Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 24, No. (1) : 2022 : 31-34

ISSN-0972-3005

http://doi.org/10.53550/AJMBES.2022.v24i01.006

EVALUATION OF ACUTE ORAL TOXICITY OF A HERBAL UTERINE RESTORATIVE AND CLEANSER

SUNIL HAJARE[1], RANJIT SURESH INGOLE[1], MAHESHKUMAR VITTHAL INGAWALE[1], VIVEK BOREKAR[1], DAVID KUMAR [2], RAVIKANTH KOTAGIRI[2] AND BHASKAR GANGULY[2]*

[1]Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola 444 104, India
[2]Research and Development Unit, Ayurvet Limited, Baddi 173 205, India

(Received 7 September, 2021; Accepted 17 November, 2021)

Key words: *Acute oral toxicity, Exapar™ Liquid, Herbal uterine cleanser, Post-partum, Safety*

Abstract–Maintaining optimum reproductive health of animal is crucial for profitability in livestock enterprises and boosting the economy of farmers. However, the reproductive efficiency of animals is commonly reduced by problems such as puerperal metritis, clinical endometritis, pyometra, subclinical endometritis, early embryonic mortality, repeat breeding, retention of foetal membranes, and post-partum anestrus. Exapar™ Liquid (M/s Ayurvet Limited, India) is a polyherbal uterine cleanser and restorative that helps to achieve and restore better post-partum reproductive efficiency. This study aimed to evaluate the potential of Exapar™ Liquid to induce acute oral toxicity in mice as per OECD 423 guidelines. Nine healthy and adult nulliparous, non-pregnant Swiss albino female mice, weighing 24-28 g, 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, signs of toxicity, gross and histological appearances of vital organs, and blood biochemistry. Exapar™ Liquid was found safe for oral use as no toxic effects or mortalities were observed up to the completion of experiment.

INTRODUCTION

Postpartum uterine health may be compromised by the outcomes of bacterial infection like puerperal metritis, clinical endometritis, pyometra and subclinical endometritis (Sheldon *et al.*, 2006). These are the most common reproductive diseases in dairy cows, which can delay the regeneration of the endometrium and interrupt the resumption of cyclic activity, resulting in delay of the first artificial insemination (AI), increasing the number of AI per conception and, thus, prolong the calving interval and lower the calving rate (Hussain and Daniel, 1991). Nutritional status, lactational stress, management practices and climatic conditions are among factors that can negatively influence postpartum recovery, thereby exerting detrimental effects on reproductive performance. Retention of fetal membranes is a condition in which the cow fails to release the placenta up to 12h after calving, which is a major risk factor for metritis, delay in involution of uterus, and repeat breeding (Narasimhan and Deopurkar, 1994; Drillich *et al.*, 2006).

Exapar™ Liquid (M/s Ayurvet Limited, India) is a scientific combination of potent medicinal herbs, which helps in cleaning up the uterus, its involution, expulsion of placenta, induction of post-partum oestrus and improving the reproductive efficiency of cows and buffaloes (Khanna *et al.*, 1996; Singal, 1996; Sahatpure *et al.*, 2012). Its key ingredients, which include *Plumbago zeylanica*, *Gloriosa superba*, and *Aloe barbadensis* among others, have antibacterial, antifungal, anti-inflammatory, immunomodulatory, antioxidant, and healing properties (Ashwini *et al.*, 2020). The present study aimed to determine the acute oral toxicity potential of Exapar™ Liquid.

