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Evaluation of acute oral toxicity of a polyherbal respiratory tonic for poultry

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Abstract

Poultry is commonly confronted with infectious diseases; among them respiratory tract pathogens are of major concern, causing heavy economic losses both in terms of decreased production and the increased costs of treatment. Infected birds express respiratory and other signs such as cough and dyspnea, complications due to secondary pathogens, and poor growth. Pulmofarm™ D Liquid is a complete herbal solution for respiratory distress in different respiratory diseases of viral and bacterial origin in poultry. A study was undertaken to evaluate the potential of Pulmofarm™ D liquid (M/s Ayurved Limited, India) to elicit acute oral toxicity as per 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 day 14 and Pulmofarm™ D liquid was found safe for oral use.

Keywords: poultry, respiratory tonic, Pulmofarm D, herbal, safety, acute oral toxicity

Introduction

Respiratory infections are a common cause of increased mortality in poultry and diseases of the respiratory tract are a significant component of the overall disease incidence in poultry. In many cases, respiratory disease observed in a flock maybe a component of a multi-systemic disease or it may be the predominant disease with lesser involvement of other organ systems. Various pathogens may initiate respiratory disease in poultry, including a variety of viruses, bacteria, and fungi. These infections result in high morbidity and mortality in the infected chicken. Environmental factors may exacerbate the signs and lesions produced by these pathogens. The most frequent sign and symptoms of respiratory distress recorded at the farms include sneezing, coughing, nasal discharge, and tracheal rales [1]. Pulmofarm™ D Liquid is a complete herbal solution for respiratory distress in different respiratory diseases of viral and bacterial origin. It is highly effective in alleviating symptoms associated with chronic respiratory disease (CRD) such as sniffing, rattling, sneezing, coughing and other signs of respiratory distress. It is used in the prevention and management of coryza, bronchitis, fowl cholera and for minimizing secondary bacterial complications. Its key ingredients, including herbal extracts of *Glycyrrhiza glabra*, *Curcuma longa*, *Ocimum sanctum*, etc. are reputed for their demulcent², anti-asthmatic, anti-allergic [3], antioxidant, expectorant [4], antimicrobial, anti-inflammatory and immunomodulatory action [5]. The present study aimed at determining the acute oral toxicity potential of Pulmofarm™ D liquid.

Materials and Methods

The present study was undertaken at the Department of Pharmacology and Toxicology, Krantisingh 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). OECD 423 guidelines [6] were followed for the evaluation of acute oral toxicity; six (3 male and 3 female) healthy adult 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 the 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 libitum*. 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; LD₅₀ value was also assessed. The observations included those for 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).

At 2000 mg/Kg body weight *i.e.* the maximum dose which can be administered by oral route, Pulmofarm™ D liquid did not cause any mortality in any of the mice and hence, the LD₅₀ 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 Pulmofarm™ D liquid 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 gonads in any of the animals as shown below in figure (1a and 1b).

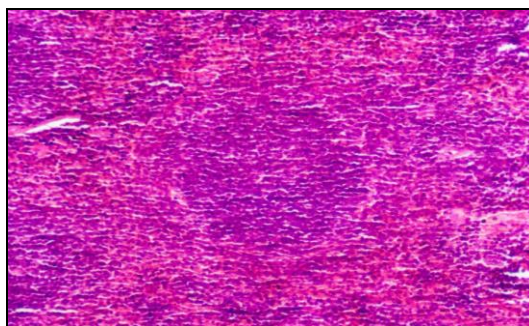


Fig 1a: Histopathology of spleen showing normal spleen cells (10X)

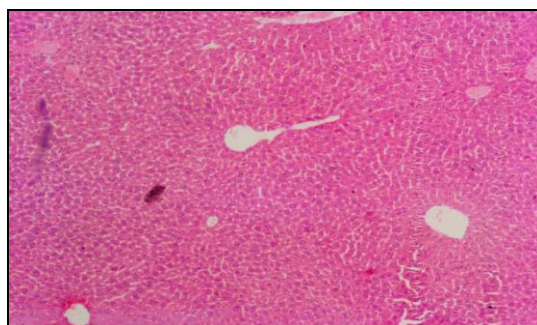


Fig 1b: Histopathology of liver showing normal hepatocytes (10X)

Pulmofarm™ D liquid is prepared from parts of plants like *Glycyrrhiza glabra*, *Curcuma longa*, *Ocimum sanctum*, etc. that belong to the Generally Regarded as Safe (GRAS) category. *Glycyrrhiza glabra* has demulcent, expectorant, and anti-inflammatory activity and is useful in irritable conditions of bronchial tubes, sore-throat and asthma, making it one of the most popular remedies for coughs and respiratory affections [7]. Curcumin, the main active component of *Curcuma longa*, is responsible for its therapeutic action. Curcumin modulates the inflammatory response and inhibits subsequent fibrosis in viral-induced acute respiratory distress syndrome in mice. In addition, curcumin also significantly inhibited the expression of α -smooth muscle actin and Tenascin-C, key markers of myofibroblast activation⁸. In catarrhal cough, sore throat, and throat infection, the decoction of turmeric rhizome is used as a gargle and also a

piece of rhizome is slightly burnt and given for chewing. The fresh juice of rhizome is useful in bronchitis. The chemical constituents of *Curcuma longa* like tumerones, curcuminoids, curcumin and tetrahydrocurcumin have an anti-asthmatic action. Curcumin also exhibits anti-inflammatory action by inhibiting several molecules involved in inflammation including phospholipase, lipoxygenase, COX-2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, MCP-1, interferon-inducible protein, tumor necrosis factor, and interleukin-12 [9]. Treatment of ovalbumin-induced asthmatic rats with hydro-ethanolic extract of *O. basilicum* leaves resulted in decreased inflammation markers such as interleukin-4 (IL-4), immunoglobulin E (IgE), phospholipase A₂ (PLA₂) and total protein levels and also ameliorated the pathological changes of rat lungs [10]. Pulmofarm™ D liquid can exert diverse

benefits, including minimizing secondary bacterial complications, relieving different symptoms associated with

respiratory diseases and, in turn, improving productivity and FCR of birds.

Table 1: Individual body weights of experimental mice

Formulation and Dose	Mice No.	Body Weight (g) on Day		
		0	7	14
Pulmofarm™ D liquid @ 2000 mg/Kg b.wt. orally (Group I: Females)	1	22.5	23.0	23.5
	2	21.0	22.0	25.0
	3	22.0	22.0	24.0
Pulmofarm™ D liquid @ 2000 mg/Kg b.wt. orally (Group II: Males)	1	23.5	24.0	23.0
	2	21.0	24.0	24.0
	3	22.5	24.0	23.5

Conclusion

Pulmofarm™ D liquid did not produce acute oral toxicity, evident as the absence of mortality, signs of toxicity, 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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