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Evaluation of acute oral toxicity of a calcium and phosphorus supplement for poultry

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Abstract
The current study was designed to evaluate the acute oral toxicity potential of AV/CSP/29 (M/s Ayurvet Limited, Baddi, India) according to OECD 423 guidelines. AV/CSP/29 is calcium and phosphorus supplement with vitamin D_3 for 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, AV/CSP/29 was found to be safe for use.

Keywords: acute oral toxicity, AV/CSP/29, OECD 423, safety, limit test

Introduction
Calcium and Phosphorus are necessary for the formation and maintenance of the skeletal structures of the poultry birds and in layers, have an additional function in eggshell formation. The main functions of calcium in poultry nutrition are skeleton formation, fat translocation from blood to egg yolk, blood clotting, eggshell formation and muscular contraction. On the other hand, phosphorus plays an important role in energy transfer (ATP), bone structure, enzyme system, DNA and RNA linkages, acid base balance and fat translocation [1]. AV/CSP/29 is recommended for supplementation during high demand periods of egg production in layers and, growth and weight gain in broilers, for better egg shell strength, for improving carcass and meat quality. AV/CSP/29 offers a promising solution for the problems associated with calcium and phosphorus. The present study aimed at determining the acute oral toxicity potential of AV/CSP/29.

Materials and Methods
The animals for the current study were procured from CPCSEA-registered breeding source *viz*. laboratory animal resource section of Department of Pharmacology and Toxicology, PGIVAS, Akola. Nine healthy, adult, nulliparous and non-pregnant female Swiss albino mice (20-25g) were used. Animals were kept in the cages for five days for acclimatization. The animals were fasted over-night, 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. 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 ℃ and relative humidity of 70%. The animals were exposed to 12 hour light-dark cycle and provided with standard pelleted diet and water *ad lib*[2]. After the administration of the test substance @ 300 mg/Kg (P.O.) in normal saline and 2000 mg/Kg with maximum volume 2 mL/ 100 g body weight, food was withheld for 1-2 hours. The animals were observed for 24 h, then for further 14 days for 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

Individual body weight of mice was recorded on days 0, 7 and 14 of the study and body weight of 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
AV/CSP/29@ 300 mg/Kg b.wt. orally (Group I)	1	25	27	28
	2	21	23	25
	3	24	25	26
	Mean ± SE	23.33±1.52	25.00±1.46	26.33±1.11
AV/CSP/29@ 2000 mg/Kg b.wt. orally (Group II)	1	20	21	24
	2	21	23	25
	3	25	26	27
	4	20	22	24
	5	21	22	23
	6	20	21	24
	Mean ± SE	21.17±0.54	22.50±0.76	24.50±0.56

No mortality was observed throughout the period of observation. In the six mice receiving the limit dose of AV/CSP/29 at 2000 mg/Kg, *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 AV/CSP/29 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).

Table 2: Biochemical findings in experimental mice

Dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (mg/dL)
300 mg/Kg	73.33	82.96	84.4	0.75
2000 mg/Kg	103.36	90.36	104.83	0.66

Discussion and Conclusion

AV/CSP/29 contains time tested herbs like *Cissus quadrangularis*, *Uraria picta* and *Lepidium sativum* that fall under the category of Generally Regarded as Safe (GRAS), and increases the absorption and utilization of both calcium and phosphorus in the body. A composition based on these GRAS constituents is least likely to be toxic in practical doses. AV/CSP/29 did not produce acute oral toxicity, evident as absence of mortality or any toxic clinical symptoms, when administered up to limit dose (2000mg/Kg) in mice. Based on this study, the formulation was found safe for oral use.

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