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Evaluation of acute oral toxicity of herbal rumenotoric and stomachic

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ABSTRACT

Maintaining optimum digestive functions is crucial for achieving health and profitability in livestock enterprises. However, the efficiency of these functions is upset by common problems such as indigestion, anorexia, flatulence, ruminal stasis and impaction. Pachoplus™ (M/s Ayurvet Limited, India) is a polyherbal rumenotoric, carminative and stomachic that helps to achieve and restore optimum digestive functions in livestock. A study was undertaken to evaluate the potential of Pachoplus™ to elicit acute oral toxicity as per OECD 423 guidelines. Nine non-pregnant, nulliparous, adult female Swiss albino mice were used for the study. The animals were observed for the manifestation of toxic effects and mortality after the oral administration of the test substance. 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. Pachoplus™ was found safe for oral use as no toxic effects or mortalities were observed till day 14.

Keywords: Livestock, Indigestion, Herbal, Safety, Acute oral toxicity, Pachoplus.

INTRODUCTION

Proper digestion is one of the keystones of health in all species of animals. Optimum digestibility and assimilation of feed is also crucial for maintaining farm profitability as feeding costs alone tend to account for up to 70% of the total costs of a livestock enterprise [1]. However, various problems like indigestion, anorexia, flatulence, ruminal stasis and impaction commonly affront digestive functions in farm animals. Pachoplus™ (M/s Ayurvet Limited, India) is a scientific combination of potent medicinal herbs which exerts rumenotoric, carminative and stomachic activity to ensure normal digestive functions. Its key ingredients, including *Allium sativum*, *Zingiber officinale*, *Trachyspermum ammi*, *etc.*, are reputed for their abilities to stimulate enzyme release, improve digestion, expel gases, and restore appetite [2-4]. In ruminants, it also stimulates ruminal motility, regulates ruminal pH and corrects ruminal stasis [5]. The present study was aimed at determining the acute oral toxicity potential of Pachoplus™.

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 24-29g, 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* [6]. 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

Mice were weighed individually 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).

Blood biochemistry revealed significant differences in the values of AST, ALT, ALP and creatinine 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.

Pachoplus™ did not cause any mortality in any of the mice at 2000 mg/Kg body weight, *i.e.*, the maximum dose which can be administered by oral route, 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 Pachoplus™ 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).

Pachoplus™ is prepared from parts of plants like *Allium sativum*, *Zingiber officinale*, *Trachyspermum ammi*, *etc.* that belong to the Generally Regarded as Safe (GRAS) category. *A. sativum* improves digestive functions both directly and indirectly. Directly, it exerts stomachic, anti-methanogenic, carminative, and appetizer effects. Its oil improves fiber digestibility in the rumen. Indirectly, it modulates the microbiome to improve feed utilization [3, 7]. *Z. officinale* stimulates rumen and gastric functions. It enhances palatability, digestive enzyme secretions and overall nutrient utilization efficiency [2, 8, 9]. *T. ammi* reduces ruminal and enteric methane production to improve nutrient utilization and performance. Supplementation of buffalo diet with *T. ammi* seeds at 2 per cent of dry matter intake was shown to reduce methanogenesis significantly by 18.38% [10]. *T. ammi* is also an excellent appetizer; the oils are carminative and anti-spasmodic [4]. Therefore, Pachoplus™ can be expected to improve digestive functions in livestock without exerting any toxic effects.

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	26	26	27	No
	2	28	28	29	No
	3	27	28	29	No
	Mean±SD	27±0.58	27.33±0.67	28.33±0.67	-
2000 mg/kg b.wt. orally (Group II)	1	24	25	27	No
	2	26	27	27	No
	3	27	28	29	No
	4	26	27	28	No
	5	26	26	28	No
	6	29	29	30	No
	Mean±SD	26.33±0.67	27.00±0.58	28.17±0.48	-

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)	50.48 ± 0.52^{b}	42.03 ± 0.83^{b}	118.01 ± 0.85^{b}	0.44 ± 0.009^{b}
2000 mg/Kg (Group II)	54.53 ± 0.21^{a}	46.05 ± 0.14^{a}	125.26 ± 1.22^{a}	0.55 ± 0.003^{a}

