American Journal of Pharmacology and Toxicology 5 (1): 42-47, 2010
ISSN 1557-4962

Safety Assessment of Herbal Formulations, Rumbion™ and Tyrel™ in Albino Wistar Rats

A.J. Joshua, K.S. Goudar, N. Sameera, G. Pavan Kumar, B. Murali, N. Dinakar and A. Amit
R and D Centre, Natural Remedies Pvt. Ltd., No. 5B, Veerasandra Industrial Area,
19th KM Stone, Hosur Road, Bangalore-560 100, India

Abstract: Problem statement: Herbal remedies form one of the effective strategies for management of livestock healthcare. Despite the availability of extensive pharmacological information, the toxicological data on herbs and herbal preparations seem to be scanty. The objective of the present investigation was to evaluate the acute oral toxicity of some herbal veterinary preparations in albino Wistar rats. **Approach:** In the sighting study, the investigational substances (Rumbion™ and Tyrel™) were orally administered in sequential manner to one animal each at 2000 and 5000 mg kg^{-1} body weight followed by four animals at 5000 mg kg^{-1} body weight in the main study. The treated animals were observed for mortality, adverse clinical signs, changes in body weight gain and necropsy findings during the study. **Results:** The results of the present study revealed that the treated rats survived throughout the study period and did not exhibit any treatment related abnormal clinical signs at the tested dose levels. Overall, the percent body weight gain in rats treated with the herbal preparations was found to be normal during the 14 day observation period. Postmortem examination of rats did not reveal any major abnormalities. **Conclusion:** In summary, acute oral toxicity testing of herbal veterinary formulations did not cause any treatment-related adverse effects up to the dose level of 5000 mg kg^{-1} body weight and hence the tested products were labeled unclassified in the hazard category according to Globally Harmonised System.

Key words: Rumbion™, Tyrel™, herbal formulations, acute oral toxicity, rats

INTRODUCTION

Traditional medicinal systems of many countries contain rich knowledge on phytomedicines. Several scientific reports reviewed in detail the therapeutic potentials of medicinal plants in alleviating animal diseases (Akhtar *et al*., 2000; Srivastava *et al*., 2000; Viegi *et al*., 2003; Fajimi and Taiwo, 2005; Wynn and Fougère, 2007). As modern medicines are increasingly unaffordable for the rural poor and due to lack of availability of animal healthcare professionals in many of the developing countries, use of veterinary herbal medicines is on the rise, especially in areas where livestock play a pivotal role in rural economy (Sharma and Singh, 1989; Rao and Varma, 2008; Weldegerima *et al*., 2008). Alternatively, herbal medicines are also used in countries where conventional medicine is predominant in the national healthcare system (World Health Organization, 2000).

Despite the popular use of herbal preparations for livestock disorders, there is limited scientific data available regarding safety aspects of these remedies (Aniagu *et al*., 2005). Published literature indicated the possible adverse effects and drug-herb interactions on use of herbal remedies (Brinker, 2000). Evaluation of safety of veterinary medicines for the target animals is a requirement of some international regulatory authorities and pertinent guidelines have been framed towards use of different approaches to acquire toxicological information on veterinary medicinal products (European Agency for the Evaluation of Medicinal Products, 1994).

As regards the safety evaluation of herbal medicines, the regulatory requirements in different countries are seem to be not uniform. Several countries have developed their own national regulations for traditional medicines (World Health Organization, 1998).

Assessment of acute oral toxicity of a test substance forms the primary step in the characterization of its toxic potential. Different test methods have been devised by the Organization for Economic Co-operation

Corresponding Author: J. Joshua Allan, R and D Centre, Natural Remedies Pvt. Ltd., No. 5B, Veerasandra Industrial Area, 19th KM Stone, Hosur Road, Bangalore-560 100, India Tel: +91-80-4020 9824/4020 9726 Fax: +91-80-4020 9817

and Development (OECD) for acute toxicity evaluation (Organization for Economic Co-Operation and Development, 2001a; 2001b; 2001c). Taking into account this background, the present study was aimed at evaluation of safety of some herbal preparations on acute oral exposure as per OECD guideline 420-Fixed dose procedure that utilizes non-lethal endpoints to determine the toxicity of the test material.

