Introduction

Mycotox Solutions Inc. (“MSI”), a Winnipeg (Canada) based Biotech Company, has a patented (pending) revolutionary technology to eliminate the fatal toxic and carcinogenic effects of mycotoxins (fungal toxins) in humans and animals caused by the consumption of toxin- contaminated food and feed, respectively. The annual losses in the US and Canada due to the impact of mycotoxins on the feed and livestock industries are estimated at US$5 billion. The market for feed mycotoxin detoxifiers in North America, Europe and Asia Pacific, was estimated at $1.8 billion USD in 2015 and $2.1 billion USD in 2020.

MSI’s Technology

Currently marketed mycotoxin binders are nonspecific and only partially effective at absorbing mycotoxins. Furthermore, they absorb important nutrients (vitamins, minerals, antioxidants, etc.) in animal feed, which causes side effects on the nutritional health of livestock, poultry and pets. These adverse side effects include reduced feed intake, less weight gain, decreased milk yield, and increased susceptibility to infectious diseases.

Thus, there is an unmet market need for developing mycotoxin detoxifying products that not only prevent gut absorption of toxin, but also deactivate absorbed toxin in the body for excretion. MSI’s unique two-pronged approach to detoxify mycotoxins in feed involves the use of: (i) specific aptamer-based toxin binders to prevent absorption of toxins in the gut; and (ii) phytochemical-based toxin deactivators to transform absorbed toxin into nontoxic or less toxic compounds in the body for excretion. The combination of these approaches will enable MSI to develop a unique, safe and highly effective product that will be superior to competing products that are currently on the market. Furthermore, MSI’s products will not only greatly reduce gut absorption of mycotoxin, but will also deactivate absorbed mycotoxin by activating the detoxifying enzyme system in the body. MSI’s products will therefore prevent the transfer of mycotoxin contamination via milk, eggs and meat to humans.

Experimental Data

The goal of this study was to assess the neutralizing effect of an Aflatoxin B1 (AFB1) specific aptamer on AFB1 toxicity induced lethality in zebrafish. The study was contracted out to Phylonix Pharmaceuticals Inc., Cambridge, MA, USA.

PROCEDURE: Zebrafish embryos were generated by using a Mass Embryo Production System. Embryos were cleaned by removing dead embryos and sorted by developmental
stage. As embryos receive nourishment from an attached yolk sac, no feeding was required for 6-days post fertilization (dpf). Aflatoxin B1 stock solution was prepared in DMSO and added to the fish water as shown in Table 1 at a final conc. of 0.25 µM AFB1 & 0.1% DMSO. Aflatoxin B1 specific aptamer Master Stock (MS) solution was prepared by dissolving a known quantity (~350 nmoles) of aptamer powder in 2.8 ml fish water to generate a 125 µM AFB1-aptamer MS. 40 µl of 125 µM AFB1-aptamer MS solution was pre-incubated with 4 µl of 0.25 mM AFB1 in 6-well microplates containing 1956 µl fish water/well for 15 min. After pre-incubation, 2 ml fish water containing 2 dpf self-hatched zebrafish (N = 30) was added to the pre-incubated solution to yield a final volume of 4 ml (Final conc. of AFB1-aptamer was 1.25 µM, 5 fold higher than final AFB1 conc). 2 dpf zebrafish were exposed continuously to each condition for 96 hr. Final treatment conditions in 4 ml fish water are shown in Table 1. Final DMSO concentration was 1% for each condition.

Table 1: Final Conditions for AFB1Toxicity Induced Lethality Test

<table>
<thead>
<tr>
<th>Condition</th>
<th>Final Conc. of AFB1 (µM)</th>
<th>Final conc. of AFB1-Aptamer (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% DMSO</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AFB1-Aptamer + 0.1% DMSO</td>
<td>0</td>
<td>1.25</td>
</tr>
<tr>
<td>AFB1 + 0.1% DMSO</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>AFB1+AFB1-Aptamer + 0.1% DMSO</td>
<td>0.25</td>
<td>1.25</td>
</tr>
</tbody>
</table>

1Dead zebrafish were counted daily and removed. After treatment for 96 hr, total lethality was calculated. To obtain mean and Standard Deviation (SD) for each condition, experiments were performed 3 times.

TEST RESULTS: After treatment for 96 hrs, 0% lethality was observed when treated with 0.1% DMSO alone, validating the assay. 0% lethality was also observed after treatment with AFB1-specific aptamer alone + 0.1% DMSO confirming that AFB1-specific aptamer did not induce lethality in zebrafish. However, 82.2 ± 1.9% lethality was observed in zebrafish after treatment with AFB1 alone + 0.1% DMSO. In contrast, 0% lethality was observed after treatment with AFB1 and AFB1-specific aptamer combination (AFB1+AFB1-Specific Aptamer) + 0.1% DMSO, indicating that AFB1-specific aptamer neutralized 100% AFB1 toxicity induced lethality in zebrafish (Table 2; FIG. 1).
Table 2: Results of Testing the Neutralizing Effect of Aflatoxin B1 (AFB1) Aptamer on AFB1 Toxicity Induced Lethality in Zebrafish¹

<table>
<thead>
<tr>
<th>Final Conc. (µM)</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% DMSO</td>
<td>0 (0/30)</td>
<td>0 (0/30)</td>
<td>0 (0/30)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AFB1-Aptamer + 0.1% DMSO</td>
<td>0 (0/30)</td>
<td>0 (0/30)</td>
<td>0 (0/30)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AFB1 + 0.1% DMSO</td>
<td>80 (24/30)</td>
<td>83.3 (25/30)</td>
<td>83.3 (25/30)</td>
<td>82.2</td>
<td>1.9</td>
</tr>
<tr>
<td>AFB1 + AFB1-Aptamer + 0.1% DMSO</td>
<td>0 (0/30)</td>
<td>0 (0/30)</td>
<td>0 (0/30)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Numbers in Parentheses: Number of dead zebrafish divided by number of zebrafish per well.

FIG. 1: Evaluation of Neutralizing Effect of Aflatoxin B1 (AFB1)-Specific Aptamer (Apt) on AFB1 Toxicity Induced Lethality in Zebrafish (ZF)¹.
Desirable Features

- Specific to a selected mycotoxin
  - Complete neutralization of a selected mycotoxin toxicity
  - Does not bind to or absorb nutrients, such as vitamins, minerals & antioxidants
  - Unlikely to cause adverse side effects, such as reduced feed intake, less weight gain, decreased milk yield, and increased susceptibility to infectious diseases
  - Can deactivate mycotoxin in the body (if absorbed) when aptamer binder is used in combination with a phytochemical (an antioxidant)
  - Prevents the transfer of mycotoxin contamination via milk, eggs and meat to humans as it could completely neutralize the toxicity of selected mycotoxin

Tech Advantages

- Aptamers are future replacements for antibodies as they have many advantages over them
  - Simpler and lower cost of production with a very low lot to lot variability
  - They are small molecules (mostly less than 30 kDa) and are readily produced by chemical or biological synthesis
  - They possess desirable storage properties as they are capable of withstanding a wide range of temperatures.
  - They can be optimized for persistence under physiological conditions, such as a low or high pH or the presence of hydrolytic enzymes, during selection
  - Aptamers are essentially nonimmunogenic
  - Universal antidotes are available
  - This technology has multiple applications as specific aptamer binders can be developed for all mycotoxins