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Evaluation of acute oral toxicity of a natural growth promoter for poultry

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Abstract
Minimizing the use of antibiotics is a major concern of poultry producers, largely due to increase in antibiotic resistance, and phytobiotics are gaining popularity as natural replacer of antibiotic growth promoter in broilers. Nbiotic™ (M/s Ayurvet Limited, India) is a complete herbal growth promoter for poultry. This study aimed to evaluate the potential of Nbiotic™ to elicit acute oral toxicity as per OECD 423 guidelines. Three female Wistar rats 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 of administration and Nbiotic™ was found safe for oral use.

Keywords: Growth promoter, herbal, phytobiotics, toxicity

Introduction
Poultry nutritionists are looking for safer and natural alternatives to antibiotic growth promoters, due to increase in antimicrobial resistance in pathogens. Furthermore, many countries have banned antibiotic growth promoters. Alternatives to antibiotic growth promoters include prebiotics, probiotics, organic acid, etc. Phytogenics have also been investigated in this direction and have demonstrated positive effects on growth performance, carcass quality, and intestinal traits in poultry (Demir *et al*., 2003) [2]. Nbiotic™ is a complete herbal preparation for promoting intestinal microbiocenosis, stabilizing gut mucosa, and inhibiting pathogenic bacteria. Its key ingredients, including herbal extracts of *Allium sativum*, *Zingiber officinale*, *Thymus vulgaris*, *etc.* are reputed for their broad spectrum antibacterial (Aarti & Khusro, 2020) [1], antioxidant, growth promoter (Khan *et al.*, 2012) [5], anti-inflammatory, and antifungal (Gholami-Ahangaran *et al.*, 2020) [3] activities. Previous studies on Nbiotic™ have reported growth promoter effect in broilers but the oral toxicity potential of Nbiotic™ has not been determined. Thus, the present study was undertaken to determine the acute oral toxicity potential of Nbiotic™.

Materials and Methods
The present experiment was conducted from 6 to 25 March, 2013 at the Department of Pharmacology and Toxicology, Bombay Veterinary College, Mumbai, India. Three healthy female Wistar rats, weighing 190-200g, were used. The animals were procured from CPCSEA-registered breeding source *i.e.* Central Laboratory Animal House of Bombay Veterinary College, Mumbai. The animals were kept in the cages five days prior to the test for acclimatization. All animals were maintained as per the SOPs outlined in the CPCSEA guidelines. The animals were identified by color marking and provided with standard commercial rat feed, except on the day prior to dosing, and water *ad libitum* (OECD 423). Thereafter, the animals were weighed and the test substance was administered orally @ 2000 mg/Kg body weight. 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 somatic activity and behavior. Clinical signs like muscular tremors, convulsions, salivation, and diarrhea, if observed, were recorded. After 14 days of observation, the animals were euthanized and necropsy was performed.

Results and Discussion

Individual body weights of rats were recorded on days 0, 7 and 14 of the study and no significant change in body weights were observed in any of the animal throughout the study period (Table 1).

Table 1: Individual body weights of experimental rats receiving Nbiotic™ @ 2000 mg/Kg b.wt. Orally

Rat No.	Body Weight (g) on Day		
	0	7	14
1	200	220	220
2	210	225	220
3	190	200	208

At 2000 mg/Kg body weight, *i.e.* the maximum dose which can be administered by oral route, Nbiotic™ did not cause any mortality in any of the rats and hence, the LD_{50} was inferred to be beyond this limit. Similarly, no abnormal signs, including muscular tremors, convulsions, salivation, and diarrhea were observed up to 14 days of Nbiotic™ administration (Table 2). 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. Nbiotic™ is prepared from parts of plants like *Allium sativum, Zingiber officinale, Thymus vulgaris, etc.* that belong to the Generally Regarded as Safe (GRAS) category. *Allium sativum* has a bioactive compound, allicin, which promotes the growth performance of intestinal flora, enhancing digestion, utilization and optimum feed conversion (Helen *et al.*, 2020) [7]. *Zingiber officinale* has various pharmacological properties like anti-inflammatory, gut modulant, antimicrobial, and antioxidant (Khan *et al.*, 2012) [5]. Synergistic action of these and many other herbs in Nbiotic™ helps to improve the growth, performance, carcass traits, and intestinal morphology of broilers.

Table 2: Clinical observations

Signs	Rat #		
	1	2	3
Skin and Fur	Normal	Normal	Normal
Eyes and mucous membranes	Normal	Normal	Normal
Behaviour	Normal	Normal	Normal
Somatomotor activity	Normal	Normal	Normal
Tremors / convulsions	Absent	Absent	Absent
Salivation	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent
Death	No	No	No
Other signs	Nil	Nil	Nil

Conclusion

Nbiotic™ did not produce acute oral toxicity, evident as the absence of mortality and signs of toxicity, when administered up to limit dose (2000 mg/Kg) in rats. Based on this study, the formulation was found safe for oral use.

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