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Evaluation of acute oral toxicity of a herbal egg quality enhancer

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Abstract
Achieving and maintaining egg quality and production levels is essential for a successful poultry layer enterprise. Layer egg quality and production levels are widely affected by a variety of factors, including inadequate management, nutritional status, and various diseases. AV/EFE/19 is a phytogenic herbal egg quality enhancer that helps to achieve optimum egg quality in layers. A study was conducted to assess the acute oral toxicity potential of AV/EFE/19 (M/s Ayurvet Limited, India) according to OECD 423 recommendations. Nine adult female Swiss albino mice, weighing 24-29 g, were used for the study. Following the oral administration of AV/EFE/19, the animals were observed for the manifestation of toxic effects and mortality. Toxicity was evaluated on the basis of changes in body weight, signs of toxicity, histology of vital organs (heart, liver, kidney, and lungs), and blood biochemical parameters (AST, ALT, ALP and creatinine). No toxic effects or mortalities were noticed until day 14 and AV/EFE/19 was found safe for oral consumption.

Keywords: acute oral toxicity, AV/EFE/19, egg quality, OECD 423, herbal

Introduction
Poultry eggs are an essential source of nutrition in many countries, and poultry layer businesses employ a large number of people in various parts of the world. Maintaining egg quality as well as bird productivity and collecting a good crop of eggs is crucial for achieving both profitability and nutritional security. AV/EFE/19 (M/s Ayurvet Limited, India) is a phytogenic egg quality enhancer that helps to achieve and sustain better egg quality in layer birds. AV/EFE/19 is a mixture of essential oils (EOs) designed to increase the quality of poultry eggs. The components in these EOs, such as thymol and cinnamaldehyde, exhibit antioxidative activity in the body, which ensures better egg qualities (Amer *et al.*, 2018; Gholami-Ahangaran *et al.*, 2021) [1, 5]. The potential of EOs to avoid systemic stress also helps to alleviate environmental stresses and sustain egg quality in hot summer conditions (Cabuk *et al.*, 2006) [2]. EOs also helps in decreasing the total saturated fatty acids and yolk cholesterol concentration and increasing the omega-3 fatty acids (Escobar *et al.*, 2020; Yalcin *et al.*, 2020) [3, 10]. The present study aimed at determining the acute oral toxicity potential of AV/EFE/19.

Materials and Methods
The study was performed at the Department of Veterinary Pharmacology and Toxicology at the Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India. Institutional Animal Ethics Committee (IAEC) of PGIVAS, Akola, approved the trial protocol (Approval number 312/4/14/2000/20, dated-06/03/2020).
Nine adult nulliparous non-pregnant Swiss albino female mice, weighing 24-29 g, were used in this study. The animals were obtained from the animal resource unit, Department of Veterinary Pharmacology and Toxicology, Akola. IAEC SOPs and CPCSEA regulations were followed for all animals. Picric acid staining was used for animal identification. The number of animals per cage was limited to three for simple monitoring and adequate housing facilities. The animals were subjected to a 12h light and 12h dark cycle with constant temperature (25 ± 2 °C) and relative humidity (70%). The animals were fed a regular pelleted feed and had access to ample water (OECD, 2001) [7].
Animals were housed in cages for five days to adapt to the laboratory setting. Thereafter, the animals were fasted for three to four hours without food or water. After a fasting period, the animals were weighed and the test substance was administered. Three mice in Group I were given the test drug at a dose of 300 mg/Kg body weight.

If no evidence of toxicity appeared in Group I, the remaining six mice in Group II were administered the maximum dose of the test substance, which was 2000 mg/Kg of body weight. In both groups, feeding was withheld for 1-2 h after the test drug was administered.

The animals were monitored continuously at least once for the first 30 minutes and periodically for first 24 h, and then further for a period of 14 days for the manifestation of toxic effects and fatalities as well as the LD_{50} value. The observations recorded included those for changes in eyes, skin and coat; and changes in respiratory, circulatory, central nervous systems, 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 histological investigations of different organs, was performed. Blood was collected and biochemical estimations of Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and creatinine were made. The data of biochemical parameters were analyzed statistically using one way ANOVA followed by complete randomized design.

