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Assessment of Acute Oral Toxicity Study of Trasina®, an Ayurvedic Herbal Formulation on Experimental Models

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ABSTRACT

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. It may also provide early information on the mode of toxic action of a substance. The study was aimed to assess the possible toxic effects and to identify the preliminary safety profile of Trasina®, a polyherbal capsule (M/S Dey's Medical Stores Manufacturing Limited, Kolkata, West Bengal, India) after single oral administration in mice according to the 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 swiss albino mice. During the study period the mice were observed for general appearance, behavior, body weight, adverse effects, mortality and necropsy up to 14 days post-treatment. No toxicological changes or mortalities related to the test substance were also observed after the administration in experimental animals. No changes in general appearance and mortality was observed. Trasina was found to be safe at dose of 2000 mg/kg. In the conclusion these results demonstrate that the extract may not have any single dose toxicity.

1. Introduction

Medicinal plants have been used in various folk medications since ancient years and have been shown to be very effective by modern medical science. According to the World Health Organization (WHO), "a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis." Such plants are in great demand by pharmaceutical companies for their active ingredients [1-3]. However, few studies have addressed the toxicity of medicinal plants, so that many questions have been raised regarding their safety and efficacy [4-6]. Despite the widespread use of plants for treatment of several ailments there is a little known about their toxicity and safety. The concept of polyherbal formulation is well documented in the ancient literature. Compared to the single herb, the ayurvedic formulation has better and extended therapeutic potential. To determine the safety of drugs and plant products for human use, toxicological evaluations are conducted in experimental animals in order to predict toxicity and establish guidelines [7, 8].

Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms prior to the use as drug or chemical in clinical use [9, 10]. The toxic effects of chemicals, food substances, pharmaceuticals, etc., have attained great significance in the 21st century [11]. According to OECD guidelines, acute toxicity is defined as the toxicity produced by a pharmaceutical when it is administered in one or more doses during a single period. Results of acute toxicity tests can be used to screen for the toxicity of a pharmaceutical or to determine whether a compound is toxic or not. Therefore, acute toxicity studies in animals are generally necessary before human use.

Ayurvedic medicines based on natural ingredients are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal preparations prepared from medicinal plants can bring about a solution to certain disorders, mostly in developing countries where the commercial drugs are expensive and not easily available to the poor people [12]. A significant majority of the populace still rely on traditional sources of medicine for cure and in most instances as an alternative to allopathic pharmacotherapy. Recently herbal formulations have growing demand in the world market. The concept of polyherbal formulation (PHF) is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential [13].

Trasina is an herbal formulation of some Indian medicinal plants classified in Ayurveda, the classic Indian system of medicine, as Medhyarasayanas or drugs reputed to improve memory and intellect. Trasina is a combination of Shilajit, *Withania somnifera*, *Tinospora cordifolia*, *Eclipta alba*, *Ocimum sanctum* and *Picrorrhiza kurroa*. Bhattacharya et al in 1997 reported that the formulation has a memory-facilitating action. Research stated that after sub chronic administration of Trasina for 21 days on two rodent models had simulate some biochemical features known to be associated with Alzheimer's disease (AD) [14, 15].

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. It is the toxicity produced by a pharmaceutical when it is administered in one or more doses during a certain period. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. The objective of the current experiment was to study the Acute Toxicity of Trasina, an Ayurvedic formulation on murine model.

2. Experimental Methods

2.1 Experimental Animals

Swiss albino wister mice (30-40 g) were obtained from the animal house of the organization Dey's Medical Stores (Mfg.) Limited. 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).

2.2 Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sufficient sawdust in a temperature controlled environment (24 ± 2 °C). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24 h period. Each cage was identified by a card mentioning, cage number, animals weight, test substance code, administration route and

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dose level. The animals were fed with standard laboratory animal food pellets with water *ad libitum*.

2.3 Assignment of Animals

The animals were identified by the markings using a yellow stain. One mouse was unmarked and the others were marked on head, body, tail, head and body, body and tail, to ease the observation.

2.4 Composition of Herbal Formulation

The composition of each capsule of Trasina (Fig. 1) composed of *Withania somnifera* 80 mg; *Ocimum sanctam* 190 mg; *Tinospora cordifolia* 10 mg; *Picrorrhiza kurroa* 10 mg and *Eclipta alba* 10 mg.

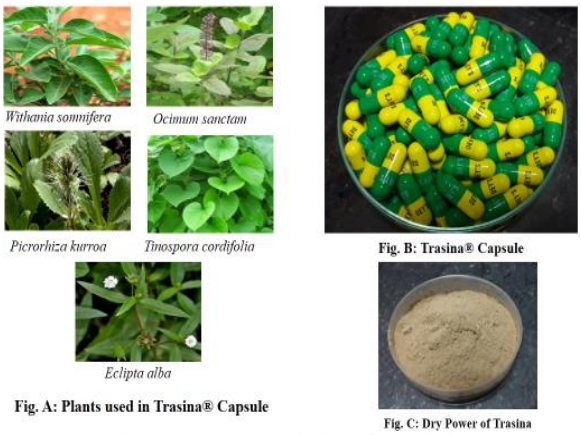

Fig. 1 Composition of Trasina®, an ayurvedic formulation (AF)

2.4 Acute Toxicity Test

Acute toxicity study was performed in healthy swiss albino mice (30-40 g) as per guidelines (AOT 425) suggested by the Organization for Economic Co-operation and Development (OECD). The animals were randomly assigned into two groups of 6 mice each and kept overnight fasting prior to extract administration. Group 1 served as the control and the mice were orally administered with 2 mL distilled water. Single concentrations of the polyherbal extract 2000 mg/kg (Trasina®) body weight was constituted in 2 mL distilled water through a mice gavage. Food was withheld for further 3 hours.

