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# Evaluation of acute oral toxicity of a polyherbal broad-spectrum mould inhibitor and mycotoxin binder

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**Abstract**
Mycotoxins are biologically active, toxic metabolites produced by toxigenic fungi mainly belonging to genus Aspergillus, Fusarium and Penicillium. They are ubiquitous in poultry feeds. Mix-02-Tox is a broad-spectrum mould inhibitor and mycotoxin binder, recommended for complete protection against multiple mycotoxins and for better production in poultry, cattle and pigs. A study was undertaken to evaluate the acute oral toxicity potential of Mix-02-Tox (M/s Ayurvet Limited, India) according to OECD 423 guidelines. Six (3 male and 3 female) Swiss albino mice were used for the study, where each animal served as its own control. Following the oral administration of the test substance, the animals were observed for the manifestation of toxic effects and mortality. No toxic effects or mortalities were observed till 14 days and Mix-02-Tox was found to be safe for oral use.

**Keywords:** Acute oral toxicity, Mix-02-Tox, OECD 423, safety, limit test

## Introduction
Mycotoxins are commonly present in animal feeds. Consumption of feeds contaminated with these mycotoxins may cause a variety of harmful effects, depending on the type of mycotoxin, quantity and duration of exposure, and the health status of the animal at the time of exposure [1]. Mycotoxicosis is a disease caused by secondary metabolite of fungi-mycotoxins. Some mycotoxins commonly encountered in animal feeds are aflatoxins, ochratoxins, zearalenone, fumonisins and trichothecenes. Aflatoxin (AF) and ochratoxin (OA) are two main toxins affecting the domesticated animals. Aflatoxin B1 (AFB1), the most toxic of all AF, is the most potent hepatocarcinogen [2]. In poultry, aflatoxicosis leads to jaundice, generalized edema, hemorrhages, periportal necrosis with bile duct proliferation, and fibrosis and depletion of lymphoid organs, ultimately causing poor nutrient absorption, poor growth rate, higher FCR, production problems and mortality [3]. Ochratoxicosis is primarily a disease of kidney in poultry and intoxication of birds by ochratoxins results in impaired feed efficiency, reduced weight gain, reduced egg production and reduced egg quality [4]. Furthermore, it causes reduction in total blood proteins [5], impairment of blood coagulation [6] and suppression of immune functions [7]. Ochratoxin a (OTA) induces degenerative changes and an increase in the weight of kidney and liver, as well as a decrease in the weights of the lymphoid organs [8]. Mix-02-Tox is a polyherbal broad-spectrum mould inhibitor and mycotoxin binder, recommended for complete protection against multiple mycotoxins and for better production in poultry, cattle and pigs. It contains herbs like *Emblica officinalis*, *Azadirachata indica*, *etc*. reputed for their hepatoprotective and alexiteric activities in Ayurveda [9]. The present study aimed at determining the acute oral toxicity potential of Mix-02-Tox.

## Materials and Methods
The present study was conducted at the Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science (KNPCVS), Shirwal, District Satara, India. The experimental protocol of the study was got approved by the Institutional Animal Ethics Committee of KNPCVS (Approval number: IAEC/16/KNPCVS/05/2019; dated: 23/08/19). Six healthy adult (3 males and 3 females) Swiss albino mice, weighing 20-25g, were used. The animals were procured from CPCSEA-registered breeding source *i.e.* National Institute of Biosciences, Pune. All animals were maintained as per the SOPs outlined in

CPCSEA guidelines. The animals were identified by appropriate means. The number of animals per cage was kept at three for clear observation of each animal; housing conditions were conventional. The ambient temperature was 25 °C and relative humidity was 70%. The animals were exposed to 12 hour light-dark cycle and provided with standard pelleted feed and water *ad lib* [10]. After procurement, the animals were kept in the cages for seven days for acclimatization. Thereafter, the animals were fasted overnight; food but not water was withheld for 3-4 hours. Following the period of fasting, the animals were weighed and the test substance was administered orally. After the administration of the test substance @ 2000 mg/Kg body weight, food was withheld for 1-2 hours. The animals were observed intensively for first 24 h, and then further for a period of 14 days for the manifestation of toxic effects and deaths; $LD_{50}$ value was also assessed. The observations included changes in skin, coat and eyes; and changes in respiratory, circulatory, CNS, autonomic, somatic activity and behavior. Clinical signs like muscular tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma, if observed, were recorded. After 14 days of observation, the animals were euthanized and necropsy, along with the histopathological investigations of the liver, kidneys, spleen, heart, lungs, and reproductive organs, was performed.

