



Evaluation of Acute Oral Toxicity of Ayucee Premix Polyherbal Poultry Formulation Containing Natural Vitamin C and Bioflavonoids

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Authors' contributions

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

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ABSTRACT

A study was conducted for the assessment of acute oral toxicity and safety analysis of Ayucee Premix (M/s Ayurvet Limited Baddi, India) according to OECD-423 guidelines. Six young adult Wistar rats, weighing 126-136 gm, were used for the study. After Oral administration of test compound the animals were observed for manifestation of toxic effect and death. Each animal served as its own control. Evaluation of safety analysis and toxicity was performed on the basis of presence of mortality or any clinical signs like lethargy, tremor, abdominal breathing and

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piloerection. Results showed no toxic effects or mortality during the experimental period. In blood biochemical parameters (AST, ALT, ALP) and histopathological examination no significant pathological alterations were observed. Thus, Ayucee Premix was found to be safe for oral consumption.

Keywords: Ayucee premix; acute oral toxicity; OECD 423; poultry; poultry production; bioflavonoids; biological properties.

1. INTRODUCTION

“Indian poultry industry is booming and emerging as the world’s second largest market. India is world’s third largest egg producer and fifth largest meat producer” [1]. Looking at the growth and scope in poultry, Indian poultry industry poised to become a global leader in this sector. The feeding programme for poultry has a major influence on profitability as feed typically accounts for much of poultry production costs [2]. “Nutrition is the most important factor affecting development, health status, growth performance and profitability of poultry production. Feeds for poultry constitute up to 70–75% of total production costs” [3]. “Lack of feed quality and an increase in their cost stimulates the need to search for opportunities to further increase the biological value of basic feeds and the texture of compound feed, in which the addition of biologically active substances and feed additives would be the most effective. In order to attain optimum immunity and growth rate which ultimately increases productivity, various types of feed additives or premix are incorporated in poultry feed” [4]. “Ayucee Premix is a polyherbal formulation containing natural vitamin C and bioflavonoids and claimed for their anti-oxidant, growth promotion and immunity boosting activities. Flavonoids have been shown to possess range of biological properties which mainly includes antiviral, boosting of immune response, prevention of lipid peroxidation and free radical scavenging activity” [5]. “The product Ayucee Premix contains herbal ingredient such as *Withania somnifera* (Ashwagandha), *Phyllanthus emblica* (Amla), *Terminalia chebula* (Haritaki) and *Shilajit* extract known for their antistress, immunomodulatory, hepatoprotective, adaptogenic properties” [6]. “*Withania somnifera* contains alkaloids, steroidal lactones, and flavonoids which is found to improve erythrocytic index, biochemical parameters, bursal weight index, lymphocyte stimulation indices and reduced histopathological insults in the infected birds. It also alleviates virus induced stress and histological and immunological alterations and reduced viral persistence in the host” [7]. “*Phyllanthus*

emblica can improve broiler performance and provide antistress, adaptogenic, immunogenic and growth-stimulating advantages. The presence of gallic acid and quercetin are Amla’s major constituents” [8]. “*Terminalia chebula* are anti-inflammatory and increase the survival rate of chickens challenged with *Escherichia coli*” [9]. “*Shilajit* supplemented birds showed improvement in overall growth of broiler chickens” [10]. Thus, Ayucee Premix can be expected to improve growth rate, immunity and ultimately the production performance in poultry. As the product has commercial utility, it is essential to evaluate its safety potential or adverse effects if any. Therefore, in the present study acute oral toxicity test was conducted according to OECD-423 guidelines to evaluate the safety and toxicity potential of this oral formulation [11].

2. MATERIALS AND METHODS

The present study was performed at the Department of Veterinary Pharmacology and Toxicology at the Post-Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, India. It is located at latitude 20.7° N and longitude 77.07° E and located 287-316 m above sea level and has a tropical climate. Six healthy young adult female Wistar rats, weighing 126-136 gm, were used in this study. The animals were procured from the Laboratory Animal Resource Section of PGIVAS, Akola. A total of four animals in one cage with corn cob as bedding material were used for the ease of monitoring. Picric acid was used for animal identification. The ambient temperature of 25±2 °C was maintained and humidity was 50-70% during the experiment. The animals were exposed to 12- hour Light-dark cycle and provided with standard pelleted feed and water *ad libitum*. The animals were housed in polypropylene cages 5 days prior to experimentation as a period of acclimatization.

