International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 28-05-2020; Accepted: 13-06-2020; Published: 29-06-2020
www.pharmacyjournal.in
Volume 5; Issue 3; 2020; Page No. 26-28

Preliminarey single dose toxicological investigation of livina capsule in mice model

Soumendra Darbar[1*], Shyamaprasad Chattopadhyay[2]
[1, 2] Research and Development Division, Dey's Medical Stores (Mfg.) Ltd, 62, Bondel Road, Kolkata, West Bengal, India

Abstract
Background: Toxicology is the important part of pharmacology which deals with the undesirable effect of phytocompounds on living organisms previous to the use as drug or chemical in clinical use. Acute toxicity studies are conducted to determine the short-term adverse effects of a drug when administered in a single dose, or in two or three times per day. It is used as a part of occupational health environmental impact assessments and batch release testing.
Aims and Objectives: The basic objective of the study is to evaluate the toxic effect of Livina capsule in mice.
Study Methods: The study was conducted according to the guidelines of OECD. Swiss albino mice weighing between 20 and 35 gm were selected for the study.
Results: Livina capsule in single oral dose (2000mg/kg) supplemented to all mice. The parameters like general appearance, behavior, body weight, mortality and necropsy were studied. No changes in general appearance and mortality was observed.
Conclusion: Livina capsule was found to be safe at dose of 2000mg/kg.

Keywords: Acute oral toxicity; Herbal Formulation; Livina capsule; OECD guidelines, Necropsy

Introduction

Safety of medicines and treatments has always been on high priority in the tradition of Ayurveda. Ayurveda has taken pride in claiming itself to be the *śuddha* approach to treatment that pacifies diseases without causing new problems. Ayurveda has looked into toxicity of so many things under the sun that can affect human health adversely. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. The toxic effects of chemicals, food substances, pharmaceuticals, etc., have attained great significance in the 21st century. Toxicity tests are mostly used to examine specific adverse events or specific end points such as cancer, hepatotoxicity, abnormal toxicity and skin/eye irritation [1]. Toxicity testing also helps calculate the No Observed Adverse Effect Level (NOAEL) dose and is helpful for clinical studies. Organisation for Economic Co-operation and Development (OECD) and the International Conference on Harmonization (ICH) brought out the guidelines for toxicity testing of pharmaceutical substances [2].

Most of the world's population relies on traditional medicine for their healthcare needs.[1] However, many of the traditional medicinal plants in use have not been well studied. Therefore, to develop safe natural plant products, preliminary studies are necessary to evaluate possible risks such as undesirable side effects and to determine appropriate dosage levels and regimens to avoid overdosing or poisoning of patients [3, 4].

In the modern era, herbal formulations have gained greater importance than ever before, mainly due to their efficacy and easy availability as well as less side effects as compared to the synthetic drugs [5]. A World Health Organization survey indicated that 70 to 80% of the global population depends on alternative medicine, predominantly herbal in nature, in their primary health care [6]. The uses of medicinal plants as a source of drugs in primary health care have become popular universally, particularly in developing countries as a safe because of natural source [7, 8].

Ayurvedic medicines based on Natural ingredients are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Recently herbal formulations have growing demand in the world market. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to evaluate a polyherbal formulation using a plant having known antidiabetic activity and its therapeutic effects in rodents [9].

Studies have indicated that many medicinal plants used in traditional medicine showed adverse effects also [10, 11]. In order to increase the confidence in their safety to humans, acute and sub-acute toxicity studies are warranted in suitable experimental subjects. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds [12]. Although the herbal formulations are in use for wide variety of clinical applications, the toxicological evaluation of herbal ingredients or combinations is still in infancy. Livina, a polyherbal formulation is very useful as an natural hepatoprotective medicine, which compose of a number of Indian medicinal plants [13, 14]. Our previous work established that Livina protect gastric mucosal damage and maintain mucosal lipid profile [15]. With the above considerations, the present study was aimed to assess the single dose acute oral toxicity of Livina capsule.

Material and Methods

Experimental Study

Animals

Female Swiss albino mice of 6-8 weeks age were used for the acute oral toxicity. All animals were bred and reared at animal house of Dey's Medical Stores (Mfg.) Limited, India. The females used were nulliparous and non-pregnant.