MATERIALS AND METHODS

Test materials: Two polyherbal veterinary preparations viz. Rumbion™ and Tyrel™ (M/s Natural Remedies Pvt. Ltd., Bangalore, India) were assessed for toxicity effects on acute oral exposure. The test substances have been intended for use on gastro-intestinal functions in small and large ruminants.

Rumbion™ bolus (Batch No.: 121/Mar'2007) consists majorly of crude powder of *Zingiber officinale*, *Andrographis paniculata*, *Terminalia chebula*, *Eclipta alba* and *Boerhaavia diffusa*. The plant materials used were analyzed for respective bioactive constituents by High Performance Liquid Chromatography (HPLC) method (Indian Pharmacopoeia, 2007). Tyrel™ liquid (Batch No.: 021N/May'2008) contains spearmint oil from *Mentha spicata* and Oleogum resin from *Ferula assa-foetida*. The oil and resin used were analyzed for respective bioactive constituents by Gas Chromatography/High Performance Thin Layer Chromatography (GC/HPTLC) methods (British Pharmacopoeia, 2007; Gupta *et al*., 2008).

Standardization of Rumbion™: The crude drug powder obtained from the standardized plant materials were mixed in appropriate proportions to prepare Rumbion™ product. The Thin Layer Chromatography (TLC) profile of product was compared with the reference material using HPTLC analysis. Product and reference standard weighing 2 g were refluxed with 50 mL of methanol separately on a water bath for 30 min and filtered after cooling. The filtrate was concentrated to 25 mL. Equal volumes (20 µL) of sample and standard were spotted on Silica 60 F_{254} plate of 0.2 mm thickness as bands. The plate was developed in a mobile phase consisting of Toluene: Ethyl acetate: Acetic acid (55:45:2). The dried plate was scanned at 254 and 366 nm. The plate was sprayed with anisaldehyde sulphuric acid reagent and dried in oven at 100°C. The fingerprint of product sample was compared with reference standards. The reference standard was prepared in the laboratory by mixing the herbs in the required proportions.

Standardization of Tyrel™: The spearmint oil and oleogum resin obtained from the standardized plant materials of *Mentha spicata* and *Ferula assa-foetida*, respectively were mixed in appropriate proportions to prepare Tyrel™ liquid. The TLC profile of product was compared with the reference material using HPTLC analysis. Product and reference standard measuring 10 mL was refluxed with 50 mL of chloroform separately on a water bath for 30 min and filtered after cooling. The filtrate was concentrated to 25 mL. Equal volumes (10 µL) of sample and standard were spotted on Silica 60 F_{254} plate of 0.2 mm thickness as bands. The plate was developed in a mobile phase consisting of Toluene: Ethyl acetate (93:7). The dried plate was scanned at 254 and 366 nm. The plate was sprayed with anisaldehyde sulphuric acid reagent and dried in oven at 100°C. The fingerprint of the product sample was compared with reference standard. The reference standard was prepared in the laboratory by mixing the spearmint oil and oleogum resin in the required proportions.

Test animals: Female albino Wistar rats of 8-12 weeks age were selected for the testing. They were bred and maintained at Central Animal Facility, Research and Development Centre, Natural Remedies Pvt. Ltd., Bangalore, India. They were housed in individual polypropylene cages with stainless grill top provided with clean bedding of paddy husk. The temperature of the experimental room was 25±2°C, relative humidity between 30 and 70% with optimal air changes per hour and illumination set to 12 h each of dark and light cycle. The animals were fed with standard pelleted rat feed (M/s Gold Mohur Foods and Feeds Ltd., Bangalore, India) and UV treated water was provided *ad libitum*.

