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PRELIMINARY ACUTE ORAL TOXICITY STUDY OF A NEWLY DEVELOPED HERBAL FORMULATION

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ABSTRACT

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. Acute toxicity studies are conducted to evaluate the effects of a single substances on animal model. The present study was designed to evaluate the acute oral toxicity study of herbal formulation (HF) according to OECD guidelines. In the present study, a single administration of the poly herbal extract at a dose of 2000 mg/kg, respectively, was given to the mice. Mice were observed for general appearance, behavior, body weight, adverse effects, mortality and necropsy up to 14 days post-treatment. In this study we could not find any mortalities during the experimental period. No changes in general appearance and mortality was observed. HF was found to be safe at dose of 2000mg/kg. In the conclusion these results demonstrate that the extract may not have any single dose toxicity.

KEYWORDS: Acute toxicity; Herbal formulation; OECD guidelines; Necropsy.

INTRODUCTION

Toxicology may be defined as the study of harmful /poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings. After gaining relevant information on the harmful effects of a compound, the levels for its safe usage or the degree of its safety is established, this is known as its (compound) Biosafety level.[1] Acute

toxicity testing in animals is typically the initial step in the assessment and evaluation of the health effect characteristics of a test substance, and its primary purpose is to provide information on potential health hazards that may result from a short term exposure.

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesse. It has attracted increasing public attention over the past 20 years as these type of medicines are easily accessible in some regions.[2] Medicinal plants contribute great importance in daily life by providing wide range of nutrients, vitamins and other compounds which widen the therapeutic arsenal. In general, natural products play a dominant role in the development of novel drug which leads for the treatment and prevention of diseases.[3] Medicinal plants behave as authentic medicines because the chemical substances of which they are formed can have a biological activity in humans. Determination of efficacy and safety of herbal remedies is necessary because many people use these agents as self-medication. Since, there is limited data available about the safety of the commonly used herbal remedies, therefore, efforts to elucidate health benefits and risks of herbal medicines should be intensified. It is the need of the hour to evaluate acute and chronic toxicities of herbal drugs.[4]

Herbal formulations available with a wide range of indications like protective to liver, appetite and growth promoters, gastrointestinal and hepatic regulator, as treatment for hepatic dysfunction, for hepatic regeneration as well as liver stimulant and tonic. Despite the widespread use, there is a lack of scientific evidence on their efficacy and safety. In fact, there is lack of evidence on quality, safety and efficacy of many herbal preparations. Although many herbal preparations are non-toxic, many plants currently used for medicines have been shown to be highly toxic when given either acutely or sub-chronically.[5,6] The increasing number of plant based medication users around the globe and lack of experimental reports on their safety make it basic to direct toxicological investigation on natural herbal products.[7,8]

There is now growing evidence that many herbal medicines do cause serious toxicity to their users. Therefore, much more scientific attention is now being given to assess the potential toxicity of herbal medicines than before.

The present study aims to determine the toxicity of newly developed herbal formulation (HF) using an acute oral toxicity test in animal models.[9] The acute oral toxicity testing was

carried out on both sexes of animals under the Organization for Economic Cooperation and Development (OECD) guidelines.[10]

MATERIAL AND METHOD

Experimental animals

Swiss albino mice (30– 40 g) were obtained from the animal house. The room was well ventilated and maintained on light for 12 hours and 12 hour darkness. Temperatures were maintained at 27– 30 °C. The mice were provided with the standard pellets and clean water *ad libitum*. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee's (IAEC) rules and regulation of this institute and the experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Composition of herbal formulation

The composition of each 5ml of Herbal Composition compose of *Azadirachta indica* (Neem) 50mg; *Curcuma longa* (Turmeric) 20mg; *Terminalia chebula* (Chebulic Myrobalan) 20mg; *Aloe Barbadensis Miller*(Aloe Vera) 20mg; *Tinospora cordifolia* (guduchi) 20mg; *Citrus limon* (Lemon) 10mg; *Trigonella foenum-graecum* (Methi) 10mg; *Piper nigrum* (Black pepper) 10mg; *Elettaria cardamomum* (cardamom) 10mg.

Assessment of Acute toxicity test

Acute toxicity study was performed in healthy swiss albino mice (30-40gm) as per guidelines (AOT 425) suggested by the Organization for Economical Co-operation and Development (OECD). The animals were randomly assigned into two groups of 6 mice each and kept 3h fasting prior to extract administration. Group 1 served as the control and the mice were orally administered with 2ml distilled water (Group 2).

Mode of Administration

Single concentrations of the polyherbal extract 2000 mg/kg body weight was constituted in 5ml distilled water through a mice gavage. Animals were fasted 3h prior to dosing (only food was withheld for 3h but not water) and 3 hours further after drug administration.

The mice were observed after every 30 minutes post extract administration for the first 2 hours and latter once a day up to the 14 th for changes in skin and fur, eyes and mucus membranes, behavior pattern, tremors, salivation, diarrhea, sleep, coma, mortality, moribund,

ill health or any visible reaction to treatment. Weight recording was done before combination extract administration, a t 24 hours, 48 hours, day 7 and day 14 using a sensitive balance.

Clinical Observation

The treated animals were observed for mortality (twice daily) and the clinical signs were recorded to note the onset, duration and reversal (if any) of toxic effect at 2, 4, 6 and 8 hours after the administration of last substances and once daily thereafter for 14 days. The routine cage side observation s included changes in skin and fur, eye and mucus membrane, somatomotor activity, general behavior pattern were noted. Miscellaneous sings like arching of the back, alopecia, wound, nasal discharge, lacrimation and loose stool were also recorded during the observation.

Body weight and food intake

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.

