Uttar Pradesh Journal of Zoology

Volume 45, Issue 20, Page 459-464, 2024; Article no.UPJOZ.4242
ISSN: 0256-971X (P)

Acute Oral Toxicity Assessment of Repchol in Wistar Rats: A Liver-protective Herbal Premix for Poultry

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i204602

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4242

Original Research Article

Received: 28/08/2024
Accepted: 01/11/2024
Published: 07/11/2024

ABSTRACT

The present study was conducted for the assessment of acute oral toxicity and safety analysis of Repchol Premix (M/s Ayurvet Limited) according to OECD-423 guidelines. Six adult Wistar rats, weighing 113-134 g, were used for the study. After oral administration of Repchol@ 300 and 2000mg/kg body weight, the animals were observed for manifestation of toxic effect and death upto14 days. Evaluation of safety analysis and toxicity was assessed on the basis of presence of mortality or toxicity symptoms. Every animal in group served as its own control. At the end of

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Cite as: *M., Tripura, Vaidya M.G., Belkhede S.A., Damekar S.C., Gupta S., and Hajare S.W. 2024. "Acute Oral Toxicity Assessment of Repchol in Wistar Rats: A Liver-Protective Herbal Premix for Poultry". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (20):459-64. https://doi.org/10.56557/upjoz/2024/v45i204602.*

experiment blood biochemical parameters and histopathological changes in heart, liver, kidney and lung were studied. The results showed that Repchol did not produce toxic effects or mortality during the experimental period. The body weight changes observed for two weeks were in the normal range. In Repchol treated rats biochemical parameters like AST, ALT, ALP and creatinine were observed within normal range and in histopathological examination no significant microscopic alterations were observed. Thus, the observations indicated that Repchol Premix did not produce toxic effect or acute oral toxicity and the LD50 of compound is beyond 2000mg/kg.

Keywords: Repchol; acute; OECD-423; poultry; Wistar rat.

1. INTRODUCTION

Livestock is a key component of India's agricultural economy, providing food and nutritional security and supporting livelihoods. Among all animal industries, the Indian poultry sector has demonstrated the most rapid growth, with an increase of approximately 6% in egg production, 10% in meat output, and 8.35% in broiler production over the last decade. Within the livestock sector, the poultry industry represents about 1% of the national GDP and 14% of the animal GDP (Pathak et al., 2022). Over the span of forty years, India's poultry industry has transitioned from a modest backyard activity to a significant commercial agricultural enterprise (Biradar et al., 2011). India holds the 2nd position globally in egg production and ranks 5th in poultry meat production (PIB, 2023). The liver is a crucial organ in poultry, playing a significant role in digestion, metabolism, and various functions that regulate growth and productivity (Hajare et al., 2020, Zaefarian et al., 2019). Repchol is a scientific combination of potent medicinal herbs which includes natural analogues of choline and biotin. Choline is a part of B-complex vitamins which is crucial in fat mobilization and many physiological processes, including methionine and phospholipids formation (Gangane et al., 2010). Biotin acts as an antioxidant as well as helps in cell growth andmetabolism of proteins and fats (Hasan Kadhim et al., 2022).

Repchol Premix is a polyherbal formulation of key herbs like *Citrullus colocynthis*, *Trigonella foenum-graecum, Sida cordifolia, Nigella sativum, Achyranthus aspera. Citrullus colocynthis and Trigonella foenum-graecum* aid in the synthesis of Betaine, leading to improved growth, performance, and carcass quality as well as it decreases triglycerides, total cholesterol (TC), high-density lipoprotein (HDL) (Sharma et al., 2021. The entire plant and roots of *Achyranthes aspera* have been widely utilized to treat neurological conditions, including epilepsy and stroke (Viswanatha et al., 2019). *Nigella sativum* is a rich source of choline and contains thymoquinone which protects liver due to its antioxidant and anti-inflammatory effects (Mollazadeh and Hosseinzadeh, 2014, Kanter et al., 2005). Thus, Repchol can be expected to maintain growth, improve egg production, hatchability and liveability in poultry while it prevents fatty liver syndrome, perosis and liver dysfunction.

Since the product has commercial value, it is important to assess its safety potential or any possible adverse effects. Hence, in this study, an acute oral toxicity test was carried out as per OECD-423 in Wistar rats (OECD, 2001).

