

Acute and repeated dose oral toxicity study of Ayurvedic coded formulation Ayush SL

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The present investigation was conducted to evaluate the safety profile Ayush SL, a polyherbal Ayurvedic formulation indicated for the management of lymphatic filariasis (LF), through acute, 28-day, and 90-day toxicity studies. In the acute oral toxicity study, the test drug was administered at a single dose of 3250 mg/kg to Swiss albino mice. For the 28-day and 90-day repeated-dose studies, rats were administered Ayush SL at doses of 225, 1125, and 2250 mg/kg. No abnormal behavioural signs and mortality were observed in the mice that received 3250 mg/kg of the test drug during the acute toxicity study. Similarly, no pre-terminal death and no significant changes in hematological and biochemical parameters were observed in rats that received three doses for 28 & 90 days during sub-acute and sub-chronic toxicity studies. Histopathological examination of major organs also did not show any treatment-related pathological changes. These studies revealed that Ayush SL was safe at a dose of 3250 mg/kg in mice upon single-dose administration. In repeated dose studies, the drug was found to be safe up to 2250 mg/kg and no adverse effects were observed.

Keywords: Ayush SL, Acute toxicity, Ayurveda, NOAEL, Sub-acute toxicity study, Sub-chronic toxicity study

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Lymphatic filariasis (LF) is a neglected tropical disease caused by parasitic nematodes, primarily *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, transmitted to humans through mosquito vectors. Globally, LF affects over 657 million people across 39 countries, with India bearing nearly 40% of the global burden and an estimated 350-400 million individuals at risk, particularly in endemic states such as Bihar, Jharkhand, Telangana, Kerala, Uttar Pradesh, Odisha, West Bengal, Andhra Pradesh, Tamil Nadu and Assam. Despite substantial progress achieved through mass drug administration (MDA) programs, LF continues to pose a major public health challenge, especially in rural and socioeconomically vulnerable populations.

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Abbreviations: CCRAS (Central Council for Research in Ayurvedic Sciences), NOAEL (No Observed Adverse Effect Level), therapeutic dose (TD), OECD (Organization for Economic Cooperation and Development), SEM (Standard Error of Mean), SGOT (Serum Glutamic-Oxaloacetic Transaminase Test), SGPT (Serum Glutamic Pyruvic Transaminase).

Clinically, LF manifests as chronic lymphoedema, elephantiasis, and hydrocele, leading to permanent disability, disfigurement, psychological distress, social stigma, and significant economic loss. Globally, more than 36 million individuals live with chronic LF-related morbidity, underscoring the urgent need for effective therapeutic and management strategies. Current elimination efforts rely on annual MDA using albendazole, ivermectin, and diethylcarbamazine (DEC), supported by morbidity management and vector control interventions. However, challenges such as incomplete coverage, treatment non-compliance, persistent transmission hotspots, limited access to morbidity care, and social stigma continue to impede elimination goals, highlighting the necessity for improved surveillance, healthcare delivery, and community engagement to achieve the World Health Organization's (WHO) 2030 elimination targets¹.

In classical Ayurvedic literature, LF is described under conditions analogous to *Shlipada*, and comprehensive management strategies encompassing

both internal and external therapeutic modalities have been documented. *Charaka Samhita* recommends Siravedhana (therapeutic venesection), while *Sushruta Samhita* advocates Raktamokshana (bloodletting) with site-specific approaches based on disease predominance. *Bhavaprakasha* outlines therapeutic principles including Langhana (fasting), Swedana (fomentation), Virechana (purgation), Raktamokshana, Kapha-hara lepa (topical applications), and Ushna upacharas (heat therapies). *Sharangadhara Samhita* further recommends Shakhotaka valka kwatha (decoction of *Streblus asper*) with gomutra for alleviating *Shlipada* symptoms.

Classical Ayurvedic formulations used in the management of LF include Kashayas, Curnas, Tailams, Ghritas, and compound formulations, such as *Noonganadhi Kashayam*, *Guggulu Tiktaka Kashayam*, *Bharngyadi Kashayam*, *Shakhotaka Kashayam* (with gomutra), *Rajani Curna*, *Vridha Daruka Curna*, *Pippalyadi Curna*, *Sudarshana Curna*, *Noongandhai Eranda Tailam*, *Panchanan Tailam*, *Vidangadi Tailam*, *Panchanan Ghrita*, *Vridha Daruka Ghrita*, *Saureswara Ghrita*, and compound preparations including *Nityanandha Rasa*, *Sleepadari Loha*, *Krishna Moodakam*, *Shakhotaka Vati*, and *Guda Haridra*².