[a, b] Values bearing different superscripts differ significantly within columns

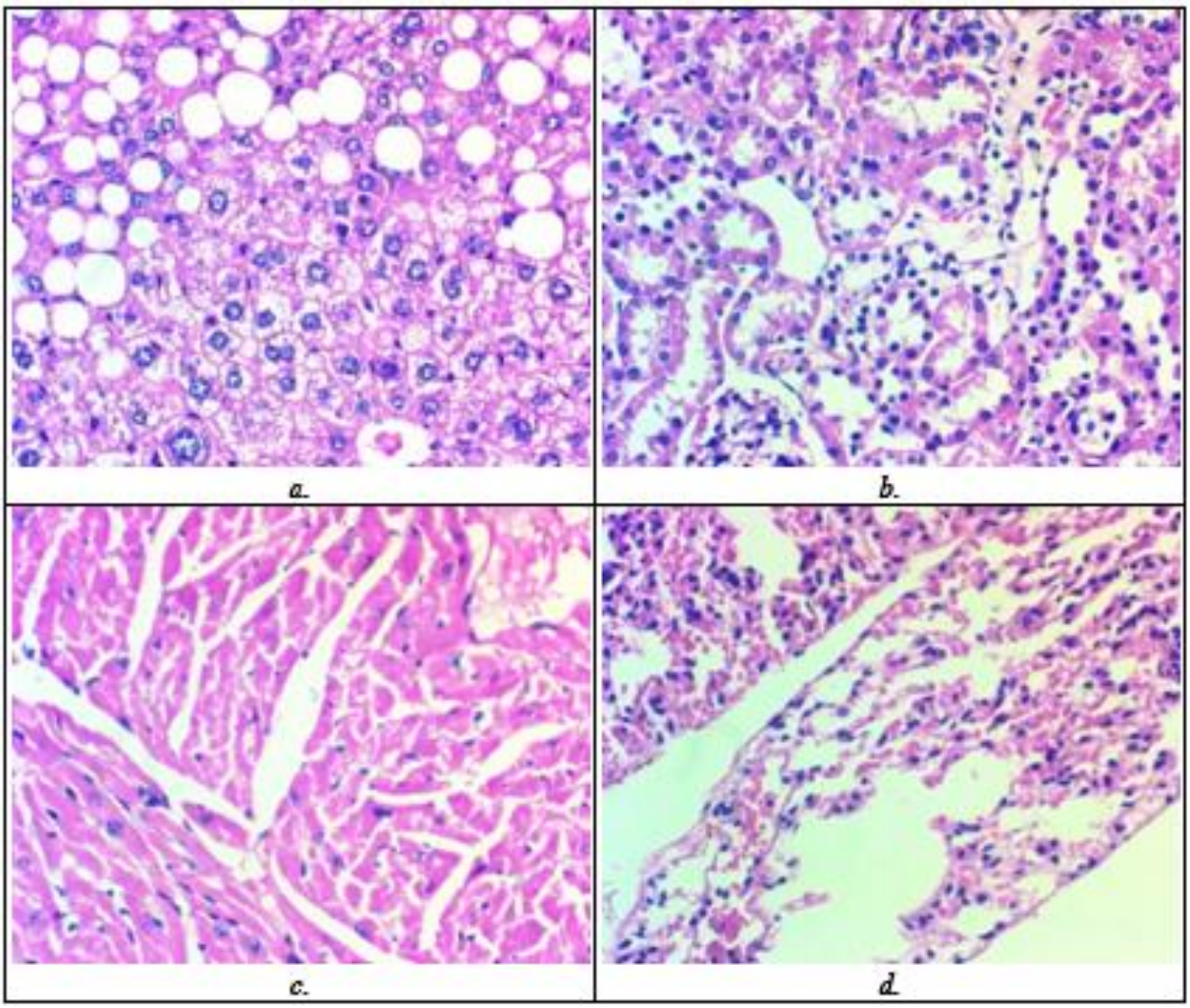

Figure 1: Histological appearance of *a.* liver, *b.* kidneys, *c.* heart and *d.* lungs of mice receiving 2000 mg of Pachoplus™ per Kg of body weight

CONCLUSION

Pachoplus™ did not produce acute oral toxicity, evident as the absence of mortality, any remarkable signs of toxicity, and gross or histopathological alterations, when administered at the limit dose of 2000 mg/Kg in mice. Based on this study, Pachoplus™ was found safe for oral use.

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