RESEARCH PAPER

OECD 423-Based Acute oral toxicity assessment of Sitramutti Kiyazham-I A Siddha polyherbal formulation

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ABSTRACT

Background: Acute oral toxicity assessment remains a fundamental component of preclinical safety evaluation, particularly for newly developed formulations intended for oral administration. OECD Test Guideline 423 is widely used for such studies because it provides a structured approach for identifying acute toxic effects while minimizing animal use.

Objective: The present study aimed to evaluate the acute oral toxicity profile of Sitramutti Kiyazham-I in Swiss albino mice under controlled laboratory conditions.

Materials and Methods: Female Swiss albino mice were maintained under standard environmental, dietary, and hygienic conditions and administered a single oral dose of the test formulation prepared as a suspension in 1.5% carboxymethylcellulose. Animals were observed for clinical signs, mortality, body weight changes, and gross pathological findings over a 14-day period in accordance with OECD 423.

Results: No mortality occurred in any dose group during the study. Clinical observations did not reveal overt signs of acute toxicity such as lethargy, piloerection, or abnormal respiration. Body weight gain remained within the expected range in all animals, and gross pathological examination revealed only one swollen left kidney in one mouse from the 2000 mg/kg group. The oral LD50 was estimated to be greater than 1000 mg/kg body weight.

Conclusion: SK-I demonstrated a low acute oral toxicity profile in Swiss albino mice under the experimental conditions employed. The findings support further preclinical evaluation of the formulation.

Keywords: acute oral toxicity, OECD 423, Swiss albino mice, Sitramutti Kiyazham-I, safety evaluation, Siddha medicine

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INTRODUCTION

Siddha medicine is among the classical traditional systems of medicine practiced in India, particularly in South India, and is founded on a holistic concept of health [1] [2]. It emphasizes the balance of body, mind, environment, dietary practices, and daily routine rather than focusing only on disease symptoms. In Siddha practice, internal medicines, external therapies, and regulated lifestyle measures are used together to restore physiological equilibrium [3].

Safety assessment is a prerequisite for the development of any therapeutic preparation intended for human use. Acute toxicity studies are generally conducted at an early stage to estimate the degree of harmful effect that may occur after a single exposure or short-term exposure to a substance [4]. These studies are particularly relevant for medicinal products, herbal formulations, and traditional preparations because they provide preliminary evidence regarding systemic tolerance, organ sensitivity, and the approximate lethal dose range.

Among the commonly used experimental models, Swiss albino mice are considered suitable for acute toxicity testing because they are small, reproducible, cost-effective, and responsive to the toxic effects of a broad range of substances [5]. Their use is consistent with the principle of employing the minimum number of animals required to generate meaningful toxicological data. In this context, oral toxicity studies are especially important when the route of administration in clinical use is expected to be oral, since they help align the experimental safety assessment with the anticipated exposure route [6].

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The present study was designed to assess the acute oral toxicity of SK-I in Swiss albino mice using OECD Guideline 423, which is recognized internationally for the acute toxic class method. The protocol involved dose escalation with observation of mortality, clinical condition, body weight trends, and gross pathology. The data generated provide the basis for preliminary safety interpretation and dose selection in future pharmacological or toxicological investigations [7].

SK-I is a test formulation that was evaluated for its immediate toxic potential after oral exposure. Because oral administration is the likely clinical route, the toxicological profile obtained through this study is directly relevant to its possible use [6]. The study therefore has practical value in preclinical development and in identifying whether the formulation can advance to repeated-dose testing [8].

SK-I [9] is a Siddha polyherbal formulation intended for Varmam-related conditions [10-15]. Since the quality and safety of such preparations may vary depending on ingredient purity, processing method, and storage conditions, scientific standardization and toxicity assessment are important. Acute oral toxicity testing provides preliminary safety information and supports further pharmacological and clinical development of traditional formulations.

Composition of the formulation

Table 01 presents the ingredients used in SK-I. The formulation consisted of thirteen raw drugs in equal proportion, comprising roots, rhizomes, root tubers, and resin. The ingredients were selected in purified form before preparation.

