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Acute Oral Toxicity Evaluation of Some Polyherbal Formulations in Albino Wistar Rats

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Abstract: The present study was conducted to evaluate the acute oral toxicity potentials of certain herbal veterinary preparations in albino Wistar rats. In the sighting study, the test substances were 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; whereas the test materials with well documented traditional use were evaluated at 5000 mg kg^{-1} body weight. The treated animals were observed for mortality, untoward clinical/toxic signs, alterations in body weight gain and necropsy findings during the study. The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the tested dose levels for all the products. The overall percent body weight gain in rats treated with the herbal products was found to be normal during the 14 day observation period. On necropsy, no abnormalities were observed. In conclusion, acute oral toxicity testing of screened herbal veterinary products did not produce any treatment-related adverse effects upto the dose level of 5000 mg kg^{-1} body weight.

Key words: Polyherbal formulations, acute oral toxicity, albino rats

INTRODUCTION

Medicinal plants, for several centuries, have been widely used as a primary source of prevention and control of livestock diseases (Sharma and Singh, 1989). The global trend towards the increased demand and use of herbal medicine in the animal healthcare sector has resulted primarily due to the increasing cost of livestock maintenance, stressful transit for seeking professional veterinary care and the introduction of new technology in the production of plant-based therapeutics (Hoareau and DaSilva, 1999; Sikarwar, 1997). In 2004, the global market for natural animal healthcare products was estimated to be around $ 444 million (Natesh, 2007), while in India, the total market of veterinary products was approximately Rs. 1500 crores in which about Rs. 225 crores was the share of herbal products during the same period (DBT, 2004).

Despite their growing popularity as naturally safe with pharmacologically active principles (Mabeku *et al*., 2007), only little information is available regarding the regulations on quality and requirements for demonstrating efficacy and safety of herbs and herbal veterinary products. Non-existence of detailed regulations, especially in many developing countries, has been a matter of concern for the sustainable and appropriate use of medicinal plants and their products in veterinary health care (ICS-UNIDO, 2007). This necessitates the generation of adequate safety data in approved model systems proposed by the regulatory agencies (Agaie *et al*., 2007; Bulder and Noordam, 2008; Jegede *et al*., 2006).

In the safety evaluation of a test substance, determination of acute oral toxicity is generally the initial step and provides information on health hazards that may arise from an acute exposure by the oral route. Data from such acute studies can serve as a basis for classification and labeling of the test substance and establishing the dosage regimen of repeated dose and other related studies. It may also provide early information on the mode of toxic action of a substance (Rispin *et al*., 2002).

The present study was carried out to assess certain popular veterinary products for their acute toxic potential by fixed dose procedure adopted by the Organisation for Economic Co-operation and Development (OECD). This approach avoids using death of animals as an endpoint and relies instead on the observation of clear signs of toxicity after administration of test material at a series of fixed dose levels. The method also renders information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS).

MATERIALS AND METHODS

Test materials: A total number of five herbal veterinary products (M/s Natural Remedies Pvt. Ltd., Bangalore, India) were evaluated for acute oral toxicity study. The

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products viz. Becknor® for anti-diarrhoeal, Hygest™ to prevent ketosis, Zigbo® to optimize liver functions, Involon™ as an uterine tonic and Zigup™ syrup for its growth promoting effects are recommended for their favourable gastro-intestinal and reproductive effects in livestock and pet animals.

Experimental animals: Female albino Wistar rats of 8 to 12 weeks age were chosen for the study. The animals were received from Central Animal Facility, Research and Development Centre, Natural Remedies Pvt. Ltd., Bangalore, India. They were kept in individual polypropylene cages provided with clean bedding of rice husk. They were acclimatized for five days prior to dosing under standard housing conditions (temperature: 25±2°C, relative humidity: between 30 and 70% with optimal air changes per hour and 12 h each of dark and light cycle) and provided with standard pelleted feed and U.V. treated water *ad libitum*.

Study design: Healthy adult female rats were used in this experiment. Animals were randomly assigned to the cages and each animal was identified by cage card number and individual picric acid marking on fur. The females were nulliparous and non-pregnant. Typically, one animal each at 2000 and 5000 mg kg^{-1} body weight and four animals at 5000 mg kg^{-1} body weight were selected, respectively, for the sighting and main studies for products evaluated by main test, whereas a total of six female rats at the dose level of 5000 mg kg^{-1} body weight were assigned for products tested under limit test. Consideration of limit test for products was mainly based on certain previous information available about the test materials. The rats were deprived of feed overnight before and 3 h after the administration of the test substance. Water was not withheld during this period. Demineralised water was used as vehicle for dosing of test substance. The concentration of the test substance was varied so as to maintain the dose volume constant at 10 or 20 mL kg^{-1} body weight as per the nature of the product. The doses were prepared fresh on the day of dosing.