## Results and Discussion

Individual body weights of mice were recorded on days 0, 7 and 14 of the study and body weights in both the groups (I and II) continued to increase throughout the study period (Table 1).

**Table 1:** Individual body weights of experimental mice

| Formulation and Dose | Mice No. | Body Weight (g) on Day | | |
|---|---|---|---|---|
| | | 0 | 7 | 14 |
| Mix-02-Tox @ 2000 mg/Kg b.wt. orally (Group I: Females) | 1 | 20 | 21 | 23 |
| | 2 | 22 | 23 | 24 |
| | 3 | 20 | 22 | 25 |
| Mix-02-Tox @ 2000 mg/Kg b.wt. orally (Group II: Males) | 1 | 20 | 21 | 23 |
| | 2 | 22 | 24 | 25 |
| | 3 | 22 | 24 | 25 |

No mortality was seen throughout the period of observation. In the six mice receiving the limit dose of Mix-02-Tox at 2000 mg/Kg body weight *i.e.* the maximum dose which can be administered by oral route, no mortality occurred and hence, the $LD_{50}$ was inferred to be beyond this limit. Similarly, no abnormal symptoms, including lethargy, tremor, abdominal breathing or piloerection, were observed up to 14 days of Mix-02-Tox administration. Necropsy after day 14 did not reveal any remarkable alterations in the gross appearance of the liver, kidneys, spleen, heart, lungs, and reproductive organs in any of the animals. Similarly, no abnormalities were detected in the histopathological appearances of the liver, kidneys, spleen, heart, lungs, and gonadal organs in any of the animals.

Mix-02-Tox contains parts of plants like *Emblica officinalis*, *Azadirachta indica*, *etc*. that fall under the category of Generally Regarded as Safe (GRAS). *Emblica officinalis* is a rich source of vitamin C and tannins, and, hence, is a good source of antioxidants. The fruit helps to purify blood, strengthens the liver, helps in the removal of toxins from the body and supports the functions of the liver. Also, a hydroalcoholic extract of the fruit was shown to reduce the severity of hepatic fibrosis induced by carbon tetrachloride and thioacetamide. The fruit extract was found to afford hepatoprotection through its membrane stabilizing, antioxidant and CYP2E1-inhibitory properties [11]. The plant and its extracts have shown remarkable abilities to ameliorate mycotoxin-induced liver injury in different species [12, 15].

*Azadirachta indica* leaf extract causes 23% and >90% inhibition of aflatoxin biosynthesis at 1.56% and 50% (v/v) concentrations, respectively. Addition of aqueous neem leaf extract above 10% (v/v) inhibits the production of aflatoxins due to suppression in glutathione S-transferase (GST) activity [16]. It also promotes growth [17], and improves performance, hematological parameters [18] and immune response [19] in broilers. It has antifungal and anti-inflammatory properties [20], thus optimizing nutrient utilization and production in mycotoxicated broilers. A composition based on GRAS constituents like *Emblica officinalis*, *Azadirachta indica*, is least likely to be toxic in practical doses. Mix-02-Tox exerts multifarious benefits, including protection of vital organs against adverse effects of mycotoxins and improvement of growth and production in broilers and layers, due to the presence of multiple active ingredients.

## Conclusion

Mix-02-Tox did not produce acute oral toxicity, evident as absence of mortality, toxic clinical symptoms, and gross and histopathological alterations, when administered up to limit dose (2000 mg/Kg) in mice. Based on this study, the formulation was found safe for oral use.

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