Acute oral toxicity study of polyherbal formulation AV/KPC/10

Gatne MM¹, Adarsh² and Ravikanth K²

¹Department of Pharmacology and Toxicology, Bombay Veterinary College, Parel, Mumbai, India
²Clinical Research & Development, Ayurvet Limited, Baddi, 173205, Himachal Pradesh, India

*Correspondence Info:
Adarsh
Clinical Research & Development,
Ayurvet Limited, Baddi, H.P. India
E-mail: clinical@ayurvet.in

Abstract
The current study was designed to study acute oral toxicity study of polyherbal formulation AV/KPC/10 (M/S Ayurvet Limited, Baddi, India) according to OECD guidelines. For new substances it is the recommended stepwise testing approach for developing scientifically sound data on the safety of the substance. Three wistar rats (2 females and 1 male) were used for the study. AV/KPC/10 in single oral dose (2000mg/kg) supplemented to all rats. The parameters like general appearance, behavior, body weight, mortality and necropsy were studied. No changes in general appearance and mortality was observed. AV/KPC/10 was found to be safe at dose of 2000mg/kg.

Keywords: AV/KPC/10, OECD guidelines, Necropsy

1. Introduction
Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various diseases[1][2]. Despite the widespread use of plants for treatment of several ailments there is a little known about their toxicity and safety. The evaluation of the toxic action of the plant extracts or herbal formulations is important in order to consider them safe before used as medicines[3]. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models[4]. The acute oral toxicity test aims at establishing the therapeutic index, i.e. defined as the ratio LD₅₀: ED₅₀. In general, the narrower this margin, the more likely it is that the drug will produce unwanted effects, the greater the index the safer the compound. However, the term acute oral toxicity is most often used in connection to lethality and lethal dose determinations[5]. The objective of the current experiment was to study the Acute Toxicity of AV/KPC/10 (M/S Ayurvet Limited, India), an herbal formulation containing herbs viz. Phyllanthus niruri, Tephrosia Purpurea, Glycyrrhiza glabra etc. recommended for the treatment of ketosis in cattle.

2. Materials & methods
Acute Toxicity study of “AV/KPC/10” was performed following OECD Guideline 423 in the Department of Pharmacology and Toxicology, College of Veterinary & Animal Sciences, Bombay Veterinary College, Mumbai, Maharashtra, India

2.1 Experimental animals
The animals for the current study were approved by Institutional Animal Ethical Committee of Bombay Veterinary College, approval no: BVC/IAEC/50/2012. Wistar rats (2 females and 1 male) of age 9 weeks and average weight of 250 gms were used for the study.

2.2 Experimental design
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. The rats were identified by color marking. Rats had free access to standard commercial rat feed, except on the day prior to dosing. Water was provided ad libitum. The rat feeding needle was used to dose the animals. Weights were recorded prior to dosing (2ml/animal/single oral dose) (2000 mg/kg) on day 7 and prior to sacrifice on
day 14. All the procedures were carried out at ambient temperature (27°C).

3. Results and discussion

3.1 General appearance and behavioral observations

The appearance and behavioral parameters of animals after drug administration is indicator of the toxicity of the test drug[6][7]. The behavioral patterns of animals were observed first 6 h and followed by 14 h after the administration. No significant changes were observed in wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucus membrane, behavioral pattern, salivation and sleep pattern parameters of the treated animals were found to be normal (table 1). No toxic symptom or mortality was observed in any animal. All treated animals lived up to 14 days after the administration of AV/KPC/10.

| Signs                        | Rat 1         | Rat 2         | Rat 3         |
|------------------------------|---------------|---------------|---------------|
| Skin and Fur                 | Normal        | Normal        | Normal        |
| Eyes And mucus membranes     | Normal        | Normal        | Normal        |
| Behavior                     | Normal        | Normal        | Normal        |
| Somatomotor activity         | Normal        | Normal        | Normal        |
| Tremors/convulsions          | Absent        | Absent        | Absent        |
| Salivation                   | Absent        | Absent        | Absent        |
| Diarrhoea                    | Absent        | Absent        | Absent        |
| Death                        | No            | No            | No            |
| Other symptoms               | Nil           | Nil           | Nil           |

