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Short Communication

Evaluation of Acute Oral Toxicity of a Phytogenic Essential Oil-Based Anti-Diarrhoeal and Gut Function Modulator

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Abstract

A study was undertaken to evaluate the acute oral toxicity potential of Liqbiotic™ (M/s Ayurvet Limited, India) according to OECD 423 guidelines. Liqbiotic™ is an anti-diarrhoeal and gut function modulator for poultry and pigs. Six (3 male and 3 female) Swiss albino mice were used for the study, where each animal served as its own control. The animals were observed for the manifestation of toxic effects and mortality following the oral administration of the limit dose of test substance @ 2000 mg/kg body weight. No toxic effects or mortalities were observed till 14 days and Liqbiotic™ was found to be safe for oral use.

Keywords: Acute Oral Toxicity, Limit Test, Liqbiotic™, OECD 423, Safety

Introduction

Diarrhoea is a common problem in poultry occurring due to various reasons like coccidiosis, viral infections (Marek's disease, lymphoid leukosis, fowl cholera, avian tuberculosis, velogenic Newcastle disease, infectious bronchitis), bacterial diseases (infectious coryza), worms, overindulgence, excess of proteins in the feed, intoxications and heat stress **(**Jackson, 2012). These etiological agents, causing damage of variable grade to the intestinal tract, result in poor feed conversion efficiency and decreased rate of body weight gain in poultry flocks (Yegani and Korver, 2008). Healthy gut microbiota is a key determinant in the maintenance of intestinal health due to its ability to modulate host physiological functions required to maintain intestinal homeostasis, mainly through competitive exclusion of detrimental microorganisms and pathogens, preventing colonization and, thereby, decreasing the energy expense that birds normally invest in keeping the immune system active against these pathogens (Diaz *et al*., 2019). Commensal gut microbiota are also important inducers for the development and maturation of both innate defence mechanisms and adaptive immune responses of chicken (Sugiharto, 2016). A "healthy" intestinal microbiota implies energy saving for the host which translates into an improvement in productive performance and strengthening of the immune system of the birds (Celi *et al*., 2017). Therefore, enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (Patterson and Burkholder, 2003). Liqbiotic™ (M/s Ayurvet Limited, India) is one such anti-diarrhoeal and gut function modulator. It is rich in essential oils (EO), such as eugenol, thymol and cineol known for their stomachic, anti-bacterial, antiviral, antioxidant, immunomodulator, growth promoter and anti-toxigenic properties (Adorjan and Buchbauer, 2010; Gopi *et al*., 2014). Liqbiotic™ is recommended for supporting gut health and natural immune system, prevention of diarrhea, optimizing digestion, stabilizing intestinal microflora, stimulating appetite, improving FCR and weight gain. The present study aimed at determining the acute oral toxicity potential of Liqbiotic™.

Materials and Methods

The present study was undertaken at the Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science (KNPCVS), Shirwal, District Satara, India. The experimental protocol of the study was got approved from the Institutional Animal Ethics Committee of KNPCVS (Approval number: IAEC/16/KNPCVS/05/2019; dated: 23/08/19). Six healthy adult (3 males and 3 females) Swiss albino mice, weighing 20-25g, were used. The animals were procured from CPCSEA-registered breeding source *i.e.,* National Institute of Biosciences, Pune. All animals were maintained as per the SOPs outlined in CPCSEA guidelines. The animals were identified by picric acid markings on the back. 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 feed and water *ad lib* (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. After the administration of the test substance @ 2000 mg/kg body weight, food was withheld for 1-2 hours. 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 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 the liver, kidneys, spleen, heart, lungs, and reproductive organs, was performed.

