The Pharma Innovation Journal 2021; SP-10(8): 664-666

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(8): 664-666

www.thepharmajournal.com
Received: 22-06-2021
Accepted: 24-07-2021

Sunil Hajare
Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India

Ranjit Suresh Ingole
Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India

Maheshkumar Vitthal Ingawale
Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India

Vivek Borekar
Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India

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

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

Evaluation of acute oral toxicity of a phytogenic egg-laying stimulant

Sunil Hajare, Ranjit Suresh Ingole, Maheshkumar Vitthal Ingawale, Vivek Borekar and Bhaskar Ganguly

Abstract
Achieving and maintaining optimum egg production levels is important for successful poultry layer enterprise. However, layer egg production is commonly affected due to various reasons such as endocrine or nutritional exhaustion, poor management and different types of diseases. Goldeg® (M/s Ayurvet Limited, India) is a phytogenic egg-laying stimulant that helps to achieve and restore optimum production levels in layers. A study was undertaken to evaluate the potential of Goldeg® to elicit acute oral toxicity as per OECD 423 guidelines. Nine non-pregnant, nulliparous, adult female Swiss albino mice were used for the study. Following the oral administration of the test substance, the animals were observed for the manifestation of toxic effects and mortality. Toxicity was evaluated on the basis of changes in body weight, overt signs of toxicity, gross and histological appearances of vital organs, and blood biochemistry. No toxic effects or mortalities were observed till day 14 and Goldeg® was found safe for oral use.

Keywords: poultry, egg, egg-laying stimulant, safety, acute oral toxicity, Goldeg

Introduction
Poultry eggs are an important source of nutrition in several societies and poultry layer enterprises also provide employment to considerable populations in different regions of the world. Obviously, maintaining the productivity of the birds and obtaining a good crop of eggs is essential for achieving both profitability and nutritional security (Kleyn and Ciacciariello, 2021) [5]. Goldeg® (M/s Ayurvet Limited, India) is a phytogenic egg-laying stimulant that helps to achieve and sustain peak egg laying in layer birds. It is also helpful in restoring optimum production levels in layers with non-specific drop in egg production (Sunidhi *et al.*, 2019) [9]. Its key ingredients, including *Leptadenia reticulata*, *Cissus quadrangularis*, *Citrullus colocynthis*, *etc.* are reputed for their abilities to overcome endocrine exhaustion, stimulate hormone release, and promote homoeostasis (Hussain *et al.*, 2014; Mohanty *et al.*, 2017; Bafna *et al.*, 2021) [4, 6, 1]. The present study aimed at determining the acute oral toxicity potential of Goldeg®.

Materials and Methods
The present study was undertaken at the Department of Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, India (20.7°N and longitude 77.07°E; 287-316 above msl). The experimental protocol of the study was got approved by the Institutional Animal Ethics Committee (IAEC, 312/GO/ReBi/2000/CPCSEA) of PGIVAS (Approval number: 312/4/14/2000/20, dated 06.03.2020).
Nine healthy non-pregnant nulliparous adult female Swiss albino mice, weighing 25-28g, were used. The animals were procured from the Laboratory Animal Resource Section of PGIVAS, Akola. All animals were maintained as per the SOPs outlined in the CPCSEA guidelines. The animals were identified by picric acid staining. 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±2 °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* (OECD 423). 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.

The test substance was administered to three mice, comprising Group I, at 300 mg/Kg of body weight. If no signs of toxicity appeared in Group I, the remaining six mice, comprising Group II, were administered the limit dose of the test substance i.e. at 2000 mg/Kg of body weight.

Food was withheld for 1-2 hours after dosing of test substance in both groups I and II. 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 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 different organs, was performed. Blood was collected and biochemical estimations of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and creatinine were made.

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 during the study period (Table 1).