Table 01. Composition of SK-I

S. No.	Tamil name	Botanical name	Family	Part used	Ratio
1	Ciṟṟāmuṭṭi	Sida cordifolia	Malvaceae	Root	Equal
2	Iśaṅku / Icaṅku	Clerodendrum inerme	Lamiaceae	Root	Equal
3	Kaṇṭaṅkattiri	Solanum surattense	Solanaceae	Root	Equal
4	Senceṭṭi / Ciṟukāñcoṟi	Tragia involucrata	Euphorbiaceae	Root	Equal
5	Vilvam	Aegle marmelos	Rutaceae	Root	Equal
6	Tāṟā / Naṭṭaisūri	Spermacoce hispida	Rubiaceae	Root	Equal
7	Ciṉṉi	Acalypha fruticosa	Euphorbiaceae	Root	Equal
8	Āṭāthōṭai	Adhatoda vasica	Acanthaceae	Root	Equal
9	Suṇṭai	Solanum torvum	Solanaceae	Root	Equal
10	Arattai	Alpinia galanga	Zingiberaceae	Rhizome	Equal
11	Amukkūram	Withania somnifera	Solanaceae	Root tubers	Equal
12	Atimaturam	Glycyrrhiza glabra	Leguminosae	Root tubers	Equal
13	Mīṟai / Vellaipōḷam	Commiphora myrrha	Burseraceae	Resin	Equal

Preparation of the formulation

Equal quantities of the purified ingredients were coarsely powdered, combined, and boiled with 1.3 L of water. The mixture was simmered until the volume reduced to one-eighth of the original amount, yielding the final decoction. The prepared formulation was stored in sterile, dry glass containers before use.

MATERIALS AND METHODS

Study design

This was a single-dose acute oral toxicity study conducted in female Swiss albino mice. The study design followed OECD Guideline 423, which is based on sequential dosing and careful observation of animals after a single oral exposure. The objective of this design is to determine the approximate toxicity range while using as few animals as possible.

The animals were maintained under standard laboratory conditions with controlled temperature, humidity, and ventilation. They were housed individually in polypropylene cages fitted with stainless steel grid tops and bedded with autoclaved corn cob material. Bedding was replaced at least twice each week. The room was cleaned regularly, and sanitation procedures were maintained before occupancy and throughout the study. [8]

Animals

The study used female Swiss albino mice, 4–5 weeks of age, weighing approximately 30–35 g. Three nulliparous, non-pregnant animals were included in the study. The animals were selected after physical examination to ensure that only healthy mice free from visible abnormalities were enrolled. The source of animals was Mass Biotech. [5]

Female mice were selected for the study in line with the common OECD approach, which frequently uses females because they are often slightly more sensitive to toxic effects. The use of a small number of animals was consistent with the ethical objective of minimizing animal use while still generating interpretable results.

Environmental conditions and husbandry

The animal room was maintained at a temperature of 21–26°C, relative humidity of 60–67%, and a light/dark cycle of about 12 hours with 14 fresh air changes per hour. Animals received certified laboratory rodent diet ad libitum. Drinking water was supplied ad libitum through sipper tubes as freshly prepared potable RO water filtered through an Aquaguard system.

Feed and water quality were monitored according to routine laboratory procedures. The rodent diet was

analyzed by the supplier for nutritional composition and environmental contaminants, and incoming batches were also screened for microbial load. Water was subjected to annual analysis for microbiological parameters, heavy metals, organophosphates, and chlorinated hydrocarbons. Bedding material was also subjected to periodic contaminant evaluation. No contaminants expected to interfere with the study were identified in the bedding, feed, or water.

Ethical approval and animal welfare

All animals were handled according to accepted laboratory animal welfare standards and CPCSEA requirements. The study protocol number was MB/IAEC/2024/02/17. Care was taken to minimize distress and to ensure that animals were housed, fed, and observed under uniform conditions throughout the study.

Test substance preparation

The required quantity of SK-I was received for testing. A fresh suspension was prepared using 1.5% carboxymethylcellulose as the vehicle. The suspension was vortexed until a uniform mixture was obtained. The oral dose volume was maintained at 10 mL/kg body weight for all treated animals.

Dose administration

The test substance was administered once by oral gavage on day 0 using a stainless-steel blunt-tipped cannula (18G) attached to a graduated borosilicate glass syringe. The oral route was chosen because it matches the expected clinical route of administration and is appropriate for evaluating oral toxicological behavior. The study included dose levels of 5, 50, 300, and 2000 mg/kg body weight.