2. MATERIALS AND METHODS

The study was conducted at the Department of Veterinary Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India. The institute is situated at a latitude of 20.70° N and a longitude of 77.070° E, with an elevation between 287 and 316 meters above sea level, and experiences a tropical climate. Six healthy young adults female Wistar rats, weighing 113-134 g, were used in this study.The study was conducted from 13th April to 26th April 2022. The animals were acquired from the Laboratory Animal Resource Section at PGIVAS, Akola. A total of four animals in one cage with corn cob as bedding material were used for ease of monitoring. Picric acid was used for animal identification. The experiment was conducted with the ambient temperature set at 25±2°C and humidity was in the range of 50-70% during experiment. The animals were kept under a 12-hour light-dark cycle and had access to standard pelleted feed and water ad libitum. The animals were housed in polypropylene cages 5 days prior to experimentation as a period of acclimatization (OECD, 2001).

Table 1. Animal groups in sighting study

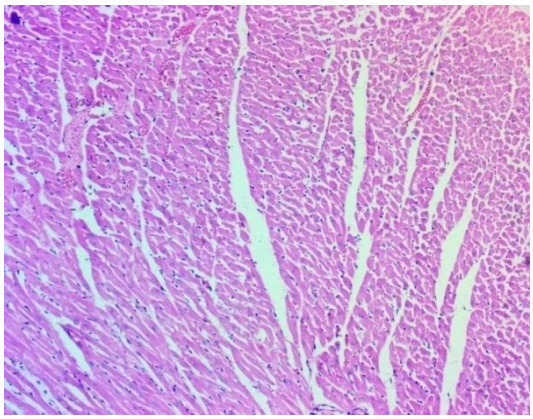
Group No.	Type of Study Used	Number of Animals	Dose (mg/kg body weight)
I	Step 1: Sighting study	01	300
II	Step 2: Sighting study	01	2000
III	Main study	04	2000

Rats were arranged into three different experimental groups (Table 1) and were fasted prior to dosing overnight, with food but not water. After the fasting period, the weight of all rats was measured. The suspension of test substance Repchol Premix (M/s Ayurvet Limited), was prepared in distilled water and administered to one rat each from Group I and Group II, @ 300 mg/kg and 2000 mg/kg of body weight, respectively. The observation of any toxicity symptom was recorded. As no toxicity signs appeared in the sighting study of Group I and Group II, the main study was performed in which all four rats from Group III were administered with test substance @ 2000 mg/kg of body weight. All rats from Groups I, II, and III were withheld from food for 1-2 hours after dosing. Animals were monitored continuously for any toxic effects and mortality for the first 30 minutes, periodically for 24 hours, and then intermittently for a total of 14 days.

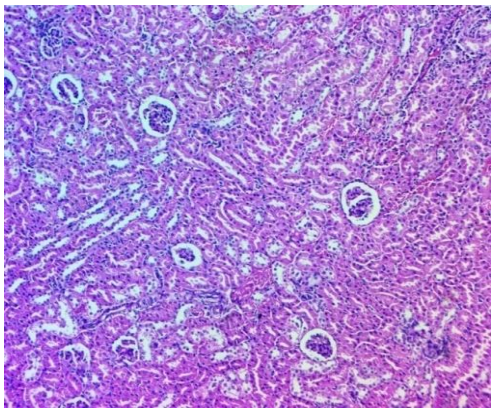
Animals were observed for mortality, convulsions, tremors, abdominal breathing, piloerection, salivation, lethargy, coma, or any other abnormal symptoms. Changes in body weight, skin coat, eyes, mucous membranes, and behavior, if any, were recorded. After 14 days of observation, the animals were euthanized using diethyl ether and vital organs (heart, liver, kidney, and lung) were collected in 10% neutral buffered formalin for histopathological examination after necropsy. Sections of 4-6 µmwere cut on a rotary microtome and stained with H&E stain for recording histopathological observations (Luna, 1968). 1-2 ml of blood was collected from the retro-orbital plexus using a capillary tube in EDTA vials for biochemical estimations of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine.

3. RESULTS AND DISCUSSION

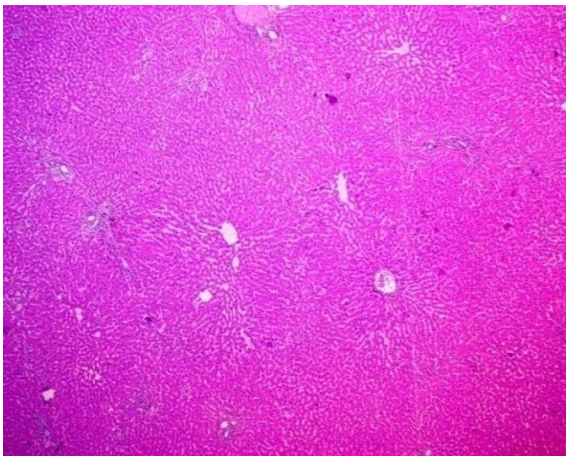
The body weights of each rat were individually recorded on days 0, 7, and 14 of the study. During the study period, the body weight of all four rats from the main test group (III) was increased within the normal range (Table 2). Biochemical analysis revealed that the values of ALT, AST, ALP, and creatinine were within the respective normal ranges (Table 3). Oral administration of Repchol Premix did not cause any mortality in any of the rats when given @2000 mg/kg, and hence, the LD50 of the compound is greater than 2000 mg/kg of body weight. Also, no abnormal symptoms including mortality, tremors, abdominal breathing, piloerection, convulsions, salivation, lethargy, and coma were observed up to 14 days. Similarly, no significant gross or histological abnormalities of the heart, liver, kidney, and lung were noticed that could be associated with the toxicity of the test substance (Fig. 1).