Table 1: Individual body weights and mortality of experimental mice

Dose	Animal No.	Body Weight (g) on day			Mortality
		0	7	14	
300 mg/kg b.wt. orally (Group I)	1	25	26	27	No
	2	25	27	28	No
	3	27	27	28	No
	Mean±SD	25.66±0.67	26.67±0.33	28.0±0.33	-
2000 mg/kg b.wt. orally (Group II)	1	24	25	27	No
	2	25	26	27	No
	3	27	27	29	No
	4	28	28	29	No
	5	27	28	29	No
	6	28	29	30	No
	Mean±SD	26.50±0.67	27.17±0.60	28.50±0.50	-

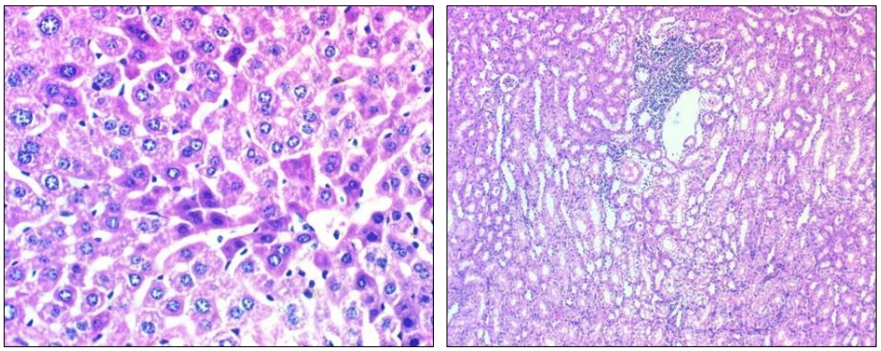
Blood biochemistry revealed significant differences in the values of AST, ALT, and creatinine but not ALP between groups I and II (Table 2). However, the values of all of these analytes in both the groups were well within their respective normal ranges.

Table 2: AST, ALT, ALP and creatinine values in experimental mice

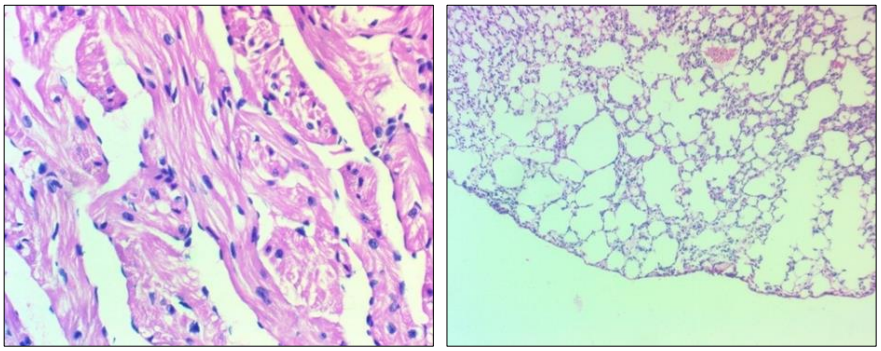
Dose	**AST** (U/L)	**ALT** (U/L)	**ALP** (U/L)	**Creatinine** (mg/dL)
300 mg/Kg (Group I)	50.74 ± 0.48^{a}	42.01 ± 0.84^{a}	118.34 ± 1.99	0.46 ± 0.009^{a}
2000 mg/Kg (Group II)	55.51 ± 0.57^{b}	46.72 ± 0.27^{b}	125.26 ± 1.50	0.54 ± 0.006^{b}

Values bearing different superscripts differ significantly within columns

At 2000 mg/Kg body weight *i.e.* the maximum dose which can be administered by oral route, Goldeg® did not cause any mortality in any of the mice and hence, 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 Goldeg® administration. Necropsy after day 14 did not reveal any remarkable alterations in the gross appearance of the liver, kidneys, heart, or lungs in any of the animals. Similarly, no abnormalities were detected in the histopathological appearances of the liver, kidneys, heart, or lungs that could be associated with toxicity of the test substance (Figure 1).



a. b.



c. d.