Results and Discussion

The body weights of mice were recorded separately on days 0, 7, and 14 of the study. During the study period, body weights of both groups (I and II) continued to increase (Table 1). After oral administration of AV/EFE/19 at 300 mg/Kg b.wt., and 2000 mg/Kg b.wt. to Group I and II mice, respectively, no mortality or abnormal signs were observed. The LD_{50} of AV/EFE/19 was greater than 2000 mg/Kg as no deaths were observed at this threshold dose, which is the maximum dose that can be given orally.

The organs *viz.* liver, heart, lungs, and kidneys showed no significant changes in appearance after 14 days of necropsy. Similarly, no histological abnormalities of the liver, heart, lungs, and kidneys were detected in any of the mice (Figure 1). While the values of AST, ALT, and creatinine in blood biochemistry differed significantly in both groups (Table 2), the results were well within normal ranges, indicating no liver or kidney damage.

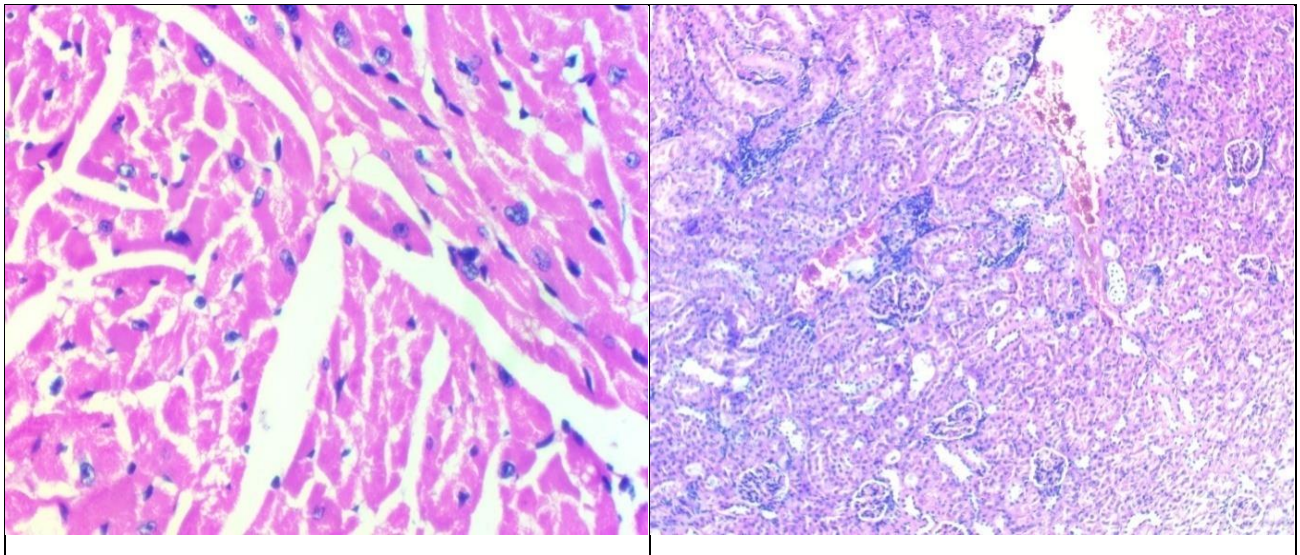
Table 1: Individual weekly body weights and mortality of experimental mice treated with AV/EFE/19

Dose	Animal No.	Body Weight (g) on day			Mortality
		0	7	14	
(Group I) AV/EFE/19 300 mg/Kg orally	1	25	25	26	No
	2	27	28	28	No
	3	27	28	29	No
	Mean±SD	26.33±0.67	27.00±1.00	27.66±0.88	-
(Group II) AV/EFE/19 2000 mg/Kg orally	1	24	26	26	No
	2	25	27	27	No
	3	26	28	28	No
	4	27	27	28	No
	5	27	27	29	No
	6	29	30	30	No
	Mean±SD	26.33±0.72	27.50±0.56	28.00±0.58	-

