Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 1431-1433

Received: 16-11-2020
Accepted: 18-12-2020

Vikas Vasant Karande
Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Maharashtra, India

Vaishnavi Sanjay Gagare
Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Maharashtra, India

Sunidhi
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Ravikanth Kotagiri
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Bhaskar Ganguly
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Corresponding Author:
Sunidhi
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Evaluation of acute oral toxicity of a broad-spectrum anti-mycotoxin and hepato-protective formulation

Vikas Vasant Karande, Vaishnavi Sanjay Gagare, Sunidhi, Ravikanth Kotagiri and Bhaskar Ganguly

DOI: https://doi.org/10.22271/j.ento.2021.v9.i1t.8338

Abstract
Mycotoxins are biologically active, toxic metabolites produced by toxigenic fungi mainly belonging to the genera *Aspergillus*, *Fusarium* and *Penicillium*. They are ubiquitous in poultry feeds. Mix-01-Vil 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-01-Vil (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-01-Vil was found to be safe for oral use.

Keywords: acute oral toxicity, mix-01-Vil, OECD 423, safety, limit test

Introduction
Mycotoxins are highly diverse secondary metabolites produced in nature by a wide variety of fungi which cause food contamination, resulting in mycotoxicoses in animals and humans. The genera of mycotoxin-producing fungi are *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Phomopsis*, *Emericella*, *Cephalosporium*, *Trichoderma*, *Trichothecium*, *Neotyphodium* and *Claviceps* [1]. Aflatoxin (AF), Ochratoxin (OA) and T-2 toxin are the main toxins affecting the domesticated animals. Aflatoxin B1 (AFB1), the most toxic of all AF, is the most potent hepatocarcinogen [2]. Ochratoxicosis is primarily a disease of the kidneys. 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 [3]. Aflatoxicosis and ochratoxicosis result in a rubbery condition of the bones, apparently related to increased tibial diameters and perhaps poor mineralization of the bones in young broiler chicks. In poultry, the T-2 toxin has been the causative agent for mouth and intestinal lesions, in addition to the impairment of immune responses, destruction of the hematopoietic system, decrease in serum protein concentrations, increase in prothrombin, declining egg production, thinning of egg shells, refusal of feed, weight loss and altered feather patterns, abnormal positioning of the wings, hysteroid seizures or an impaired righting reflex [4].
Mix-01-Vil 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 *Andrographis paniculata*, *Emblica officinalis*, *Azadirachata indica*, *Allium sativum*, *etc*. reputed for their hepatoprotective, alexiteric and immunomodulatory activities in Ayurveda [5-7]. The present study aimed at determining the acute oral toxicity potential of Mix-01-Vil.

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* [8]. 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-01-Vil @ 2000 mg/Kg body weight orally (Group I: Females)	1	22.0	23.0	24.0
	2	20.0	21.0	23.0
	3	22.0	22.0	24.0
Mix-01-Vil @ 2000 mg/Kg body weight orally (Group II: Males)	1	22.0	22.0	23.0
	2	22.0	24.0	24.0
	3	23.0	24.0	23.5
Mean ± S.E.		21.83±0.37	22.67±.45	23.58±0.18