In-life clinical observations: The treated animals were observed for mortality (twice daily) and clinical signs were recorded to note the onset, duration and reversal (if any) of toxic effects at 10, 30 min, 1, 2, 4 and 6 h after the administration of the test substance and once daily thereafter for 14 days. The routine cage side observations included changes in skin and fur, eyes and mucous membrane and also respiratory, autonomic and central nervous systems and somatomotor activity and behaviour pattern. The behavioural profile studied included 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 taken into consideration. The criteria for autonomic profile included findings on pupil size, palpebral opening, exophthalmos, salivation, piloerection and skin colour. Miscellaneous signs like arching of the back, alopecia, wound, nasal discharge, lacrimation and loose stool were also recorded during the observations. The clinical signs were graded by a scoring system wherein scores for normal, abnormal, subnormal and supernormal responses were assigned as 4, 0, <4 and >4, respectively and the maximum score for any response was assigned as 8.

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 on day 15. Weekly changes in body weight gain were calculated and recorded.

Gross pathology and histopathology: All the rats in the study, dying during the observation period, sacrificed moribund for humane reasons or sacrificed terminally were subjected to a complete necropsy and the gross pathological changes, if any, were recorded. Histopathology examination of organs and tissues was considered in case of evidence of any gross pathology findings.

RESULTS

Reported here are the results of acute toxicity studies on selected herbal veterinary formulations. The reference data on scores for exhaustive profile of clinical signs and mean body weight of individual animals in the main study are not presented here for brevity of the article.

Becknor®: Animals treated at the dose level of 5000 mg kg^{-1} body weight in the sighting study and main study survived throughout the study period and did not show any major abnormal clinical signs following dosing and during the observation period of 14 days, post treatment. Body weight and percent body weight gain in dosed rats after 7 and 14 days of treatment was found to be increased during the sighting study (Table 1) and main study (Table 2). Macroscopic examination of animals sacrificed at termination revealed no abnormalities.

Table 1: Effect of herbal products on body weight and percent body weight gain in rats (Sighting study)

		Body weight (g)			Body weight gain (%)		
Products	Dose (mg kg^{-1})	Day 0	Day 7	Day 14	Day 0-7	Day 7-14	Day 0-14
Becknor®	5000	158.00	180.00	194.00	13.92	7.78	22.78
Hygest™	5000	162.00	165.00	130.00	1.85	-21.21	-19.75
Zigbo®	5000	163.00	191.00	208.00	17.18	8.90	27.61
Involon™	5000	164.00	185.00	197.00	12.80	6.49	20.12
Zigup™ syrup	5000	159.00	170.00	208.00	6.92	22.35	30.82

Table 2: Effect of herbal products on mean body weight and percent body weight gain in rats (Main study)

		Mean body weight (g)			Body weight gain (%)		
Products	Dose (mg kg^{-1})	Day 0	Day 7	Day 14	Day 0-7	Day 7-14	Day 0-14
Becknor®	5000	162.00	189.00	207.00	16.67	9.52	27.78
Hygest™	5000	163.00	186.40	188.00	14.36	0.86	15.34
Zigbo®	5000	162.60	174.60	198.20	7.38	13.52	21.89
Involon™	5000	161.40	188.40	203.80	16.73	8.17	26.27
Zigup™ syrup	5000	162.75	190.25	189.00	16.90	-0.66	16.13

Hygest™: Neither mortality nor any abnormal clinical signs were observed in rats treated at the dose level of 5000 mg kg^{-1} body weight in the sighting and main studies, respectively. Table 1 and 2 show the effect of Hygest™ on body weight and percent weight gain after single oral administration in female rats. Overall, the percent body weight gain during the complete 14 day observation period was found to be normal in all the animals except for one female rat each in sighting study and main study showed reduction in body weight gain during the 14 day observation period. Gross pathology of animals sacrificed at termination revealed no abnormalities.

Zigbo®: Treatment related death or toxic effects were not noticed in animals upto the dose level of 5000 mg kg^{-1} body weight during the experiment. Two animals in the main study showed reduced body weight gain during the first week, but regained during the second week and revealed comparable body weight gain at the end of the study period. The average body weight and percent weight gain of treated animals was found to be normal (Table 1, 2). No significant abnormalities were noticed during the postmortem examination of treated rats.

Involon™: The treatment with Involon™ was found to be non lethal at and upto the dose level of 5000 mg kg^{-1} body weight as the treated animals were alive throughout the study period and did not show any adverse clinical signs following dosing and after the period of 14 days, post treatment. Table 1 and 2 show that the treatment with Involon™ did not reveal any adverse effect on body weight gain in female rats. Autopsy of treated animals did not show any abnormalities during terminal sacrifice.

