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EVALUATION OF ACUTE ORAL TOXICITY OF A POLYHERBAL POULTRY COCCIDIOSTAT

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Abstract–The current study was designed to evaluate the acute oral toxicity potential of Coccihar™ (M/s Ayurvet Limited, Baddi, India) according to OECD 423 guidelines. Coccidiosis is recognized as a major parasitic disease of poultry resulting in loss of productivity. Coccihar™ is a herbal coccidiostat recommended for the prevention of coccidiosis, for improvement of intestinal health, growth performance and weight gain in poultry. Nine female Swiss albino mice were used for the study. Each animal served as its own control. Following the oral administration of the test substance, the animals were observed for manifestation of toxic effects and deaths. No toxic effects or mortalities were observed. The estimation of biochemical parameters (AST, ALT, ALP and creatinine) and histopathological studies also did not reveal any significant findings. Hence, Coccihar™ was found to be safe for oral use.

INTRODUCTION

Avian coccidiosis is a complex intestinal disease caused by obligatory protozoan parasites belonging to the genus *Eimeria*. The parasite causes massive destruction of the epithelial cells, which leads to bloody diarrhea, reduced weight gain and reduction in production (Masood *et al.*, 2013). Although known for many years, coccidiosis is still globally considered as the most economically important parasitic condition affecting the production and performance of poultry under intensive production systems (De Gussem, 2007). Large-scale and long-term use of anticoccidials has led to the worldwide development of resistance against all these drugs (Peek and Landman, 2011). The use of botanicals has played a major role in the control of avian coccidiosis as they are natural and new therapeutic molecules to which resistance has not yet developed. The use of botanicals as anticoccidial remedies, therefore, holds promise as an alternative in the control of coccidiosis (Abbas *et al.*, 2012). Coccihar™ is one such herbal coccidiostat for the prevention of coccidiosis in poultry. It contains herbs like *Berberis aristata, Azadirachta indica, Syzygium aromaticum* and *Ocimum sanctum* known for their strong antioxidant, analgesic and antipyretic properties (Eeyuri and Putturu, 2013). Leaves of *Azadirachta indica* can be used for feeding and reducing the parasitic load of animals. The fruit of *Azadirachta indica* also has anticoccidial activity for poultry (Malik *et al.*, 2013). Coccihar™ is recommended for the prevention of caecal and intestinal coccidiosis, for better feed conversion and increased weight gain in poultry. Coccihar™ has been shown to improve growth performance and intestinal health in infected birds (Giannenas *et al.*, 2016). In another comparative study with salinomycin, Coccihar™ was found to improve live weight gain and FCR in infected birds (Jadhav *et al.*, 2007). Coccihar™ caused degeneration and vacuolation of second generation schizonts and, additionally appeared to have inhibitory effects on gametogonic stages including oocyst (Pangasa *et al.*, 2007). The present study aimed at determining the acute oral toxicity potential of Coccihar™.

MATERIALS AND METHODS

The protocol of the study was got approved by the

Institutional Animal Ethics Committee (IAEC, 312/GO/ReBi/2000/CPCSEA) of PGIVAS, Akola. Nine healthy, adult, nulliparous and non-pregnant female Swiss albino mice, weighing 20-25g, were used. The animals were procured from CPCSEA-registered breeding source *viz*. Laboratory Animal Resource section of the Department of Pharmacology and Toxicology, PGIVAS, Akola. All animals were maintained as per the SOPs outlined in CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) 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 diet and water *ad lib* (OECD, 2001). Animals were kept in the cages for five 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 @ 300 mg/Kg body weight in normal saline and 2000 mg/Kg body weight with maximum volume 2 mL/ 100 g body weight, food was with held for 1-2 hours. The animals were observed intensively for 24 h, and then for a further period of 14 days for the manifestation of toxic effects and deaths; LD_{50} value was also estimated. The observations included changes in skin, fur 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 any, observed during study period were recorded.

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).

No mortality was observed throughout the period of observation. In the six mice receiving the limit dose of Coccihar™ at 2000 mg/Kg b.wt., *i.e.* the maximum dose which can be administered by oral route, no mortality occurred and hence, the LD_{50} was beyond this limit. Similarly, no abnormal symptoms, including lethargy, tremor, abdominal breathing, piloerection were observed up to 14 days of Coccihar™ administration. Necropsy on day 14 did not show any remarkable findings in the gross or microscopic appearance of liver, kidney, spleen, heart, lungs, and genital organs in any of the animals. Pooled serum samples were analyzed in triplicate for AST, ALT, ALP and creatinine and all were within their normal ranges (Table 2).

Coccihar™ contains parts of plants like *Berberis*

Table 1. Individual body weights of experimental mice

Formulation and Dose	Mice No.	Body Weight (g) on Day		
		0	7	14
Coccihar™ @ 300 mg/Kg b.wt. orally (Group I)	1	23	23	25
	2	22	24	26
	3	21	23	25
	Mean±SE	22.00±0.73	23.33±0.42	25.33±0.42
Coccihar™ @ 2000 mg/Kg b.wt. orally (Group II)	1	25	26	28
	2	21	23	25
	3	20	22	24
	4	21	23	25
	5	24	26	27
	6	24	25	26
	Mean±SE	22.5±0.85	24.17±0.70	25.83±0.60

Table 2. Biochemical findings in experimental mice

Dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (mg/dL)
300 mg/ Kg b.wt.	58.5	30.2	48.4	0.38
2000 mg/ Kg b.wt.	68.2	19.7	38.76	0.76

aristata, *Azadirachta indica*, *Syzygium aromaticum* and *Ocimum sanctum* that fall under the category of Generally Regarded As Safe (GRAS). *Azadirachta indica*, at a concentration of 150 g/50 kg feed, has been found to have anticoccidial effects against *E. tenella* infection by reducing shedding of oocyst per gram of faeces (Abbas *et al.*, 2006). *Syzygium aromaticum* contains active component eugenol, which acts as anappetite and digestive stimulant. As a stimulant it stimulates sluggish circulation, counter act spasmodic disorders and by promotion of enzymatic activity, promotes digestion and metabolism and relieves dyspepsia. Its anaesthetic action numbs the gullet, stomach, and stops vomiting (Deshpande, 2011a). *Berberis aristata* is rich in alkaloid berberine, which act as anti-diarrhoeal and stomachic agent. Its root bark extract, rasount or rasanjan, is useful in relieving diarrhea and dysentery (Deshpande, 2011b). A composition based on these GRAS constituents is least likely to be toxic in practical doses. Due to the presence of multiple active ingredients, Coccihar™ exerts multifarious benefits in poultry, including improvement of growth performance and intestinal health, live weight gain and FCR.

CONCLUSION

Coccihar™ did not produce acute oral toxicity, evident as absence of mortality or any toxic clinical symptoms, when administered up to limit dose (2000 mg/ Kg b.wt.) in mice. Based on this study, the formulation was found safe for oral use.

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