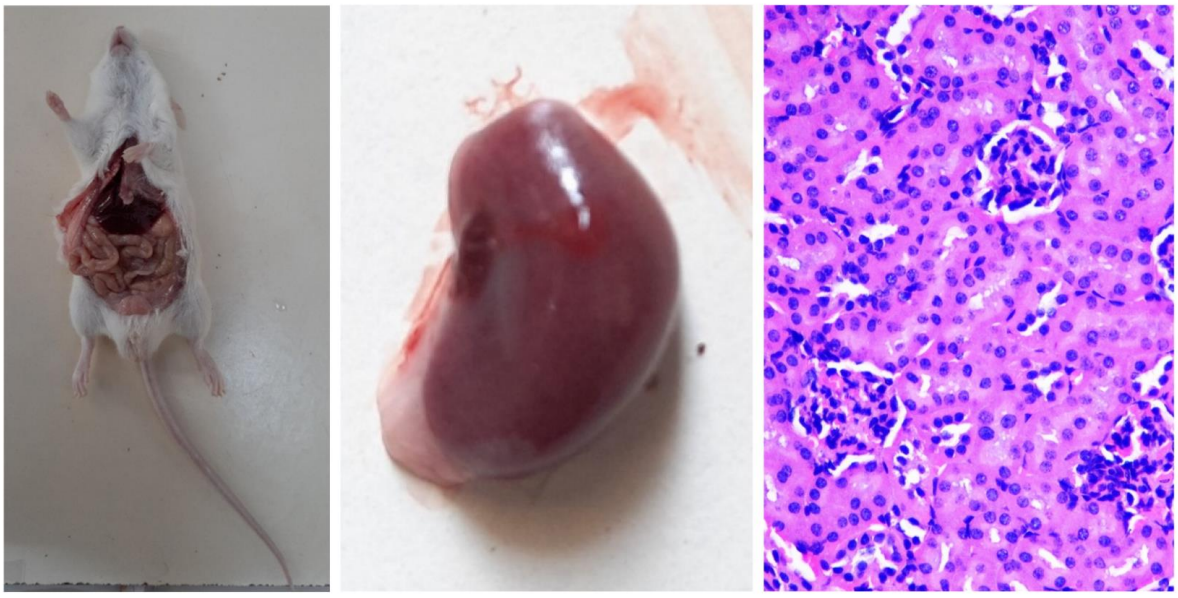
Results and Discussion

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 seen throughout the period of observation. Since no mortality occurred in the six mice receiving the limit dose of Liqbiotic™ at 2000 mg/kg body weight *i.e.,* the maximum dose which can be administered by oral route, therefore, the LD_{50} was inferred to be beyond this limit.

Table 1: Individual body weights of experimental mice

Formulation and Dose	Mice No.	Body Weight (g) on Day		
		0	**7**	**14**
Liqbiotic™ @ 2000 mg/kg body weight orally (Group I: Females)	1	22.5	23.5	24
	2	21	22.5	25
	3	21	23	25
Liqbiotic™ @ 2000 mg/kg body weight orally (Group II: Males)	1	23	24	24.5
	2	22	24	23.5
	3	23	23.5	24
Mean± S.E.		22.08±0.34	23.42±0.22	24.33±0.23

Similarly, no abnormal symptoms, including lethargy, tremor, abdominal breathing or piloerection, were observed up to after 14 days of Liqbiotic™ administration. Necropsy after day 14 (Fig. 1a) 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 (Fig. 1b). Similarly, no abnormalities were detected in the histopathological appearances of the liver, kidneys, spleen, heart, lungs, and gonadal organs in any of the animals (Fig. 1c).



a. ***b.*** ***c.***

Figure 1: Necropsy (a) or histology did not reveal any remarkable changes in the appearance of the organs. Gross (b) and histological (c) appearance of the right kidney of a representative animal has been shown for example.

The constituents of Liqbiotic™ are rich in essential oils (EO) like eugenol, thymol and cineol. EOs have wide range of activities in poultry feeding like stomachic, growth promoter, anti–bacterial, antioxidant, immunomodulator, hypolipidemic, antimycotic, antiparasitic, antitoxigenic and antiviral (Adorjan and Buchbauer, 2010; Gopi *et al.*, 2014). The antimicrobial activity of EOs may be due to the changes in the permeability of the cytoplasmic membrane to hydrogen (H^+) and potassium (K^+) ions. Their hydrophobic nature makes them more active against Gram positive bacteria as compared to Gram negative bacteria (Deans and Ritchie, 1987). Thymol inhibits the growth of *S.* Typhimurium and *E. coli*, causing disintegration of the membrane of bacteria and, hence, leading to the release of membrane-associated material from the cells to the external medium. EOs have good antioxidant role in biological system and act as effective free radical scavengers. EOs also influence the *in vivo* antioxidant defense systems such as SOD, glutathione peroxidase and Vit E (Youdim and Deans, 1999; Youdim and Deans, 2000).

In vivo studies in rabbits reveal that thyme oil significantly improved total antioxidant status (TAS) in the blood plasma and glutathione peroxidase (GPx) activity in the liver, and it decreased malondialdehyde (MDA) concentration in the duodenal tissue. It also resulted in strengthened intestinal integrity, as the essential oil supplementation significantly increased transepithelial electrical resistance (TEER) values when fed @ 0.5 g per kg

dry matter (DM) (Placha *et al*., 2013). In broilers, EO supplementation exerted a positive effect on caecal microbiota (increase in the proportions of *Lactobacillus* and *E. coli*) with a concomitant enhancement in growth performance (Tiihonen *et al*., 2010).

EOs have been documented to improve nutrient digestibility and intestinal absorptive capacity in poultry (Zeng *et al*., 2015). The improvement in nutrient absorption may be partly explained by increased secretions of saliva, bile and enhanced enzyme activity in broilers (Jang et al., 2004; Lee et al., 2003; Platel and Srinivasan, 2000). EOs also stimulate blood circulation, exert antioxidant properties, and enhance immune status (Brenes and Roura, 2010). Liqbiotic™ also exerts multifarious benefits, including strengthening of immune system, improvement in weight gain, FCR, and productive performance of the birds due to the presence of multiple EOs.

Conclusion

Liqbiotic™ did not produce acute oral toxicity as evident from the absence of mortality, toxic clinical symptoms, and gross and histopathological alterations, when administered up to a limit dose of 2000 mg/kg body weight in mice. Based on these findings, it could be concluded that the formulation is safe for oral use.

Conflict of Interests

There is no conflict of interest.

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