Experimental design: Healthy adult, virgin female rats were used for the study. Veterinary examination was carried out before allocation of animals to groups and after the completion of acclimation period. Experimental rats were randomly assigned to the individual cages and each animal was identified by cage card number. For both products, initially one animal was tested each at 2000 and 5000 mg kg^{-1} body weight for the sighting study and four animals at 5000 mg kg^{-1} body weight for the main study. The rats were acclimatized for one week prior to dosing. They were deprived of feed overnight before and 3 h after the administration of the herbal preparations. Water was not withheld during this period. Both test substances were administered by oral gavage. Demineralised water was used as vehicle for

administration of test substances. The concentration of the final formulation was varied so as to maintain the dose volume constant at 20 mL kg^{-1} body weight for Rumbion™ and 10 mL kg^{-1} body weight for Tyrel™ to administer the doses of 2000 and 5000 mg kg^{-1} body weight, respectively. The doses were prepared fresh on the day of dosing.

Observations:

Mortality: The treated animals were observed for mortality twice daily till the completion of the study period.

Clinical signs: Observations of clinical signs were made at 10 min, 30 min, 1, 2, 4 and 6 h after dosing and once daily thereafter for 14 days at approximately same time. Daily cage side observations included changes in skin and fur, eyes and mucous membrane and also signs pertinent to respiratory, autonomic and central nervous systems, somatomotor activity and behavior pattern. The behavioral profile examined were alertness, visual placing, stereotypy, passivity, grooming, vocalization, irritability, spontaneous activity, reactivity and touch response whereas neurological observations such as straub response, tremor, convulsions, staggering gait, limb tone, grip strength, corneal reflex and pinna reflex were recorded. Signs of autonomic system included findings on pupil size, palpebral opening, exophthalmos, salivation, piloerection and skin color. Miscellaneous signs like arching of the back, alopecia, wound, nasal discharge, lacrimation and loose stool, if any, were also observed and recorded.

Body weight: Body weight data of individual animals were recorded following the period of fasting on the day of dosing, weekly thereafter and at termination (after overnight fasting) on day 15. Weekly changes in body weight gain were calculated and recorded.

Gross pathology and histopathology: All the rats in the study, either died during the study period, sacrificed moribund for humane reasons or sacrificed terminally were subjected to a complete post mortem examination to identify the gross pathological changes, if any. Microscopic examination of organs and tissues was considered only in case of evidence of any gross lesions.

RESULTS

The plant materials were analyzed for respective bioactive constituents by HPLC/GC/HPTLC as shown in Table 1. The pertinent profile of Rumbion™ bolus and Tyrel™ liquid were found to be similar to that of respective reference standards.

Rumbion™: The Rumbion™ treated rats did not show any mortality or adverse signs of intoxication immediately following dosing and during the observation period of 14 days. Rumbion™ did not reveal any major adverse effect on the body weight gain except for one female rat treated at 5000 mg kg^{-1} in the main study, which showed reduced body weight gain during the first week of 14 day observation. One female rat in the sighting study and three animals in the main study treated at 5000 mg kg^{-1} revealed reduced body weight gain during the second week of 14 day observation period. Overall, the percent body weight gain during the complete 14 day observation period was found to be normal in all the animals (Table 2 and 3). On necropsy, no major gross pathological changes were observed in any of the treated rats.

Tyrel™: Treatment with Tyrel™ did not produce any deaths in rats up to the dose level of 5000 mg kg^{-1} and did not show any abnormal clinical signs during the study period of 14 days. The treated animals showed reduced body weight gain during the second week as compared to first week of 14 day observation period.

Table 1: List of plants used in the formulation of Rumbion™ and Tyrel™ with respective bioactive constituents

Products and ingredients	Bioactive constituents
Rumbion™	
Zingiber officinale	Total gingerols
Andrographis paniculata	Andrographolide
Terminalia chebula	Chebulagic acid and Chebulinic acid
Eclipta alba	Wedelolactone
Boerhaavia diffusa	Boeravinone B
Tyrel™	
Mentha spicata	Carvone
Ferula assa-foetida	Ferulic acid

Table 2: Effect of herbal preparations on body weight and percent body weight gain in rats (sighting study)