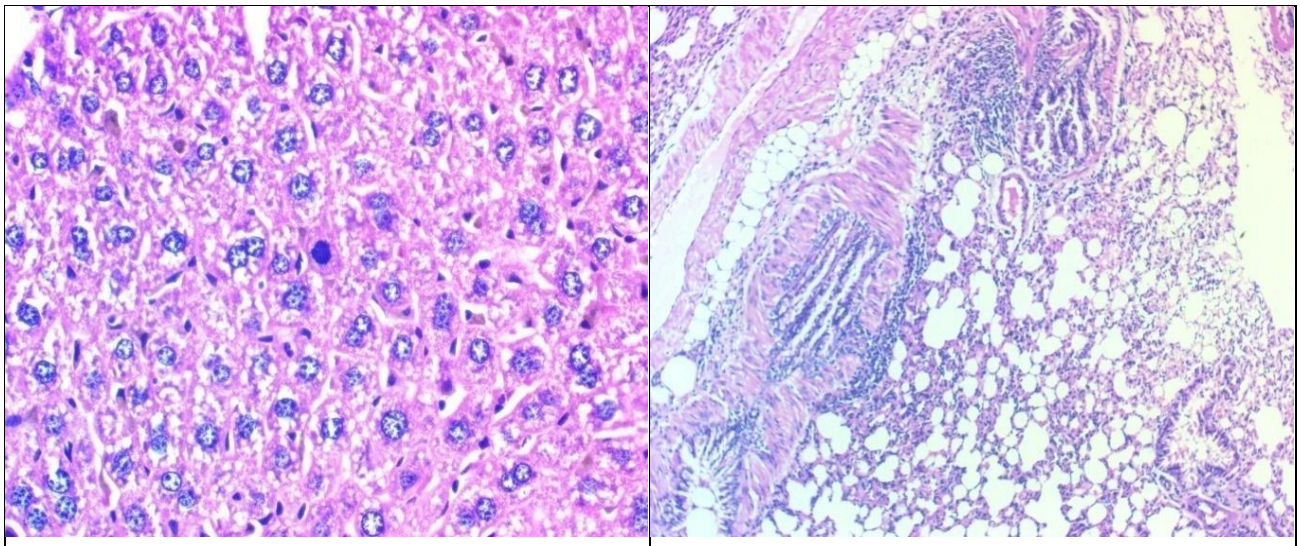
Table 2: Mean ± SD values of AST, ALT, ALP and creatinine values in experimental mice

Dose	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dL)
(Group I) AV/EFE/19 300 mg/Kg b.wt. orally	51.38 ± 0.75^{b}	42.67 ± 1.15^{b}	120.01 ± 1.38	0.47 ± 0.009^{b}
(Group II) AV/EFE/19 2000 mg/Kg b.wt. orally	55.51 ± 0.59^{a}	47.38 ± 0.75^{a}	126.59 ± 2.67	0.55 ± 0.012^{a}

$^{a-b}$ values bearing different superscripts differ significantly within columns ($p<0.05$)



A. B.



C. D.

Fig 1: Histological appearances of A. heart, B. kidneys, C. liver and D. lungs of mice receiving AV/EFE/19 (2000 mg/Kg of b.wt.)

AV/EFE/19 contains different phytogenic essential oils (EOs) which belong to the Generally Regarded as Safe (GRAS) category. Pulungan and Pane (2020) [8] reported that cinnamaldehyde helps in the lowering of total cholesterol levels after consumption of high-fat diet. Similarly, Li *et al.* (2012) [6] reported antihyperglycemic and antihyperlipidemic action of cinnamaldehyde.

Thymol is used to improve the performance and fatty acid profiles in the yolk of eggs in laying birds (Vakili and Heravi, 2016) [9]. Yalcin *et al.* (2020) [10] reported that the supplementation of 2% thymol resulted in useful effects in egg-laying birds due to the decrease in the total saturated fatty acids and yolk cholesterol concentration, and increase in omega-3 fatty acids. Thymol also provides PUFA for synthesis and deposition in membranes within the egg and act as energy-producing substrate. Additionally, thymol supplementation could be advised for the production of healthier table eggs (Fernandez *et al.*, 2017) [4]. Thus, AV/EFE/19 can be used to improve egg quality without exerting any toxic effects.

Conclusion

The present study revealed that AV/EFE/19 did not elicit acute oral toxicity, even when given at the maximum limit dose in mice (2000 mg/Kg of b.wt.), as evidenced by the lack of death, clinical toxicity, and gross or histological changes. Based on these findings it was concluded that the supplement is safe for oral use.

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