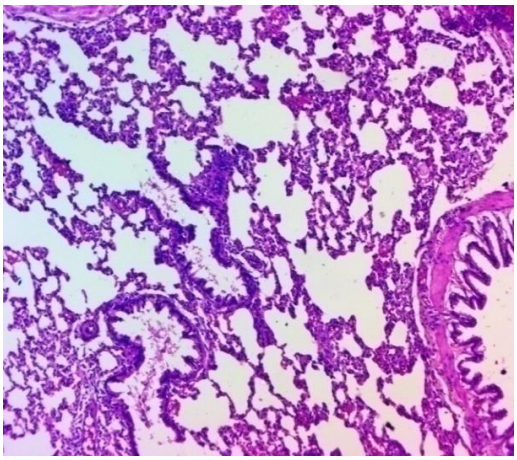
Microphotograph of Heart showing normal histoarchitecture (H & E, 100X)



Kidney showing normal histoarchitecture (H & E, 100X)



liver showing normal architecture (H & E, 40 X)



Lung showing normal architecture (H & E, 100X)

Fig. 1. Histopathological observations of tissue sections of heart, kidney, liver and lung from rats received Repchol premix formulation @2000mg/kg BW

Table 2. Weekly body weight of rats received Repchol premix@2000mg/kg

Sr. No.	Formulation	Dose	Animal Number	Sex	Body Weight (g) on Day		
					0	7	14
1	Repchol premix	2000 mg/kg b.wt. orally	1	F	134	143	158
			2	F	118	140	149
			3	F	113	123	139
			4	F	122	135	146
			Mean±SE		121.7±4.47	135.25±4.40	148±3.93

Rat No. 1, 2 3 and 4, are from main test group III, F= Female, Total values are expressed as Mean±SE (standard error)

Table 3. AST, ALT, ALP and creatinine values in experimental rats treated with Repchol premix@2000mg/kg

Dose of Repchol premix	Rat No.	Biochemical parameters			
		AST	ALT	ALP	Creatinine
2000mg/kg	1	47.91	27.00	76.26	0.31
	2	63.76	19.72	72.23	0.22
	3	45.60	26.01	89.93	0.40
	4	58.33	23.54	77.50	0.23
	Total	53.9±4.29	24.06±1.62	78.98±3.81	0.29±0.04

Rat No. 1, 2 3 and 4, are from main test group III, Total values are expressed as Mean±SE (standard error)

Repchol Premix is a polyherbal formulation containing natural analogues of choline and biotin and other herbal ingredients like *Citrullus colocynthis, Trigonella foenum-graecum, Sida cordifolia, Nigella sativum, and Achyranthes aspera* which are generally regarded as safe. Also, they are known for their array of beneficial effects. Therefore, the composition based on these constituents is likely to be non-toxic in practical doses which are several fold below the LD50 dose. Conversely, due to multiple ingredients and their proven beneficial effects, Repchol Premix may provide multifarious health benefits like improvement in growth, production, and quality of carcass in birds (Sharma et al., 2021). In related study a polyherbal formulation Ayucee Premix used for growth promotion and immunity booster in poultry caused no adverse effects when evaluated as per OECD-423 study (Hajare et al., 2024). Thus interpretation of results indicates that Repchol Premix is safe for oral feeding and there is no possibility of acute oral toxicity with its oral use.

4. CONCLUSION

The present study of the Repchol premix revealed that it did not cause acute toxicity, even when given at the maximum limit dose (2000 mg/kg b. wt.) in Wistar rats, as demonstrated by no mortality, clinical toxicity, and gross or histological changes. Based on these findings, it can be concluded that the Repchol premix is safe for oral use.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

All animals were maintained as per the SOPs of the Institutional Animal Ethics Committee (IAEC) and guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA). Before the experiment began, the study protocol was approved (approval no. 312/01/2000/03/22, dated 08.04.2022) by the IAEC (312/GO/ReBi/2000/CPCSEA) of PGIVAS, Akola.

ACKNOWLEDGEMENT

The authors express their gratitude to M/s Ayurvet Limited, India, for funding the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4242