Fig 1: Histological appearance of *a.* liver, *b.* kidneys, *c.* heart and *d.* lungs of mice receiving 2000 mg of Goldeg® per Kg of body weight

Goldeg® is prepared from parts of plants like *Leptadenia reticulata, Cissus quadrangularis, Citrullus colocynthis, etc.* that belong to the Generally Regarded as Safe (GRAS) category. Previously, significant improvements in egg yield have been demonstrated in hens receiving dietary supplementation with *L. reticulata*. Up to 15% increase in egg yield was seen with supplementation of 211 mg of *L. reticulata* per bird per day. The crude extract of the plant and stigmasterol, isolated from the extract, were also found capable of increasing egg production; 18% increase in production was achieved with 992 mg of crude extract per bird per day whereas feeding 0.084 mg stigmasterol per day per bird resulted in 39% increase in eggs (Sharma *et al.*, 2015) [8]. The egg-laying stimulant activities of *C. colocynthis* are more likely to be exerted through its ability to improve hepatic function and homeostasis – carbohydrate and lipid metabolism, in particular (Hussain *et al.*, 2014) [4]. It is also used traditionally for the treatment of female reproductive ailments in humans (Gupta and Sharma, 2008a) [3]. *C. quadrangularis* is also used traditionally for similar purposes (Gupta and Sharma, 2008b) [2] but a greater contribution to egg-laying stimulant activity of the plant is expected from its ability to improve calcium and phosphorus metabolism (Mohanty *et al.*, 2017) [6]. Therefore, Goldeg® can exert diverse benefits across different systems, improving productivity and yield of layer-purpose poultry birds. Further, it is very unlikely to cause toxicity at its recommended inclusion level of 500 g/tonne of feed, which is far below the levels tested in the current study.

Conclusion

Goldeg® did not produce acute oral toxicity, evident as the absence of mortality, any remarkable signs of toxicity, and gross and histopathological alterations, when administered up to the limit dose *i.e.* 2000 mg/Kg in mice. Based on this study, the formulation was found safe for oral use.

Acknowledgments

The authors acknowledge M/s Ayurvet Limited, India, for funding the research.

References

1. Bafna PS, Patil PH, Maru SK, Mutha RE. *Cissus quadrangularis* L: A comprehensive multidisciplinary review. Journal of Ethnopharmacology 2021, 114355.
2. Gupta AK, Sharma M (Eds.) *Cissus quadrangularis*. In: Reviews on Indian Medicinal Plants. Indian Council of Medical Research, New Delhi 2008b;6:412.
3. Gupta AK, Sharma M (Eds.) *Citrullus colocynthis*. In: Reviews on Indian Medicinal Plants. Indian Council of Medical Research, New Delhi 2008a;6:444.
4. Hussain AI, Rathore HA, Sattar MZ, Chatha SA, Sarker SD, Gilani AH. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses and nutritional potential. Journal of ethnopharmacology 2014;155(1):54-66.
5. Kleyn FJ, Ciacciariello M. Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? World's Poultry Science Journal 2021, 1-2.
6. Mohanty SK, Swamy MK, Sinniah UR, Anuradha M. *Leptadenia reticulata* (Retz.) Wight & Arn. (Jivanti): botanical, agronomical, phytochemical, pharmacological, and biotechnological aspects. Molecules 2017;22(6):1019.
7. No. OECD Test 423: Acute oral toxicity-acute toxic class method. OECD guidelines for the testing of chemicals (section 4: health effects) 2001;1:14.
8. Sharma M (Ed.) Leptadenia reticulata. In: Reviews on Indian Medicinal Plants. Indian Council of Medical Research, New Delhi 2015;14:398.
9. Sunidhi Ganguly B, Sapra S, Kotagiri R. Reverse pharmacological modeling of the mechanism of action of an herbal egg-laying stimulant. In: Compendium of abstracts, International Conference on Animal Nutrition. Kolkata, India 2019, 188.