3.2 Body Weights

An increase in body weight of the animal after test drug administration is indicator of its toxic effect[8]. Table 2 showed the change observed before and after the administration of the AV/KPC/10. Although, the body weights of all the rats were increased after the oral administration of AV/KPC/10. But, the changes of the body weights were found to be statistically insignificant. Insignificant increase in body weight of test animals indicates that the administration of the AV/KPC/10 had no toxic effect on animals.

| Rat | Weight in grams |
|-----|-----------------|
|     | Day 1 | Day 7 | Day 14 |
| 1   | 230   | 240   | 230    |
| 2   | 270   | 300   | 300    |
| 3   | 250   | 270   | 280    |

3.3 Necropsy

All limit test animals were euthanized at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No lesions were observed in all rats.

| Rat number | Observed lesions |
|------------|------------------|
| 1          | Nil              |
| 2          | Nil              |
| 3          | Nil              |

3.4 LD₅₀ Value

An LD₅₀ value is the dose at which 50 percent of the test animals can be expected to die[9][10][11]. As per calculations from Acute Oral Toxicity (Category 5 as per OECD guidelines 420, 423 & 425 for acute Toxicity Studies) the LD₅₀ value of AV/KPC/10 was found to be more than 2000 mg/kg body weight.

4. Conclusion

All the three animals survived by the end of the study; Clinical signs symptoms and gross necropsy did not reveal any major findings. The LD₅₀ of the AV/KPC/10 was greater than 2000mg/kg (Category 5 as per OECD guidelines 420, 423 & 425 for acute Toxicity Studies) and hence it is practically nontoxic.

Acknowledgement

The authors are thankful to Ayurvet Limited, Baddi, India and Department of Pharmacology and Toxicology, Bombay Veterinary College, Parel, Mumbai for providing the required facilities, guidance and support.

References

[1] Humber JM. The role of complementary and alternative medicine: Accommodating pluralism. J Am Med Assoc 2002; 288: 1655-56.
[2] Dorai AA. Wound care with traditional, complementary and alternative medicine. Indian J Plast Surg 2012; 45(2): 418-24.
[3] Nordeng H, Al-Zayadi W, Diallo D, Ballo N, Berit Smestad Paulsen. Traditional medicine practitioners’ knowledge and views on treatment of pregnant women in three regions of Mali. J Ethnobiol Ethnomed 2013, 9: 67.
[4] Sari LM, Suyatna Fd, Utami S, Chairul C, Subita GP, Whulandhary YS, Auerkauri EI. Acute oral toxicity study of areca catechu linn. Aqueous extract in sprague-dawley rats. Asian J Pharm Clin Res 2014; 7(5): 20-2.
[5] Ghosh MN. Fundamentals of Experimental Pharmacology, 3rd Edition. S.K. Ghosh & others publications. 2005: 190-7.

[6] Kumar VK, Lalitha KG. Acute oral toxicity studies of Anacyclus pyrethrum dc root in albino rats. *Int J Pharm Pharm Sci* 2013; 5(4): 675-78.

[7] Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY and Sasidharan S. Acute Oral Toxicity of Methanolic Seed Extract of Cassia fistula in Mice. *Molecules* 2011; 16: 5268-82.

[8] OECD/OCDE 423. Acute Oral Toxicity – Acute Toxic Class Method. OECD guideline for testing of chemicals. 1-14.

[9] Deora PS., Mishra C K., Paresh M. Rani A, Shrivastava B and Nema RK. Effective alternative methods of LD50 help to save number of experimental animals. *J Chem Pharm Res* 2010; 2(6): 450-53.

[10] European Chemical Industry Ecology and Toxicology Center. Acute toxicity tests, LD50, (LC50) determinations and alternatives. *Monograph* 1985; 6, Brussels, Belgium.

[11] Food and Drug Administration. LD50 Test Policy. *Federal Register* 1988; 53: 39650-51.