No mortality was seen throughout the period of observation. Since no mortality occurred in the six mice receiving the limit dose of Mix-01-Vil at 2000 mg/Kg body weight *i.e.* the maximum dose which can be administered by oral route, therefore, 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-01-Vil 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-01-Vil contains parts of plants like *Andrographis paniculata*, *Emblica officinalis*, *Azadirachta indica*, *Allium sativum*, *etc*. that are Generally Regarded as Safe (GRAS). Methanolic extracts of *Andrographis paniculata* exhibited a significant hepatoprotective activity against paracetamol-induced (500 mg/Kg) hepatic damage in mice when orally administered at doses of 10 mg/Kg and 100 mg/Kg at 24 and 72 hours time interval in each group. There was also a significant decrease ($P<0.05$) in the liver protein content of the hepatotoxicated mice after the treatments [9]. Andrographolide present in *Andrographis paniculata* was reported to have immunoregulatory activities. In tumor-bearing mice it enhanced the activity of natural killer cells [10], increased the secretion of IL-2 and IFN-γ by T-cells and, thereby, inhibited tumor growth [11]. In autoimmune encephalomyelitis in mice, it interfered with the maturation of dendritic cells [12], induced antigen-specific tolerance and, thus, prevented detrimental autoimmune responses [13]. *Emblica officinalis* tannoids emblicanin A, emblicanin B, punigluconin and pedunculagin have been reported to exhibit antioxidant activity *in vitro* and *in vivo* [14]. When an aqueous extract of *Emblica officinalis* fruits was given to alloxan-induced diabetic rats @ 0.35 g/Kg body weight for 8 weeks, the total white and red blood cells, T- and B-cells increased, while the number of monocytes and eosinophils decreased in the treatment group, thus showing immunomodulatory activity [15]. Pre-treatment with *E. officinalis* at doses of 100 and 200 mg/Kg body weight, prior to CCl_4 intoxication showed significant reduction in the levels of SGOT, SGPT, LDH, glutathione-S-transferase and DNA synthesis. There was also increase in reduced glutathione, glutathione peroxidase and glutathione reductase, suggesting that the *E. officinalis* inhibits hepatic toxicity in Wistar rats [16].

Besides their mould inhibitory action, *Azadirachta indica* and *Allium sativum* also possess profound immunomodulatory activity. Intraperitoneal injection of *Azadirachta indica* oil in mice showed increase in leukocytes after 3 days of treatment. It exerts anti-inflammatory activity by inhibiting some of the functions of macrophages and neutrophils relevant to the inflammatory response both *in vivo* and *in vitro*. Oral administration of 5-25 mg nimbidin/Kg body weight to rats for 3 consecutive days significantly inhibited the migration of macrophages to their peritoneal cavities in response to inflammatory stimuli and inhibited NO and prostaglandins E_2 (PGE_2) production by macrophages [17]. *Allium sativum* agglutinins (ASA) I and II and two major proteins QR-2 and QR-1 contribute to the immunomodulatory activity of garlic [18]. Garlic oil supplementation had desirable effects on the measured redox parameters, eliminating some of the adverse effects of feeding T-2 toxin-contaminated diet in broiler chicken [19]. In another study, dietary supplementation of garlic counteracted induced mycotoxicosis in broilers in a dose-dependent manner [20]. A study comparing the immunomodulatory effects of garlic consumption in

Sporothrix schenckii-infected mice revealed better responses in the garlic-supplemented group in terms of the functioning of peritoneal macrophages and cytokine production (NO, IL-1β, IL-10 and IL-12), which also corresponded with the ability to mount a more effective fight against the infection [21].

A composition based on GRAS constituents like *Andrographis paniculata*, *Emblica officinalis*, *Azadirachta indica*, *Allium sativum*, *etc*. is least likely to be toxic in practical doses. Mix-01-Vil exerts multifarious benefits, including protection of vital organs against adverse effects of mycotoxins and improvement of growth and production in animals, due to the presence of multiple active ingredients.

Conclusion

Mix-01-Vil did not produce acute oral toxicity as evident from the absence of mortality, toxic clinical symptoms, and gross and histopathological alterations, when administered up to a limit dose of 2000 mg/Kg body weight in mice. Based on these findings, the formulation was found to be safe for oral use.