The sequential nature of OECD 423 allows higher dose selection based on the survival of earlier animals. In this study, the starting dose was selected as 5 mg/kg because no prior toxicity information on the test substance was available. Subsequent animals were dosed at higher levels according to survival outcomes.

Observations

Animals were monitored at least twice daily for mortality and morbidity. On the day of dosing, clinical observations were performed before dosing and at 30 minutes, 1, 2, 3, and 6 hours after administration. Thereafter, animals were observed once daily for 14 days. Body weights were recorded prior to dosing and on day 7 and day 14.

The primary clinical parameters included posture, locomotor activity, lethargy, piloerection, respiration, and general appearance. Feed intake was also recorded on day 0, day 7, and day 14. At termination, all animals were euthanized using carbon dioxide asphyxiation and subjected to gross pathological examination. The examination included the external body surface, all orifices, thoracic cavity, abdominal cavity, and internal contents.

Statistical and interpretive approach

Because this was an acute toxicity screening study, the analysis was primarily descriptive. The main endpoints were survival, visible signs of toxicity, body weight evolution, and gross pathology. Based on the pattern of observed responses, the median lethal dose was estimated to exceed 1000 mg/kg body weight.

Results

Mortality and survival

No deaths were observed in any of the animals treated with SK-Iat doses of 5, 50, 300, or 2000 mg/kg body weight. All animals survived throughout the 14-day observation period. There was no evidence of delayed mortality.

Clinical signs

No obvious signs of intoxication were observed in the treated animals. Specifically, the animals did not exhibit lethargy, piloerection, abdominal breathing, or other overt clinical signs suggestive of acute toxicity. The animals remained free from visible treatment-related abnormalities during the period of observation as described on Table 02.

Table 02. Summary of clinical observations and mortality

Observation	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Normal	3/3	3/3	3/3	3/3
Lethargy	0/3	0/3	0/3	0/3
Abdominal breathing	0/3	0/3	0/3	0/3
Piloerection	0/3	0/3	0/3	0/3
Mortality	0/3	0/3	0/3	0/3

Body weight

Table 03 shows that the treated mice showed normal body weight gain after dosing. Body weights recorded on day 7 and day 14 demonstrated continued growth and no meaningful weight suppression attributable to the test substance. These findings support the absence of significant acute systemic toxicity.

Table 03. Individual body weights recorded during the study

Mouse No.	Dose (mg/kg)	Sex	Day 0	Day 7	Day 14
1	5	Female	31	32	31
2	5	Female	35	32	33
3	5	Female	30	32	30
4	50	Female	34	32	35
5	50	Female	37	37	36

6	50	Female	34	36	37
7	300	Female	31	32	32
8	300	Female	31	33	33
9	300	Female	36	38	38
10	2000	Female	32	32	32
11	2000	Female	36	38	35
12	2000	Female	34	38	35

Gross pathology

At termination, gross pathological examination identified one animal in the 2000 mg/kg group with a swollen left kidney. No additional macroscopic abnormalities were observed in the external surface, thoracic cavity, abdominal cavity, or the remaining organs. No gross lesions were found in the 5, 50, or 300 mg/kg groups, as on Table 04, Figure 01 - 04.

Table 04. Gross pathological findings

Mouse No.	Dose (mg/kg)	External findings	Internal findings
1	5	No abnormality	No abnormality
2	50	No abnormality	No abnormality
3	300	No abnormality	No abnormality
4	2000	No abnormality	Swollen left kidney

Figure 1. Gross pathology of control/low-dose animal showing no visible abnormality.

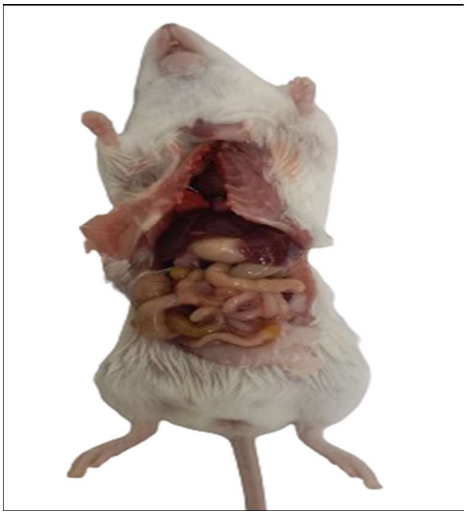

Figure 2. Gross pathology of 50 mg/kg group showing no visible abnormality.