All animals were acclimatized and maintained under standard housing conditions (temperature: 22 ± 3°C, relative humidity: between 40-60% with and 12 h light-12 h dark cycle). All animals were provided with purified water *ad libitum* and standard laboratory diet. The experiments were carried out with the approval of Institutional Animal Ethics Committee (IAEC) and in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Composition of Herbal Formulation

The composition of each capsule of Livina contain the following ingredients: *Solanum nigrum* 20mg, *Holarrhena antidysentrica* 10mg, *Tephrosia purpurea* 40mg, *Andrographis paniculata* 10mg, *Phyllanthus niruri* 20mg, *Tinospora cordifolia* 10mg, *Terminalia chebula* 10mg, *Asteracantha longifolia* 20mg, *Alstonia scholaris* 20mg, *Berberis aristata* 40mg, *Chichorium intybus* 10mg, *Picrorhiza kurroa* 20mg

Fig 1: Livina® Capsule

Acute oral toxicity study

The study was conducted in accordance with OECD guidelines for the testing of chemicals, OECD 420-Fixed dose procedure. Animals were fasted overnight and 3 h after test substance administration but water was provided *ad libitum*. The sighting study was conducted by dosing one animal at 300 mg/kg body weight (p.o.). Since the animal survived, a second animal was treated with 2000 mg/kg. In main study, six female mice were administered at 2000 mg/kg body weight [16]. The treated animals were observed carefully for presence of adverse clinical signs and mortality at 10 min, 30 min, 1, 2, 4 and 6 h after dosing and once daily for 14 days.

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

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.

Result 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 [17].

Behavioral Observations and General appearance

In this study the Table 2 shoed the behavioral parameters and appearance of animals after drug administration is indicator of the toxicity of the test drug [18, 19]. 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. No significant changes were observed in wellness parameters used for evaluation of toxicity [20]. 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 Livina.

Table 1: Clinical observations of mice at 2,000 mg/kg dose of Livina® Capsule

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

Body Weights

An increase in body weight of the animal after test drug administration is indicator of its toxic effect [21]. Table 1 showed the change observed before and after the administration of the Livina. Although, the body weights of all the rats were increased after the oral administration of Livina. But, the changes of the body weights were found to be statistically insignificant. Insignificant increase in body weight (Figure 2) of test animals indicates that the administration of the Livina had no toxic effect on animals

Table 2: Effect of Livina® Capsule on the body weight of mice at 2,000 mg/kg dose

Swiss Albino Mice	Weight in grams		
	Day 1	Day 7	Day 14
1.	25.5	27.0	30.0
2.	25.0	28.5	31.0
3.	25.0	27.0	29.5
4.	26.0	28.0	31.5
5.	25.5	27.5	29.5
6.	25.5	27.0	30.0

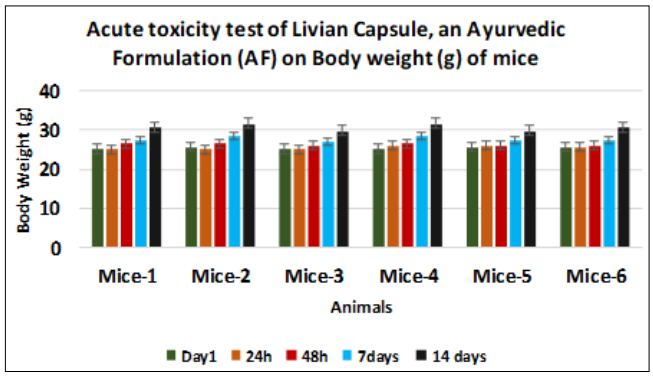

Fig 2: Assessment of Acute toxicity test of Livina® Capsule, an Ayurvedic Formulation (AF) on Body weight (g) of mice

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 (Table 3). No lesions were observed in all rats.

Table 3: Effect of Livina® Capsule on the Necropsy of mice at 2,000 mg/kg dose

Animals	Observed lesions
1.	Nil
2.	Nil
3.	Nil
4.	Nil
5.	Nil
6.	Nil

Summary and Conclusion

Therefore, it is concluded that the administration of Livina a popular marketed poly 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 Livina is practically nontoxic and has no adverse effect.