References

1. Adhikari M, Negi B, Kaushik N, Adhikari A, Al-Khedhairy AA, Kaushik NK *et al*. T-2 mycotoxin: toxicological effects and decontamination strategies. Oncotarget 2017;8(20):33933-33952.
2. Bakshi CS, Sikdar A, Johari TS, Meenakshi M, Singh RK. Effect of graded dietary levels of aflatoxin on humoral immune response in commercial broilers. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 2000;21(2):163-164.
3. Stoev SD, Anguelov G, Ivanov I, Pavlov D. Influence of ochratoxin A and an extract of artichoke on the vaccinal immunity and health in broiler chicks. Experimental and Toxicologic Pathology 2000;52(1):43-55.
4. Jewers K. Mycotoxins and their effect on poultry production. Options Méditerranéennes 1990;7:195-202.
5. Thilakchand KR, Mathai RT, Simon P, Ravi RT, Baliga-Rao MP, Baliga MS. Hepatoprotective properties of the Indian gooseberry (*Emblica officinalis* Gaertn): a review. Food & Function 2013;4(10):1431-1441.
6. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from Andrographis paniculata against carbon tetrachloride. The Indian journal of medical research 1990;92:276-283.
7. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L *et al*. Immunomodulation and anti-inflammatory effects of garlic compounds. Journal of immunology research 2015, 2015, Article ID 401630, 13 pages. https://doi.org/10.1155/2015/401630.
8. Acute oral toxicity-Acute toxic class method. Organisation for Economic Co-operation and Development. Test No. 423: Acute oral toxicity-acute toxic class method. OECD guidelines for the testing of chemicals (section 4: health effects). OECD Publishing 2001;1:14.
9. Sutha D, Jegathambigai R, Kumar P, Sivaramakrishnan S. A study on the hepatoprotective effect of *Andrographis paniculata* (Burm.F) Nees on mice. Journal of Phytology 2010;2(11):25-30.
10. Sheeja K, Kuttan G. Modulation of natural killer cell activity, antibody-dependent cellular cytotoxicity, and antibody-dependent complement-mediated cytotoxicity by andrographolide in normal and Ehrlich ascites carcinoma-bearing mice. Integrative cancer therapies 2007;6(1):66-73.
11. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide. Immunopharmacology and immunotoxicology 2007;29(1):81-93.
12. Iruretagoyena MI, Tobar JA, González PA, Sepúlveda SE, Figueroa CA, Burgos RA *et al*. Andrographolide interferes with T cell activation and reduces experimental autoimmune encephalomyelitis in the mouse. Journal of Pharmacology and Experimental Therapeutics 2005;312(1):366-372.
13. Iruretagoyena MI, Sepúlveda SE, Lezana JP, Hermoso M, Bronfman M, Gutiérrez MA *et al*. Inhibition of nuclear factor-κB enhances the capacity of immature dendritic cells to induce antigen-specific tolerance in experimental autoimmune encephalomyelitis. Journal of Pharmacology and Experimental Therapeutics 2006;318(1):59-67.
14. Bhattacharya A, Kumar M, Ghosal S, Bhattacharya SK. Effect of bioactive tannoid principles of *Emblica officinalis* on iron-induced hepatic toxicity in rats. Phytomedicine 2000;7(2):173-175.
15. Daisy P, Priya N, Rajathi M. Immunomodulatory activity of *Eugenia jambolana*, *Clitoria ternatea* and *Phyllanthus emblica* on alloxan-induced diabetic rats. Journal of Experimental Zoology, India 2004;7(2):269-78.
16. Sultana S, Ahmad S, Khan N, Jahangir T. Effect of *Emblica officinalis* (Gaertn) on CCl4 induced hepatic toxicity and DNA synthesis in Wistar rats. NISCAIR-CSIR 2005;43:430-436.
17. Mukherjee PK, Nema NK, Bhadra S, Mukherjee D, Braga FC, Matsabisa MG. Immunomodulatory leads from medicinal plants. NISCAIR-CSIR 2014;13(2):235-256.
18. Clement F, Pramod SN, Venkatesh YP. Identity of the immunomodulatory proteins from garlic (*Allium sativum*) with the major garlic lectins or agglutinins. International Immunopharmacology 2010;10(3):316-324.
19. Ancsin ZS, Erdélyi M, Balogh K, Szabó-Fodor J, Mézes M. Effect of garlic oil supplementation on the glutathione redox system of broiler chickens fed with T-2 toxin contaminated feed. World Mycotoxin Journal 2013; 6(1):73-81.
20. Ahmed SU, Baruah KK, Sapcota D, Phukan B. Effect of feeding garlic (*Allium sativum*) on carcass characteristics and nutrient utilization in broilers in induced aflatoxicosis. Indian Journal of Animal Nutrition 2007;24(2):99-103.
21. Burian JP, Sacramento LV, Carlos IZ. Fungal infection control by garlic extracts (*Allium sativum* L.) and modulation of peritoneal macrophages activity in murine model of sporotrichosis. Brazilian Journal of Biology 2017;77(4):848-855.