The mice were observed after every 30 minutes post extract administration for the first 2 hours and latter once a day up to the 14th 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 extract administration and at 48 hours, day 7 and day 14 after extract administration using a sensitive balance.

2.6 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 observations included changes in skin and fur, eye and mucus membrane, somato motor 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.

2.7 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.

3. 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].

3.1 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 2 h, 4 h, 6 h and 8 h interval and followed by 14 h after the administration. The behavioral parameters and appearance was observed according to the standard protocol [16]. 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 1) [17, 18]. No toxic symptom or mortality was observed in any mice. All treated mice lived up to 14 days after the administration of Ayurvedic capsule-Trasina.

Table 1 Clinical observations of mice at 2,000 mg/kg dose of Trasina®

Signs and symptoms	Mice 1	Mice 2	Mice 3	Mice 4	Mice 5	Mice 6
Alertness	Normal	Normal	Normal	Normal	Normal	Normal
Touch response	Absent	Absent	Absent	Absent	Absent	Absent
Pain response	Absent	Absent	Absent	Absent	Absent	Absent
Righting reflux	Present	Present	Present	Present	Present	Present
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

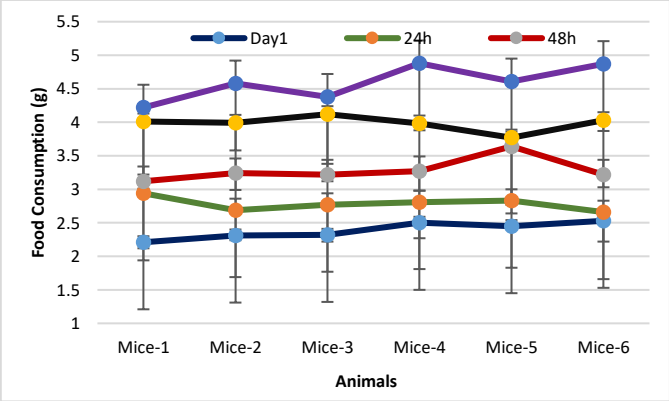

Fig. 2 Acute toxicity test of Trasina®, an ayurvedic formulation (AF) on Daily Food intake (g) of mice

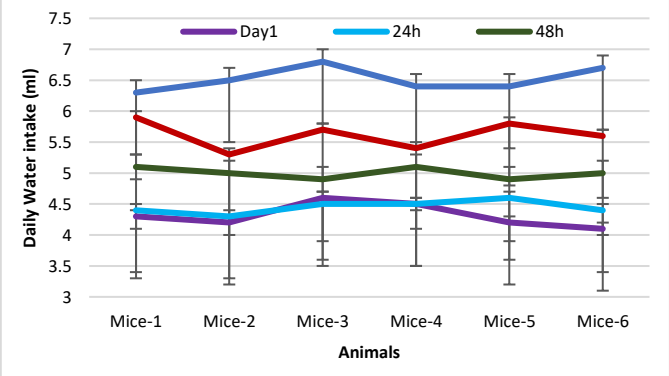

Fig. 3 Acute toxicity test of Trasina®, an ayurvedic formulation (AF) on daily water intake (mL) of mice

3.2 Body Weights

An increase in body weight of the animal after test drug administration is indicator of its toxic effect [15]. Table 2 showed the change observed before and after the administration of the Trasina. Although, the body weights of all the mice were increased after the oral administration of Trasina. But, the changes of the body weights were found to be statistically

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insignificant (Fig. 2). Insignificant increase in body weight of test animals Fig. 4 indicates that the administration of the Trasina had no toxic effect on animals. Daily food and water intake remain unchanged in comparison to control (Figs. 3 and 4).

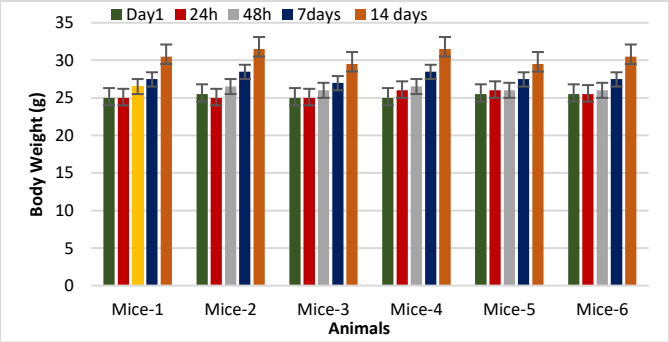

Fig. 4 Acute toxicity test of Trasina®, an Ayurvedic Formulation (AF) on Body weight (g) of mice

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

Swiss Albino	Weight in grams		
Mice	Initial Weight (Day 1)	Final Weight (Day 14)	Weight Gain
1.	25.5	30.5	5.0
2.	25.5	31.5	6.0
3.	25.0	29.5	4.5
4.	25.0	31.5	6.5
5.	25.5	29.5	4.0
6.	25.5	30.5	5.0

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 thoroughly. No lesions were observed in all mice.

4. Conclusion

Therefore, it is concluded that the administration of Trasina a popular marketed poly herbal formulation is safest and 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 Trasina is practically nontoxic and has no adverse side effects on experimental animals.

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