		Body weight (g)			Percent body weight gain		
Products	Dose (mg kg^{-1})	Day 0	Day 7	Day 14	Day 0-7	Day 7-14	Day 0-14
Rumbion™	2000	169	185	195	9.47	5.41	15.38
	5000	170	189	182	11.18	-3.70	7.06
Tyrel™	2000	154	176	197	14.29	11.93	27.92
	5000	158	181	199	14.56	9.94	25.95

Table 3: Effect of herbal preparations on body weight and percent body weight gain in rats (main study)

Products	Dose (mg kg^{-1})	Body weight (g)			Percent body weight gain		
		Day 0	Day 7	Day 14	Day 0-7	Day 7-14	Day 0-14
Rumbion™	5000	173	192	205	10.98	6.77	18.50
		168	186	194	10.71	4.30	15.48
		162	165	173	1.85	4.85	6.79
		165	179	187	8.48	4.47	13.33
Tyrel™	5000	161	192	210	19.25	9.38	30.43
		159	182	195	14.47	7.14	22.64
		161	186	197	15.53	5.91	22.36
		160	181	187	13.13	3.31	16.88

Overall body weight gain was found to be normal during the study duration in all the treated animals (Table 2 and 3). Gross pathology examination of animals sacrificed at the end of the study revealed no major abnormalities.

DISCUSSION

Safety studies on veterinary herbal formulations, as per the internationally accepted guidelines of regulatory authorities, are very rarely performed and hence only scanty toxicological data are available worldwide. Of late, the scientific and commercial importance of validating the safety aspects of animal healthcare products according to various guidelines is recognized widely and toxicological evaluations of herbal veterinary preparations have been conducted as per the regulatory testing protocols (Joshua *et al*., 2008; Rajurker *et al*., 2009).

As reported elsewhere in the article, the testing protocols for acute toxicity testing that provide the predictive link is considered to be an important aspect in the preliminary safety evaluation of any test substance. In contrast to the traditional approaches of acute toxicity assessment that use mortality of test animals as the end point (Organization for Economic Co-operation and Development, 1987), the current investigation is carried out in compliance to a recent test guideline (OECD 420-fixed dose procedure) wherein the emphasis is on the manifestation of adverse clinical signs at specific dose levels. The critical observations recommended by the guideline during conduct of the study include mortality, signs of toxicity, changes in body weight and gross pathology along with histopathological examinations in case if any gross lesions were observed (Organization for Economic Co-operation and Development, 2001a).

In single dose toxicity studies, the time and mode of death of study animals forms one of the important indications of toxic response (European Agency for the Evaluation of Medicinal Products, 1987). Different stages that lead to death of experimental animals are clearly defined in scientific literature. In addition, criteria for humane killing of moribund, impending death and other conditions are also described (Organization for Economic Co-operation and Development, 2000). In the present investigation, Rumbion™ and Tyrel™ did not produce any mortality or conditions that may warrant for humane killing of rats up to the dose level of 5000 mg kg^{-1} indicating the safety of the herbal formulations.

Depending on the nature of the study, clinical examination that correlates all aspects of physiological processes is suggested for toxicological evaluation of test substance, either immediately or at an appropriate period after administration, every day till completion of the study period (Organization for Economic Co-operation and Development, 2000). Outward manifestation of untoward toxic signs indicative of systemic toxicity have been documented for various herbs/herbal preparations (Wynn and Fougère, 2007; Brinker, 2000). However, no treatment-related adverse clinical signs were recorded for both herbal formulations immediately following dosing and during the observation period of 14 days.

As specified in various regulatory guidelines, recording of changes in body weight gain is an integral part of the conventional safety evaluation of a test material (Organization for Economic Co-operation and Development, 2000; Environmental Protection Agency, 1998). Significant body weight loss is considered to be one of the most sensitive indicators of an animal's deteriorating health status (Organization for Economic Co-operation and Development, 2000). In this acute toxicity study, although there were infrequent incidences of reduced body weight gain observed in some animals at certain time intervals, the tested herbal products did not affect the overall weight gain in rats.

Necropsy examination plays an important role in identifying the general and target organ specific toxic effects of the test substance under study (Organization for Economic Co-operation and Development, 2000; Gad, 2007; Hayes, 2007). Absence of any remarkable gross pathological lesions in treated rats at the terminal

sacrifice signifies the reasonably harmless nature of the tested products.

