µg/kg AFB1</td>
<td>C</td>
<td>F</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>100 µg/kg AFB1</td>
<td>H</td>
<td>J</td>
<td>K</td>
<td>L</td>
<td>M</td>
<td>O</td>
</tr>
</tbody>
</table>
Table 2. The effect of ADTZ on growth and AFB1 residual of AFB1 exposure broiler

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average slaughtering weight (g)</th>
<th>Feed conversion (F/G)</th>
<th>Residual AFB1 in blood (ng/g)</th>
<th>Residual AFB1 in liver (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (basal diet)*</td>
<td>1217.33 ± 48.51</td>
<td>1.93 : 1</td>
<td>0.041 ± 0.04</td>
<td>6.36 ± 0.44</td>
</tr>
<tr>
<td>B (+20 ppb AFB1)</td>
<td>1149.00 ± 18.40</td>
<td>1.95 : 1</td>
<td>0.084 ± 0.013</td>
<td>9.63 ± 0.91</td>
</tr>
<tr>
<td>D (+20 ppb AFB1 + 1% ADTA)</td>
<td>1165.67 ± 30.56</td>
<td>1.98 : 1</td>
<td>0.051 ± 0.014</td>
<td>6.53 ± 1.47</td>
</tr>
<tr>
<td>E (+20 ppb AFB1 + 3% ADTA)</td>
<td>1176.78 ± 41.94</td>
<td>1.93 : 1</td>
<td>0.044 ± 0.004</td>
<td>6.10 ± 1.65</td>
</tr>
<tr>
<td>C (+50 ppb AFB1)</td>
<td>1144.89 ± 22.11</td>
<td>1.95 : 1</td>
<td>0.051 ± 0.007</td>
<td>16.73 ± 3.02</td>
</tr>
<tr>
<td>F (+50 ppb AFB1 + 1% ADTA)</td>
<td>1177.78 ± 38.20</td>
<td>1.96 : 1</td>
<td>0.036 ± 0.009</td>
<td>9.99 ± 2.53</td>
</tr>
<tr>
<td>G (+50 ppb AFB1 + 3% ADTA)</td>
<td>1102.22 ± 27.38</td>
<td>1.95 : 1</td>
<td>0.016 ± 0.004</td>
<td>7.02 ± 1.28</td>
</tr>
<tr>
<td>H (+100 ppb AFB1)</td>
<td>1130.56 ± 39.58</td>
<td>1.96 : 1</td>
<td>0.051 ± 0.005</td>
<td>20.86 ± 4.05</td>
</tr>
<tr>
<td>J (+100 ppb AFB1 + 1% ADTA)</td>
<td>1146.44 ± 24.83</td>
<td>1.95 : 1</td>
<td>0.033 ± 0.004</td>
<td>16.82 ± 3.17</td>
</tr>
<tr>
<td>K (+100 ppb AFB1 + 2% ADTA)</td>
<td>1148.44 ± 16.42</td>
<td>1.95 : 1</td>
<td>0.028 ± 0.004</td>
<td>9.30 ± 1.81</td>
</tr>
<tr>
<td>L (+100 ppb AFB1 + 3% ADTA)</td>
<td>1182.78 ± 14.47</td>
<td>1.93 : 1</td>
<td>0.043 ± 0.007</td>
<td>9.75 ± 1.57</td>
</tr>
<tr>
<td>M (+100 ppb AFB1 + 4% ADTA)</td>
<td>1154.33 ± 14.95</td>
<td>1.95 : 1</td>
<td>0.033 ± 0.004</td>
<td>8.17 ± 1.31</td>
</tr>
<tr>
<td>O (+100 ppb AFB1 + 5% ADTA)</td>
<td>1111.22 ± 20.66</td>
<td>1.95 : 1</td>
<td>0.035 ± 0.006</td>
<td>7.33 ± 1.20</td>
</tr>
</tbody>
</table>

*Possibly at a low level of contamination

by 1.70 %. The data of residual of AFB1 in blood and liver have revealed the effectiveness of ADTZ unanimously. AFB1 residual in blood of group J, K, L, M and O showed lower than 0.04 ng/g, while in group H AFB1 residual in blood is 0.05 ng/g. When compare group F and G with group C, compare group D and E with group B, the similar result may conclude. Liver is the main detoxification organ for animal; a healthy liver is seriously for animal’s health. So the lower residual in liver is critically important for animal.

The effective of ADTZ to remove AFB1 is clearly shown when detect the liver residual of AFB1. At low contamination (20 ppb), 1% of ADTZ formula is effective, while for higher contamination (50 ppb and 100 ppb), 3% of ADTZ is suggested.

The toxicity of AFB1 may lead to an increased activities of GOT and GPT. As Table 3 shown, the addition of ADTZ up to 3% may significantly better the harmfulness: activities of GOT and GPT are lower with higher total proteins, albumin and globulin, which can almost match the data of basal diet control group A.