Zigup™ syrup: No mortality was recorded in experimental animals treated with Zigup™ syrup upto 5000 mg kg^{-1} body weight in the sighting and main studies. The observations on clinical signs after test substance administration did not show any significant effects throughout the study period. Zigup™ syrup treatment did not reveal any major adverse effect on the body weight gain except for the treated female rats in the main study group, which showed reduction in body weight gain during the second week of observation period (Table 1, 2). On necropsy, no major gross pathological changes were observed in any of the treated rats.

DISCUSSION

Continued exploration of lesser known medicinal plants provides excellent leads for development of new therapeutics (Lee, 2005). As more pharmacological and clinical information on medicinal plants become available, the toxicological database of these agents also grows increasingly more refined (Francis, 2000). Like their synthetic counterparts, toxicological studies must be performed for herbal preparations to validate their safety (Becker *et al*., 2007). Unfortunately, there is limited scientific evidence reported pertinent to safety of phytopreparations to back up the continued therapeutic application of these remedies (Aniagu *et al*., 2005).

Many official guidelines have been developed by various regulatory agencies for toxicity testing. These guidelines relate to the use of botanical products as medicinal preparations and provide standard methods for toxicological studies to assess the safety of medicinal products. Not all tests are necessarily performed for each herbal product and the need for each toxicity test should be evaluated depending on the availability of published literature related to safety, efficacy, established and traditional claims and intended uses (Schilter *et al*., 2003).

Single dose studies are conducted to define the extent of toxicity in the absence of other data. Information on acute toxicity will usually be required for botanical preparations if they contained biologically active principles (Schilter *et al*., 2003). Commenting on the disadvantages of acute toxicity testing quoted by various researchers as a parameter for assessing safety of a substance, Aniagu *et al.* (2005) supports the useful information that could be obtained from such studies in certain occasions. Apart from these, the fixed dose method also provides the minimum lethal dose data of the polyherbal products.

The experimental protocols recommended by different authorities worldwide place a great deal of emphasis on effects of test substance on mortality of treated animals (Rispin *et al*., 2002). As per fixed dose procedure, the selection of appropriate dose levels during acute toxicity testing and final classification of the test material depends on lethality/evident toxicity signs observed during the experiment. In the present study on polyherbal veterinary preparations, neither incidence of mortality nor animals found in a moribund condition was recorded at the treated dose levels throughout the observation period.

From the long-term traditional folk use and clinical applications, it is fairly understood that most herbal plants or products derived from them have an excellent safety record (Bhattacharjee, 1998; Agaie *et al*., 2007; Michael, 2007). Nevertheless, critical investigation of suspected adverse effects forms the important prerequisite in safety appraisal of herbal veterinary formulations in view of product stewardship in regulatory programmes across the world (Michael, 2007). The battery of clinical signs investigated during the acute oral toxicity studies can strengthen the foundation of knowledge on toxicity and safety issues of the substance under study. The findings of the current studies did not reveal any major abnormal behavioural and or clinical signs relevant to the screened products at the employed dose levels. The behavioural, neurological and autonomic parameters recorded in terms of graded scores in the experimental animals immediately after the administration of the herbal formulations and once daily thereafter for 14 days were well within the normal levels.

The negative influence of toxic compounds on body weight of the laboratory animal species is recognized and well documented in published literature (Aniagu *et al*., 2005; Joshi *et al*., 2007; Gorniak *et al*., 2003). The toxic nature of the administered product is generally correlated with its ability to produce a 10% or more decrement in body weight or growth rate of the selected test animals (Schilter *et al*., 2003). From the results of the current study, the overall percent body weight gain in rats treated with the herbal products was found to be normal at the end of 14 days observation period. A temporary reduction in weight gain after the administration of few products can best be explained by the altered physiological processes in test animals for transient period due to exaggerated dosages of tested products.

Postmortem toxicology of treated animals is customarily recommended in the adopted guidelines for acute toxicity testing (Dadarkar *et al*., 2007). The gross pathological finding for each animal is genuinely considered as potential source of information on the target organ/system and the toxic nature of the chosen test substance. Necropsy examination conducted at the termination of 14 day observation study on individual veterinary product was normal and did not show any significant treatment related macroscopic changes of organs or other structures.

In conclusion, acute oral toxicity testing of screened herbal veterinary products did not produce mortality, toxicity signs or any significant pathological changes upto the dose level of 5000 mg kg^{-1} body weight and an overall normal body weight gain was observed in all the treated female rats and hence resulted in tested products being labelled unclassified in the hazard category according to Globally Harmonised System.

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