MATERIALS AND METHODS

The experiment was undertaken at the Department of Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, situated in the Vidarbha region of Maharashtra state of India (20.7°N and longitude

77.07°E; 287-316 m above sea level). The protocol of the study was approved (Approval number: 312/4/14/2000/20, dated 06.03.2020) by the Institutional Animal Ethics Committee (IAEC, 312/GO/ReBi/2000/CPCSEA) of PGIVAS, Akola. A total of nine healthy and adult nulliparous non-pregnant Swiss albino female mice, weighing 24-28 g, were used. The animals were procured from the laboratory animal resource section of the Department of Pharmacology, PGIVAS, Akola. All animals were maintained as per the SOPs of IAEC and guidelines of CPCSEA. The animals were marked by picric acid staining for individual identification. Three animals were maintained per cage to allow easy observation of each animal; housing conditions were conventional. The animals were subjected to a 12-hour light-dark cycle at a temperature of 25±2 °C and a relative humidity of 70%. The animals were fed a conventional pelleted diet with free access to water (OECD test 423). The animals were kept in the cages for seven days prior to dosing for acclimatization to laboratory conditions. 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. After the dosing of test substance, food was withheld for 1-2 hours in both groups I and II. The animals were monitored at least once for first 30 minutes and periodically for first 24 h, and then further for a period of 14 days for the manifestation of toxic effects and fatalities as well as the LD_{50} value. The observations recorded included those for changes in eyes, skin and coat; and changes in respiratory, circulatory, central nervous systems, 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. The data of biochemical parameters were analyzed statistically using one way ANOVA followed by complete randomized design.

RESULTS

The body weights of mice were measured separately on days 0, 7, and 14 of the study, and body weights of both groups (I and II) continued to increase throughout the study period (Table 1). No abnormal or toxic symptoms, including lethargy, tremor, abdominal breathing, and piloerection, were observed after oral administration of Exapar™ Liquid at 300 mg/Kg body weight and 2000 mg/Kg body weight to Group I and Group II, mice, respectively. The $LD_{50}$of the Exapar™ Liquid is more than 2000 mg/Kg as no mortality occurred at this limit dose, *i.e.* the maximum dose which can be administered by oral route. Necropsy after 14 days did not reveal any significant changes in the gross appearance of the liver, kidneys, heart, or lungs in any of the animals. Likewise, no abnormalities in the

Table 1. Individual weekly 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	27	27	No
	2	27	27	28	No
	3	25	26	27	No
	Mean±SD	26.00±1.00	26.67±0.58	27.33±0.58	-
2000 mg/Kg b.wt. orally (Group II)	1	25	26	26	No
	2	28	28	29	No
	3	26	27	28	No
	4	26	26	27	No
	5	25	25	26	No
	6	26	27	27	No
	Mean±SD	26.0±1.10	26.50±1.05	27.17±1.17	-

histopathological appearances of the liver, kidneys, heart or lungs were seen that could be linked to the test substance toxicity (Figure 1). Although blood biochemistry showed significant differences in the values of AST, ALT, ALP and creatinine between groups I and II (Table 2), all of these analytes were well within normal ranges in both the groups, indicating neither liver nor kidney damage.

DISCUSSION

Exapar™ Liquid is prepared from parts of several medicinal plants, prominently including *Plumbago zeylanica*, *Gloriosa superba*, and *Aloe barbadensis*, that belong to the Generally Regarded as Safe (GRAS) category. *P. zeylanica* is a pharmacologically important plant. It exhibits broad range of pharmacological activities, which include antibacterial, antifungal, anti-inflammatory, antioxidant, and wound healing (Shweta and Dubey, 2015; Singh *et al.*, 2017; Aleem, 2020). *G. superba* shows oxytocic activity and acts as an early abortifacient, maintains labour, aids the timely removal of placenta, and minimizes post-partum bleeding. Similarly, plant extracts of *G. superba* increase the activity and contractions of the uterus (Maurya *et al.*, 2004; Malpani 2011). *A. barbadensis* shows anti-inflammatory activity due to presence of potent polysaccharides and anti-inflammatory agents and also down-regulates *TNF*-α and *Cox-2* expression (Davis *et al.*, 1994; Paul *et al.*, 2021). Moreover, *A. barbadensis* has many other functions such as wound healing, immunomodulation, antimicrobial and antioxidant activity in addition to its anti-inflammatory properties (Ashwini *et al.*, 2020). Therefore, Exapar™ Liquid can be used for