In histological study (Fig. 11), on the basal diet control group A, the liver structure is basically normal with slightly denature of liver cell and swelling. On the toxin control group H, the damages include liver cells are hyperplastic around portal (as arrow point), the various size of nuclear, management disorder of hepatic cells, spotted nerosis region and cell lysis. On the treatment group L, although the slightly disorder of the management of liver cell, hepatic cell hypertrophy, various sized spherical lipid droplet in plasma and bigger unclear, the management of hepatic cell structure is basically normal. On group M, except the slightly swelling of hepatic cell, the management of cell structure is normal.
### Table 3. Effect of ADTZ on serum enzyme activities and serum protein

<table>
<thead>
<tr>
<th>Groups</th>
<th>GOT</th>
<th>GPT</th>
<th>Total Prot.</th>
<th>Albumin</th>
<th>Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A* (basal diet)</td>
<td>194.36 ± 4.38</td>
<td>1.84 ± 0.27</td>
<td>47.39 ± 1.21</td>
<td>19.74 ± 0.41</td>
<td>27.65 ± 1.19</td>
</tr>
<tr>
<td>B (+20 ppb AFB1)</td>
<td>204.90 ± 6.69</td>
<td>2.35 ± 0.58</td>
<td>42.09 ± 1.62</td>
<td>18.96 ± 0.45</td>
<td>22.06 ± 1.53</td>
</tr>
<tr>
<td>D (+20 ppb AFB1 + 1‰ ADTZ)</td>
<td>202.90 ± 5.61</td>
<td>2.22 ± 0.46</td>
<td>42.10 ± 2.35</td>
<td>19.07 ± 0.71</td>
<td>22.70 ± 1.58</td>
</tr>
<tr>
<td>E (+20 ppb AFB1 + 3‰ ADTZ)</td>
<td>198.60 ± 5.04</td>
<td>2.12 ± 0.41</td>
<td>44.34 ± 1.69</td>
<td>19.06 ± 0.77</td>
<td>22.42 ± 1.19</td>
</tr>
<tr>
<td>C (+50 ppb AFB1)</td>
<td>207.03 ± 5.37</td>
<td>2.44 ± 0.32</td>
<td>41.33 ± 0.86</td>
<td>19.24 ± 0.52</td>
<td>20.53 ± 1.24</td>
</tr>
<tr>
<td>F (+50 ppb AFB1 + 1‰ ADTZ)</td>
<td>202.71 ± 5.85</td>
<td>2.40 ± 0.45</td>
<td>42.20 ± 1.33</td>
<td>19.23 ± 0.77</td>
<td>20.05 ± 0.62</td>
</tr>
<tr>
<td>G (+50 ppb AFB1 + 3‰ ADTZ)</td>
<td>199.23 ± 5.62</td>
<td>2.22 ± 0.39</td>
<td>43.72 ± 2.06</td>
<td>19.79 ± 0.55</td>
<td>22.54 ± 1.74</td>
</tr>
<tr>
<td>H (+100 ppb AFB1)</td>
<td>212.64 ± 2.72</td>
<td>3.16 ± 0.43</td>
<td>41.04 ± 1.53</td>
<td>18.18 ± 0.68</td>
<td>21.67 ± 1.47</td>
</tr>
<tr>
<td>J (+100 ppb AFB1 + 1‰ ADTZ)</td>
<td>207.87 ± 4.87</td>
<td>3.24 ± 0.42</td>
<td>41.63 ± 1.36</td>
<td>18.95 ± 0.76</td>
<td>22.97 ± 1.31</td>
</tr>
<tr>
<td>K (+100 ppb AFB1 + 2‰ ADTZ)</td>
<td>199.56 ± 3.49</td>
<td>2.20 ± 0.32</td>
<td>43.34 ± 2.16</td>
<td>19.95 ± 0.45</td>
<td>24.91 ± 1.92</td>
</tr>
<tr>
<td>L (+100 ppb AFB1 + 3‰ ADTZ)</td>
<td>187.80 ± 2.84</td>
<td>1.90 ± 0.60</td>
<td>45.62 ± 1.41</td>
<td>21.24 ± 0.60</td>
<td>24.66 ± 1.03</td>
</tr>
<tr>
<td>M (+100 ppb AFB1 + 4‰ ADTZ)</td>
<td>196.99 ± 5.06</td>
<td>1.91 ± 0.37</td>
<td>42.67 ± 1.54</td>
<td>21.68 ± 0.87</td>
<td>22.24 ± 0.87</td>
</tr>
<tr>
<td>O (+100 ppb AFB1 + 5‰ ADTZ)</td>
<td>198.40 ± 6.09</td>
<td>1.92 ± 0.39</td>
<td>43.67 ± 2.74</td>
<td>20.16 ± 0.91</td>
<td>24.95 ± 2.53</td>
</tr>
</tbody>
</table>

*Possibly at a low level of contamination

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**Figure 11.** Histological studies of AFB1 and ADTZ’s antitoxic influences on broiler liver
Comprehensively, the histological studies indicated the surely effective of ADTZ to lessen the damages of AFB1 (100 ppb) on hepatic cells.

**CONCLUSION**

Aflatoxin oxidase (AFO) is a copper enzyme, active with oxygen dependence. It has a high affinity to its substrate aflatoxin B1 and sterigmatocystin. AFO has been successfully used as the recognizer on biosensor to detect aflatoxin B1 and sterigmatocystin with wide linear rang, high selectivity and sensitivity. The detoxification of AFO on aflatoxin B1 has been confirmed. It can be used as feeds additive to lessen the live damages of aflatoxin exposure, protect animal’s liver and their health against the harmful aflatoxin contamination.

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