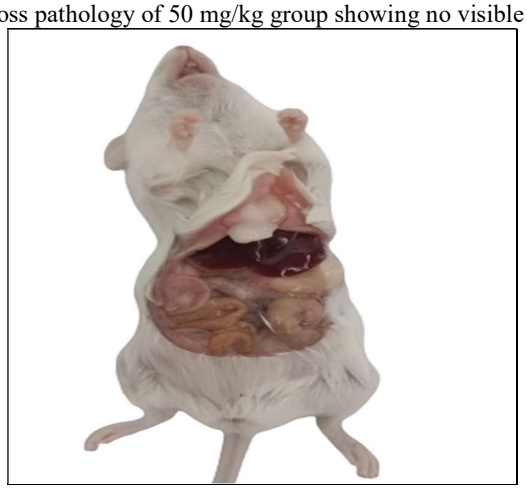

Figure 3. Gross pathology of 300 mg/kg group showing no visible abnormality**.**

Figure 4. Gross pathology of 2000 mg/kg group showing swollen left kidney.

Table 05. Feed intake/consumption recorded in the study file

Dose group	Day 0	Day 7	Day 14
5 mg/kg	32.64	32.00	31.33
50 mg/kg	35.17	32.64	36.00
300 mg/kg	32.66	34.33	34.33
2000 mg/kg	34.00	36.00	34.00

DISCUSSION

The principal aim of an acute oral toxicity study is to identify evidence of immediate toxic effects following a single oral exposure and to estimate the dose range associated with adverse outcomes. In the present study, SK-I was administered orally to Swiss albino mice and monitored carefully for 14 days [6]. The absence of mortality at all tested dose levels suggests that the formulation has a favorable acute safety profile in this animal model.

Clinical observation is one of the most informative parts of acute toxicity testing because overt toxicity often appears rapidly after administration. In this study, no signs of intoxication such as lethargy, piloerection, or abnormal breathing were recorded [16]. This suggests that the test substance did not produce obvious central nervous system depression, respiratory distress, or acute distress symptoms at the doses evaluated.

Body weight is another important indicator in short-term toxicology because severe toxicity commonly results in impaired feed utilization, decreased appetite, or reduced growth. The animals in this study showed normal weight gain during the follow-up period [17]. This outcome indicates that the formulation did not interfere with general metabolism or feeding behavior to a degree that produced measurable growth suppression.

The gross pathological finding of a swollen left kidney in one mouse from the 2000 mg/kg group is noteworthy but should be interpreted in the context of the complete dataset. A single isolated macroscopic lesion without corresponding mortality, clinical toxicity, or parallel abnormalities in other treated animals does not establish a reproducible toxic effect [17].

. Nevertheless, the kidney finding may warrant attention in future repeated-dose studies, where histopathology and

organ weight assessment may help determine whether the observation is incidental or treatment related.

The estimated LD50 of greater than 1000 mg/kg body weight is consistent with low acute toxicity under the conditions of this study. In acute oral toxicity screening, substances with no mortality at the upper test limit generally fall into a low-hazard category, although definitive classification depends on the regulatory framework used [18]. The current results therefore support the continuation of preclinical development with more extensive toxicological evaluation.

The use of Swiss albino mice as the test system is appropriate because this species is commonly employed in early safety testing and provides reliable indicators of systemic toxicity. The study design also followed good laboratory and ethical principles, including controlled housing, standardized feeding, routine sanitation, and humane termination [19-23]. These elements strengthen confidence in the reliability and reproducibility of the findings.

This study also has practical value for dose selection. When a substance produces no acute toxicity up to a high oral dose, investigators can plan repeated-dose studies and efficacy studies with greater confidence and improved safety margins. Conversely, the isolated kidney finding suggests that organ-specific monitoring would be prudent in later studies.

CONCLUSION

SK-I showed no mortality, no major clinical toxicity, and no significant body weight suppression after single oral administration in Swiss albino mice. Gross pathology showed one swollen kidney in a single animal from the highest dose group, but no consistent treatment-related lesion pattern was detected. The oral LD50 was estimated to be greater than 1000 mg/kg body weight, indicating low acute oral toxicity. Overall, the formulation appears suitable for further preclinical toxicological evaluation

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