RESULTS AND DISCUSSION

Herbal remedies positioned themselves in various forms such as dietary supplements, mono or polyherbal drugs, dietary ingredients, etc., and have become famous and safe commercial commodities. However, the herbal preparations, irrespective of the popular belief that they are safe based on ancient literature, required to be confirmed for their non-toxic/relatively less toxic effects compared to the chemical therapeutic counterparts.[11]

Behavioral Observations and General appearance

In this study the behavioral parameters and appearance of animals after drug administration is indicator of the toxicity of the test drug.[12,13] The behavioral patterns of animals were observed in 2h, 4h, 6h and 8h interval and followed by 14 h after the administration. The behavioral parameters and appearance was observed according to the standard protocol.[14] No significant changes were observed in wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation and sleep pattern parameters of the treated animals were found to be normal (table 2). No toxic symptom or mortality was observed in any mice. All treated mice lived up to 14 days after the administration of herbal formulation (HF).

Body Weights and food intake

An increase in body weight of the animal after test drug administration is indicator of its toxic effect.[15] Figure 1 showed the change observed before and after the administration of the HF. Although, the body weights of all the mice were increased after the oral administration of HF. 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 HF had no toxic effect on animals. Intake of food during the study was normal (table 1).

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 mice (table 3).

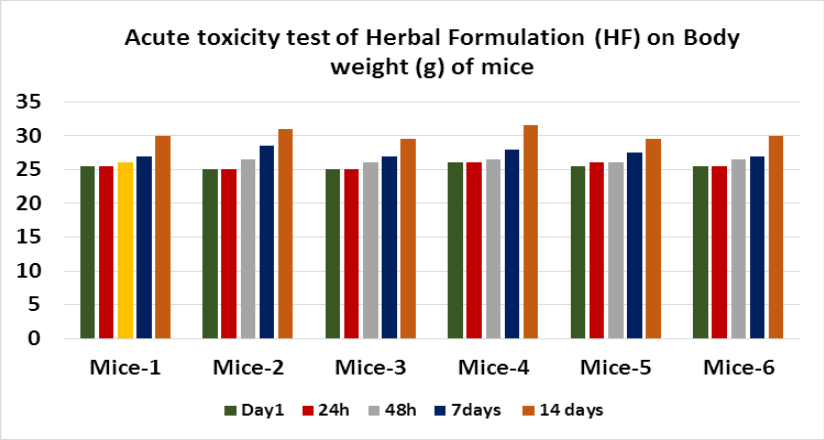

Fig. 1: Effect of Herbal Formulation (HF) on the body weight (g) of mice at 2,000 mg/kg dose.

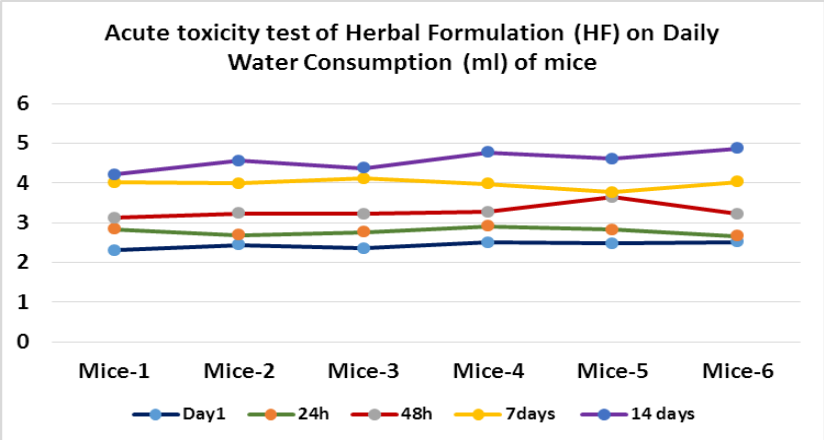

Fig. 1: Effect of Herbal Formulation (HF) on the Water consumption (ml) of mice at 2,000 mg/kg dose.

Table 1: Effect of Herbal Formulation (HF) on the food consumption of mice at 2,000 mg/kg dose.

Swiss Albino Mice	Weight in grams		
	Day 1	Day 7	Day 14
1.	5.13	5.88	5.90
2.	5.22	5.47	6.01
3.	5.28	5.63	5.83
4.	5.28	5.77	6.24
5.	5.02	5.48	6.11
6.	4.96	5.61	6.08

Table 2: Clinical observations of mice at 2,000 mg/kg dose of Herbal Formulation (HF).

Signs and symptoms	Mice 1	Mice 2	Mice 3	Mice 4	Mice 5	Mice 6
Behavior	Normal	Normal	Normal	Normal	Normal	Normal
Somatomotor activity	Normal	Normal	Normal	Normal	Normal	Normal
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes And mucous membranes	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Absent	Absent	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent
Tremors/ convulsions	Absent	Absent	Absent	Absent	Absent	Absent
Death	Nil	Nil	Nil	Nil	Nil	Nil
Other symptoms	Nil	Nil	Nil	Nil	Nil	Nil

Table 3: Effect of Herbal Formulation (HF) on the Necropsy of mice at 2,000 mg/kg dose.

Experimental Animals	Observed lesions during study
1.	Nil
2.	Nil
3.	Nil
4.	Nil
5.	Nil
6.	Nil

SUMMARY AND CONCLUSION

Therefore, it is concluded that the administration of the newly developed herbal formulation is safest & has no adverse effect on animals. All the animals survived by the end of the study; Clinical signs symptoms and gross necropsy did not reveal any major findings. Hence it may be concluded (Category 5 as per OECD guidelines 420, 423 & 425 for acute Toxicity Studies) that the developed HF is practically nontoxic, safe and has no adverse effect.

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