Rats were arranged into three different groups (Table no. 1). The test substance Ayucee Premix (suspension prepared in distilled water) was administered to one rat each from group I and group II, at the dose rate of 300 mg/kg and 2000

Table 1. Animal groups in sighting study

Group No	Type of study used	Number of animals	Dose (mg/kg bodyweight)
I	Step 1: Sighting study	01	300
II	Step 2: Sighting study	01	2000
III	Main study	04	2000

mg/kg of body weight respectively. The observation of the toxicity effect if any were recorded. As no toxicity signs were appeared in sighting study of group I and group II, the main study was performed in which all the four rats from group III were administered with test substance @ 2000 mg/kg of body weight. All rats from group I, II, III were withheld of food for 1-2 hours after dosing. "Animals were monitored continuously for any toxic effects and mortality for first 30 minutes, periodically for 24 h, and then, intermittently for a total of 14 days. Animals were observed for mortality, lethargy, tremor, abdominal breathing and piloerection or any other abnormal symptoms. Changes in body weight, eyes, skin coat and behavior, if any, were recorded. After 14 days of observation, the animals were euthanized and different organs were collected in 10% neutral buffered formalin for histopathological examination after necropsy. Blood was collected for biochemical estimations of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and creatinine".

3. RESULT S AND DISCUSSION

The body weights of individual rats were recorded separately on the days 0, 7, 14 of the

study. During the study period, body weight of all the four rats from main test group (III) was found to be increased in the normal range (Table 2). Biochemical analysis revealed values of ALT, AST, ALP and Creatinine within the respective normal ranges (Table 3). Oral administration of Ayucee Premix did not cause any mortality in any of the rat when given at the rate of 2000 mg/kg, and hence, the LD50 of compound is greater than 2000 mg/kg of body weight [11]. Also no abnormal symptoms, including lethargy, tremor, abdominal breathing and piloerection were observed upto 14 days. Similarly, no significant gross or histological abnormalities of heart, liver, lungs and kidney were noticed that could be associated with toxicity of test substance (Fig. 1). Since, Ayucee Premix is a polyherbal formulation containing natural vitamin C, bioflavonoids and other herbal ingredients like *Withania somnifera*, *Phyllanthus emblica* (Amla), *Terminalia chebula* and *Shilajit* which are generally regarded as safe. Also they are known for their array of beneficial effects [7,8,9,10]. Further it has been demonstrated that Amla powder supplementation to birds reduces the effects of aflatoxins [13]. Therefore, the composition based on these constituents is likely to be nontoxic in practical doses. Conversely due to multiple ingredients and their proven beneficial

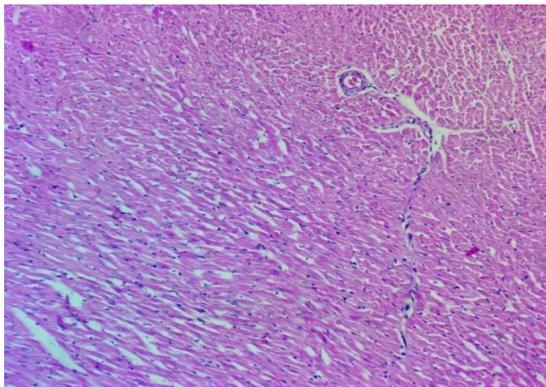
Table 2. Weekly body weight of Individual rats received Ayucee Premix@2000mg/kg

Formulation and Dose	Animal No.	Body Weight (g) on Day		
		0	7	14
2000 mg/kg b.wt. orally	1	128	142	164
	2	136	161	176
	3	126	142	159
	4	130	145	166
	Mean±SE	130±2.17	147.5±4.55	166.25±3.57

