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Acute Toxicity Study of Herbal Ovulation Inducer Product 'Estrofarm' in Wistar Rats

KEYWORDS: Estrofarm, ovulation inducer, polyherbal, toxicity, weight gain

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ABSTRACT *Estrofarm, a polyherbal ovulation inducer product (supplied by m/s Ayurvet Ltd, Baddi, India) was assessed for its acute oral toxicity in wistar rat following OECD Guideline 423. Estrofarm was administered orally in sequential manner to three male and three female rats at the limit dose level of 2000 mg/kg body weight. Animals had free access to standard commercial rat feed, except on the day prior to dosing. Water was provided adlib. The body weights of the rats were recorded prior to dosing, on day 7 and prior to sacrifice on day 14. The animals were observed for signs of convulsions, tremors, circling, depression, excitement and mortality. After the observation period of 14 days, all surviving animals were sacrificed and subjected to complete necropsy to find out any signs of systemic toxicity. All the animals survived by the end of the study without any signs of toxicity. Thus, Estrofarm was found to be safe after oral administration to wistar rat up to a dose of 2000mg/kg body weight.*

Introduction
Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human or veterinary use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and occasionally revealing delayed toxicity (CDER, 1996). The purposes of acute toxicity testing are to obtain information on the biologic activity of a product and gain insight into its mechanism of action. The information on acute systemic toxicity generated by the test is used in hazard identification and risk management in the context of production, handling, and use of product. The LD50 value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis for toxicological classification of product (Walum, 1998). The objective of current experimental trial is to evaluate safety standards of Estrofarm, a polyherbal ovulation inducer product in Wistar rats as per acute toxicity testing guidelines.

Materials and Methods
The present study was conducted in the Department of Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Bombay Veterinary College, Mumbai, Maharashtra, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

Experimental design
The study was actually performed following OECD Guideline 423 suggested by Organization for Economic Co-operation and Development 2001. The animals were bred and maintained at the Central Laboratory Animal House of Bombay Veterinary College, Mumbai. The rats were acclimatized to their surroundings 5 days prior to the test by separating them from the rest of the animals and allotting different cages to them.

Estrofarm was administered orally in sequential manner to three male and three female rats at the limit dose level of 2000 mg/kg body weight. The animals were identified by color marking. Animals had free access to standard commercial rat feed, except on the day prior to dosing and water was provided adlib. Oral gavage was used to dose the animals. All the procedures were carried out at 24^0C.

Estrofarm is a blend of selected herbal extracts, which shows affinity for bio gonadotropin and synchronizes the release of hormones in order to cause physiological estrus and regulation of ovarian function. It is polyherbal formulation with Citrullus colocynthis, Piper longum, Piper nigrum, Shilajit and many more in fixed concentration.

The body weights of the rats were recorded prior to dosing, on day 7 and prior to sacrifice on day 14. The animals were observed daily for signs of convulsions, tremors, circling, depression, excitement and mortality. After the observation period of 14 days, all surviving animals were sacrificed and subjected to complete gross necropsy to find out any signs of systemic toxicity

Results and Discussion
All the animals were observed for the period of 14 days after giving Estrofarm at the dose rate of 2000 mg/Kg body weight. The Estrofarm treated rats did not show any signs intoxication immediately following dosing and during the observation period of 14 days. The treated animals did not reveal any major adverse effect on the body weight gain also and the body weight was in the normal range. There was no mortality and no abnormal signs were observed in any of the animal fed with Estrofarm.

After the observation period of 14 days, all surviving animals were sacrificed and subjected to complete gross necropsy examination to find out any signs of systemic toxicity. All the organs were examined thoroughly for any abnormality or sign of systemic toxicity. On necropsy, no gross pathological changes were observed in any of the treated rats.

The results of the present study are in agreement with Reddy et al. 2012, Anand et al. 2010, Shah et al. 1998 and Siharat et al. 2007, who observed similar findings in acute oral toxicity studies conducted in case of Citrullus colocynthis, Linum usitatissimum, Piper longum, Piper nigrum respectively.

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Table 1: Body weight of rats after oral dosing with Estrofarm

Number and identification	Weight (g)		
	Day 1	Day 7	Day14
1	230	248	246
2	238	260	250
3	215	230	228
4	234	246	242
5	226	232	234
6	230	236	234

Table 2: Body condition and clinical signs in rats after oral dosing with Estrofarm

Signs	1	2	3	4	5	6
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes And mucous membranes	Normal	Normal	Normal	Normal	Normal	Normal
Behavior	Normal	Normal	Normal	Normal	Normal	Normal
Somatomotor activity	Normal	Normal	Normal	Normal	Normal	Normal
Tremors/ convulsions	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent
Death	No	No	No	No	No	No
Other symptoms	Nil	Nil	Nil	Nil	Nil	Nil

Conclusion

Since all the animals survived by the end of the study and clinical signs symptoms and gross necropsy did not reveal any major findings, The LD_{50} of the concerned compound is greater than 2000mg/kg (Category 5 as per OECD guidelines 420, 423 & 425 for acute Toxicity Studies) and hence the product Estrofarm is completely safe for animal usage and exert no adverse effect on animals.

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