Inspite of differences in the opinion on the utility of conducting acute toxicity studies, their significance in toxicological characterization of the test substances is still widely accepted (Aniagu *et al*., 2005; Chapman and Robinson, 2007). The single dose safety studies in laboratory rodents reveal the untoward events that may arise out of an acute exposure of test materials in target species by similar route of administration (European Agency for the Evaluation of Medicinal Products, 1987).

CONCLUSION

In conclusion, acute oral toxicity testing of Rumbion™ and Tyrel™ in the female rats did not cause deaths, toxicity signs or any significant gross pathological changes up to the dose level of 5000 mg kg^{-1} body weight. The overall weight gain was found to be normal in treated rats and hence the tested products are labeled unclassified in the hazard category according to Globally Harmonized System and can be considered relatively safe.

ACKNOWLEDGEMENT

The researchers are thankful to Sri. R.K. Agarwal, Chairman, M/s Natural Remedies Pvt. Ltd., Bangalore, India for his constant encouragement and support in completing this work successfully.

REFERENCES

Akhtar, M.S., Z. Iqbal, M.N. Khan and M. Lateef, 2000. Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo-Pakistan subcontinent. Small Rumin. Res., 38: 99-107. DOI: 10.1016/S0921-4488(00)00163-2

Aniagu, S.O., F.C. Nwinyi, D.D. Akumka, G.A. Ajoku and S. Dzarma *et al*., 2005. Toxicity studies in rats fed nature cure bitters. Afr. J. Biotechnol., 4: 72-78. http://www.academicjournals.org/AJB/PDF/Pdf2005/Jan/Aniagu%20et%20al.pdf

Brinker, F.N.D., 2000. The Toxicology of Botanical Medicines. 3rd Edn., Eclectic Medical Publications, Sandy, Oregon, ISBN: 1888483091, pp: 13-14.

British Pharmacopoeia, 2007. Spearmint Oil. British Pharmacopoeia Vol. I and II. The stationery Office, British Pharmacopoeia Commission Secretariat, London. ISBN: 01132217256, pp: 1562.

Chapman, K. and S. Robinson, 2007. Challenging requirement for acute toxicity studies: A workshop report. National Centre for the Replacement, Refinement and Reduction of Animals in Research. http://www.nc3rs.org.uk/downloaddoc.asp?id=559

Environmental Protection Agency, 1998. Health effects test guidelines, OPPTS 870. 1100, Acute oral toxicity, US Government Printing Office, Washington DC. http://iccvam.niehs.nih.gov/methods/acutetox/invidocs/EPA_870_1100.pdf

European Agency for the Evaluation of Medicinal Products, 1987. Single dose toxicity. 3BS1a. EMEA, London, UK. http://www.emea.europa.eu/pdfs/human/swp/3bs1aen.pdf

European Agency for the Evaluation of Medicinal Products, 1994. Evaluation of the safety of veterinary medicinal products for the target animals. http://www.emea.europa.eu/pdfs/vet/vetguidelines/7ae2a.pdf

Fajimi, A.K. and A.A. Taiwo, 2005. Herbal remedies in animal parasitic diseases in Nigeria: A review. Afr. J. Biotechnol., 4: 303-307. http://ajol.info/index.php/ajb/article/view/15099

Gad, S.C., 2007. The Rat. In: Animal Models in Toxicology, Gad, S.C. (Ed.). CRC Press, Boca Raton, FL, ISBN: 10: 0824754077, pp: 193-195.

Gupta, A.K., N. Tandon and M. Sharma, 2008. Quality Standards of Indian Medicinal Plants. Vol. V, Indian Council of Medical Research, New Delhi, pp: 232-237.

Hayes, A.W., 2007. Principles of Pathology for Toxicological Studies In: Principles and Methods of Toxicology, Hayes, A.W. (Ed.)., 5th Edn., CRC Press, New York, ISBN: 084933778X pp: 592.