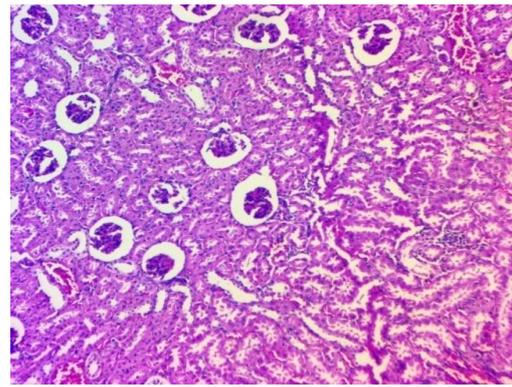
Table 3. AST, ALT, ALP and creatinine values in rats treated with Ayucee Premix@2000mg/kg

Dose of Ayucee Premix	Rat No.	Biochemical parameters			
		AST	ALT	ALP	Creatinine
2000mg/kg	1	51.58	26.52	84.67	0.19
	2	63.40	22.84	76.55	0.48
	3	54.62	13.69	68.17	0.38
	4	69.81	19.40	72.63	0.30
	Mean±SE	59.85±4.15	20.61±2.72	75.51±3.50	0.33±0.06

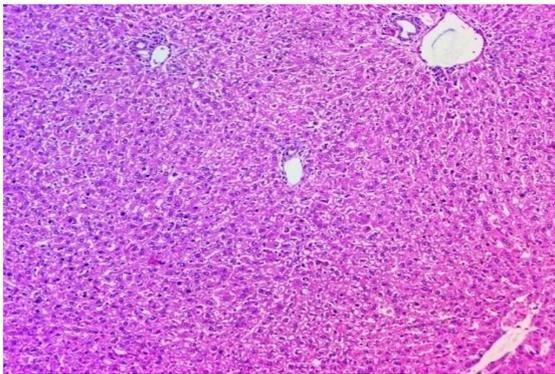
Key: Total values are expressed as Mean±SE, Rat No. 1, 2 3 and 4, are from main test group III



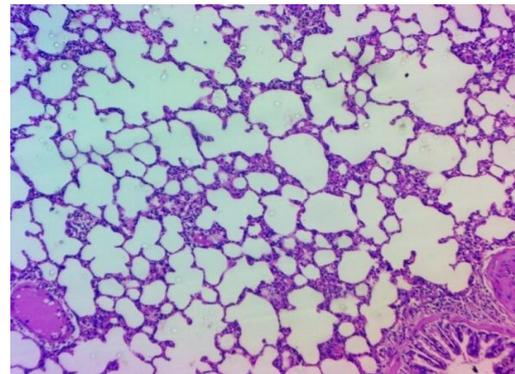
Microphotograph of heart showing normal histoarchitecture (H & E, 100X)



Kidney showing minimal congestion (H & E, 100X)



Liver showing mild vacuolar changes (H & E, x 100)



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

Fig. 1. Histopathological observations of tissue sections of heart, kidney, lung and liver from rats received Ayuceed Premix formulation @2000mg/kg body weight

effects, Ayuceed Premix may provide multifarious health benefits like improvement in digestion, stimulation of growth and production efficiency in animals and birds.

4. CONCLUSION

The present study revealed that Ayuceed Premix did not produce acute oral toxicity, even when administered at the maximum limit dose (2000 mg/kg) in rats, as it is evident from the absence of death, any clinical toxicity, and no significant gross or histological alterations. Based on these findings, it could be concluded that Ayuceed premix is safe for oral use.

ETHICAL APPROVAL

All animals maintained as per the SOPs of IAEC (Institutional Animal Ethics Committee) and guidelines of CCSEA (Committee for control and

supervision of experiments on animals). Before start of the experiment, the protocol of this study was approved (approval no. 312/01/2000/03/22 dated 08.04.2022) from IAEC (Reg. No. 312/GO/ReBi/2000/CPCSEA) of PGIVAS, Akola.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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