Conflict of Interest

The Company has no conflict of interest regarding the study.

References

1. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother. 2011: 2(2):74-79.
2. Setzer RW, Kimmel CA. Use of NOAEL, benchmark dose, and other models for human risk assessment of hormonally active substances. Pure Appl Chem. 2003; 75:2151-2158.
3. LiYihang LiGuang SongMeifang LiXuelan–ZhanXia LuJuan ChenXi, *et al.* Acute toxicity study of Aspidopterys obcordata aqueous extract in Sprague-Dawley rats. Journal of Traditional Chinese Medicine. 2016: 36(3):377-381.
4. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo ZG. Medical plants in therapy J Bulletin of the World Health Organization. 1985; 63(6):965-981.
5. Petchi RR, Chockalingam V, Parasuraman S. Antidiabetic activity of polyherbal formulation in streptozotocin nicotinamide induced diabetic Wistar rats. Afr J Tradi t Complementary Altern Med. 2014; 4:108 -17.
6. Mardi M. Algandaby. Assessment of acute and subacute toxic effects of the Saudi folk herb Retama raetam in rats. J Chin Med Assoc. 2015; 78:691-701.
7. Kifayatullah M, Mustafa MS, Sengupta P, Sarker MMR, Das A, Das SK. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of Pericampylus glaucus (Lam.) Merr. in BALB/c mice. J Acute Disease. 2015; 4:309-315.
8. Belhekar Santosh N, Chaudhari Pravin D. Acute and subacute oral toxicity assessment of the polyherbal formulation in albino wister rats. Int J Pharm Pharm Sci. 2011; 8(7):311-316.
9. Sari LM, Suyatna Fd, Utami S, Chairul C, Subita GP, Whulandhary YS, Auerkauri EI, *et al.* Acute oral toxicity study of *areca catechu* linn. Aqueous extract in sprague-dawley rats. Asian J Pharm Clin Res. 2014; 7(5): 20-22.
10. Ernst E. Harmless herbs? A review of the recent literature. Am J Med. 1998; 104:170-178.
11. Talalay P, Talalay P. The importance of using scientific principles in the development of medicinal agents from plants. Academic Medicine. 2001; 76:238-247.
12. ShettyAkhila J, Alwar M. Acute toxicity studies and determination of median lethal dose. Curr Sci. 2007; 93:917-920
13. Darbar S, Ghosh B, Chattopadhyay SP, Ghosh, B. Antioxidant and hepatoprotective activity of Livina, a polyherbal liquid formulation. Asian J Chem. 2009; 21(2):1495-1499.
14. Darbar S, Chakraborty MR, Chattopadhyay SP, Ghosh, B. Protective effect of Livina, a polyherbal liquid formulation against ethanol induced liver damage in rats. Ant sci life. 2009; 28(3):14-17.
15. Darbar S, Chattopadhyay SP, Ghosh B, Chakraborty, MR. Effect of a polyherbal liquid formulation on aceclofenac induced gastric mucosal damage in albino wistar rats. J Pharm Res. 2008; 7(2):62-65.
16. Bisht Asha, Madhav NV Satheesh, Upadhyaya Kumud. Development, Standardization and Acute toxicity testing of antilipidemic polyherbal formulations. Guru Drone Journal of Pharmacy and Research. 2014; 2:5-13.
17. Oreagba IA, Oshikoya KA, Amachree M. Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complement Altern Med. 2011; 11:117.
18. 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.
19. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Sasidharan S, *et al.* Acute Oral Toxicity of Methanolic Seed Extract of Cassia fistula in Mice. Molecules. 2011; 16:5268-82.
20. Gatne MM, Adarsh and Ravikanth K. Acute oral toxicity study of polyherbal formulation AV/KPC/10. International Journal of Biomedical and Advance Research. 2015; 6(03):281-283.
21. OECD/OCDE 423. Acute Oral Toxicity – Acute Toxic Class Method. OECD guideline for testing of chemicals, 1-14.