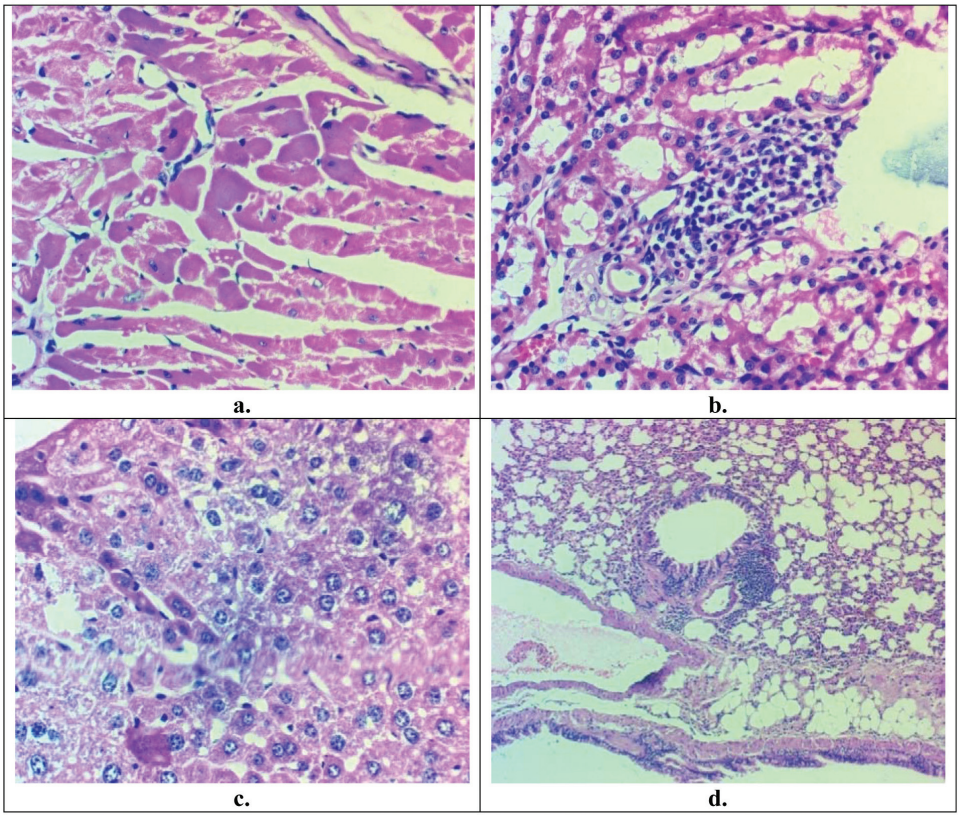

Fig. 1. Histological appearance of **a.** heart, **b.** kidneys, **c.** liver and **d.** lungs of mice receiving 2000 mg of Exapar™ Liquid per Kg of body weight

Table 2. Mean ± SD values of AST, ALT, ALP and creatinine in experimental mice

Dose	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dL)
300 mg/Kg (Group I)	50.37± 0.39[b]	41.67 ± 0.85[b]	117.68 ± 1.38[b]	0.45 ± 0.012[b]
2000 mg/Kg (Group II)	54.85 ± 0.25[a]	46.38 ± 0.40[a]	125.92 ± 2.06[a]	0.54 ± 0.009[a]

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

improving uterine health in livestock without exerting any toxic effects.

CONCLUSION

Exapar™ Liquid did not produce acute oral toxicity, evident as the absence of mortality, any toxic symptoms, and gross or histopathological alterations, when administered at the limit dose (2000 mg/Kg body weight) in mice. Based on this study, the product was found safe for oral use.

ACKNOWLEDGMENTS

The authors acknowledge M/s Ayurvet Limited, India, for funding the research.