Indian Pharmacopoeia, 2007. Ministry of Health and Family Welfare, Government of India. Vol. III, The Indian Pharmacopoeia Commission, Ghaziabad, ISBN: 81-903436-3-7, pp: 2027-2068.

Joshua, A.J., K.S. Goudar, A. Damodaran, N. Sameera and A. Amit, 2008. Acute oral toxicity evaluation of some polyherbal formulations in albino Wistar rats. Int. J. Pharmacol., 4: 388-392. DOI: 10.3923/ijp.2008.388.392

Organization for Economic Co-operation and Development, 1987. OECD guideline for testing of chemicals: Guideline 401, Acute oral toxicity, OECD, Paris, France. http://iccvam.niehs.nih.gov/docs/acutetox_docs/udpProc/udpfin01/append/AppI.pdf

Organization for Economic Co-operation and Development, 2000. Guidance document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. Environmental Health and Safety Monograph Series on Testing and Assessment No 19, OECD, Paris, France. http://www.imm.ki.se/sft/pdf/OECD19.pdf

Organization for Economic Co-operation and Development, 2001a. OECD guideline for testing of chemicals: Guideline 420, Acute oral toxicity-Fixed dose procedure, OECD, Paris, France. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL420.pdf

Organization for Economic Co-operation and Development, 2001b. OECD guideline for testing of chemicals: Guideline 423, Acute oral toxicity-Acute toxic class method, OECD, Paris, France. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf

Organization for Economic Co-operation and Development, 2001c. OECD guideline for testing of chemicals: Guideline 425, Acute oral toxicity-Up-and-Down procedure, OECD, Paris, France. http://www.oecd.org/dataoecd/17/51/1948378.pdf

Rajurker, S., D.S. Rekhe, S. Maini and K. Ravikanth, 2009. Acute toxicity studies of polyherbal formulation (Methiorep Premix). Vet. World, 2: 58-59. http://www.veterinaryworld.org/Vol.2%20No.2%20Full%20Text/Acute%20toxicity%20studies%20of%20polyherbal%20formulation%20(Methiorep%20Premix).pdf

Rao, M.L.S. and Y.N.R. Varma, 2008. Ethno-veterinary medicinal plants of the catchments area of the river Papagni in the Chittor and Ananthapur districts of Andhra Pradesh, India. Ethnobotanic. Leaflets, 12: 217-226. http://opensiuc.lib.siu.edu/ebl/vol2008/iss1/26/

Sharma, P.K. and V. Singh, 1989. Ethnobotanical studies in northwest and Trans-Himalaya. V. Ethno-veterinary medicinal plants used in Jammu and Kashmir, India. J. Ethnopharmacol., 27: 63-70. DOI: 10.1016/0378-8741(89)90078-0

Srivastava, G.N., S.A. Hasan, G.D. Bagchi and S. Kumar, 2000. Indian Traditional Veterinary Medicinal Plants. 1st Edn., Central Institute of Medicinal and Aromatic Plants, Lucknow, ISBN: 81-86943-67-6.

Viegi, L., A. Pieroni, P.M. Guarrera and R. Vangelisti, 2003. A review of plants used in folk veterinary medicine in Italy as basis for a databank. J. Ethnopharmacol., 89: 221-244. DOI: 10.1016/j.jep.2003.08.003

Weldegerima, B., T. Abula and M. Ragunathan, 2008. Ethno-veterinary use of medicinal plants by traditional healers in Dabat district, Northwestern Ethiopia. Pharm. Mag., 4: 93-99. http://www.phcogmag.com/ethno-veterinary-use-medicinal-plants-traditional-healers-dabat-district-northwestern-ethiopia

World Health Organization, 1998. Regulatory situation of herbal medicines: A worldwide review. WHO/TRM/98.1. http://whqlibdoc.who.int/hq/1998/WHO_TRM_98.1.pdf

World Health Organization, 2000. Herbal medicines. WHO Drug Inform., 14: 237. http://apps.who.int/medicinedocs/pdf/s2201e/s2201e.pdf

Wynn, S.G. and B. Fougère, 2007. Veterinary Herbal Medicine. Elsevier Health Sciences, USA., ISBN: 0323029981, pp: 4.