REFERENCES

Aleem, M. 2020. Anti-Inflammatory and Anti-Microbial Potential of *Plumbago zeylanica* L.: A Review. *J. Drug Deliv. Ther.* 10(5-s): 229-235.

Ashwini, J., Omkar, P., Sampada, K. and Mangesh, B. 2020. Review on Aloe vera is used in medicinal plant. *AJP Sci.* 10 (1) : 26-30.

Davis, R. H., Donato, J. J., Hartman, G. M. and Haas, R. C. 1994. Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. *J. Am. Podiatr. Med. Assoc.* 84 (2): 77-81.

Drillich, M., Reichert, U., Mahlstedt, M. and Heuwieser, W. 2006. Comparison of two strategies for systemic antibiotic treatment of dairy cows with retained fetal membranes: preventive vs. selective treatment. *J. Dairy Sci.* 89 (5): 1502-1508.

Hussain, A. M. and Daniel, R. C. W. 1991. Bovine normal and abnormal reproductive and endocrine functions during the postpartum period: a review. *Reprod. Domest. Anim.* 26 (3) : 101-111.

Khanna, S., Khurana, K. L., Tripathi, V. N. and Manuja, A. 1996. Effect of Exapar (herbal uterine cleanser and restorative) on some parameters of reproductive efficiency in buffaloes. *Indian J. Anim. Reprod*. 51 : 231-237.

Malpani, A.A. 2011. Effect of the aqueous extract of *Gloriosa superba* Linn (Langli) roots on reproductive system and cardiovascular parameters in female rats. *Trop. J. Pharm. Res.* 10 (2) : 169-176.

Maurya, R., Srivastava, S., Kulshreshta, D. K. and Gupta, C. M. 2004. Traditional remedies for fertility regulation. *Curr. Med. Chem.* 11 (11) : 1431-1450.

Narasimhan, K. S. and Deopurkar, V. L. 1994. Accidents and diseases incidental to parturition. *Reproduction in Farm Animals.* (2nd Edn.) Varghese Publishing House, Bombay, India.

No. OECD Test 423 2001. Acute oral toxicity-acute toxic class method. *OECD guidelines for the testing of chemicals (section 4: health effects)*, 1:14.

Paul, S., Modak, D., Chattaraj, S., Nandi, D., Sarkar, A., Roy, J., Chaudhuri, T. K. and Bhattacharjee, S. 2021. *Aloe vera* gel homogenate shows anti-inflammatory activity through lysosomal membrane stabilization and down regulation of TNF-á and Cox-2 gene expressions in inflammatory arthritic animals. *Future J. Pharm. Sci.* 7 (1): 1-8.

Sahatpure, S. K., Patil, M. S., Saxena, M. J., Ravikanth, K. and Maini, S. 2012. Prophylactic efficacy of Exapar N in prevention of post partum reproductive disorders in Nagpuri cattle. *Theriogenology.* 2 (1) : 57-62.

Sheldon, I. M., Lewis, G. S., LeBlanc, S. and Gilbert, R. O. 2006. Defining postpartum uterine disease in cattle. *Theriogenology*. 65(8): 1516-1530.

Shweta. S. and Dubey, S. 2015. Antimicrobial activity of leaves extract of *Plumbago zeylanica* plant against known drugs. *Int. J. Res. Stud. Biosci.* 3 (6): 1–6.

Singal, S. P. 1996. Efficacy of Exapar in postparturient disorders with retained placenta in bovines. *Indian J. Anim. Reprod*. 17 (2) : 109-110.

Singh, M. K., Pandey, A., Sawarkar, H., Gupta, A., Gidwani, B., Dhongade, H. and Tripathi, D. K. 2017. Methanolic extract of *Plumbago zeylanica* – aremarkable antibacterial agent against many human and agricultural pathogens. *J. Pharmacopuncture*. 20 (1): 18-22.