Ayush SL is a proprietary Ayurvedic formulation developed by the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of Ayush, Government of India, for the management of Lymphatic filariasis (LF) or *Shlipada*³. Ayush-SL is a formulation developed from medicinal plants traditionally utilized in the management of *Shlipada*, a condition analogous to lymphatic filariasis, as documented in classical Ayurvedic texts⁴.

It is imperative to systematically evaluate and scientifically document the safety and toxicity profiles of newly developed Ayurvedic-coded formulations to enhance their visibility and acceptance on a global platform. In this context, several preclinical toxicity studies have been undertaken to establish their safety. For instance, the safety of coded formulation Ayush-64, intended for use in type 2 diabetes, has been evaluated through a 90-day repeated-dose oral toxicity study, and the test drug was found to be safe up to 1500 mg/kg in rats⁵. Similarly, Liberin, an anti-diabetic polyherbal formulation, demonstrated safety up to 1000 mg/kg in rats upon 90 days of consecutive oral administration⁶. Moreover, the coded formulation AYUSH Carctol-S, developed for the treatment of cancer, was reported safe up to 1800 mg/kg in rats in a 90-day repeated-dose oral toxicity study⁷.

The safety and efficacy of Ayush SL for filarial lymphedema in different endemic areas of India, evaluated in a double-blind, placebo-controlled, multicentre clinical study, revealed that the drug is safe and effective for LF. During this study, Ayush SL was given as an adjunct to MDA and the therapeutic effect of this combination was due to its comprehensive anti-inflammatory, antimicrobial, and anti-allergic activities⁸. The current investigation was designed to evaluate the safety profile of Ayush SL by conducting acute, 28-day and, 90-day repeated dose toxicity studies.

Materials and Methods

Procurement and storage of test drug

Ayush SL was supplied by CCRAS, New Delhi, in powdered form and stored at room temperature.

Chemicals and Kits

Clinical chemistry kits were sourced from M/s Erba Mannheim, Germany, while hematological reagents were procured from Sysmex Corporation, Japan. Additionally, all other standard chemicals and reagents used were of analytical grade. Hematological and biochemical analyses of the samples were conducted at the Institute's Biochemistry and Pathology divisions using the Sysmex Hematology analyzer and Auto analyzer (RA-50, Bayer), respectively.

Animals

The animals were procured from the Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur, Kerala (Registration No. 28/PO/C/01/CPCSEA). All experimental procedures were carried out in accordance with established guidelines for the care and use of laboratory animals. Prior approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC) under Approval Nos. IAEC/CRIA/2004-05/2 and IAEC/CRIA/2006-07/1.

Dose selection

The equivalent doses for Mice and Rats were determined based on the human dose using the body surface area calculation method⁹.

Acute oral toxicity study

The study was performed as per the CCRAS protocol based on Organization for Economic Cooperation and Development (OECD) guideline 423¹⁰ and Schedule Y of the Drugs and Cosmetics

Act, 1940. A total of 40 Swiss albino mice (20 Males and 20 Females) were randomized and equally divided into 4 groups. Mice having body weights of 20-25 g were selected for the acute study. The animals in the vehicle control group received distilled water. The animals of the other group (groups II, III and IV) received the drug (Ayush SL) in TD (325 mg/kg), 5 times of TD (1625 mg/kg), and 10 times of TD (3250 mg/kg), respectively, once on the first day of the experiment. The animals were observed for morbidity, mortality and clinical signs of toxicity during the next 10 days. Body weights were measured before and after the experiment, and feed intake was recorded over 10 days.

Sub-acute oral toxicity study

The experiment was performed as per CCRAS based on OECD Guideline 408 and the provisions of Schedule Y of the Drugs and Cosmetics Act, 1940¹¹. 48 Wistar rats (24 Males + 24 Females) were randomized and equally divided into 4 groups. Male and Female rats weighing 120-150 g between 4 – 6 weeks of age were selected for the sub-acute study. Animals receiving the treatment were housed in cages with appropriate identification. The vehicle control group (Group I) animals received distilled water depending on their body weights. The animals of the other group (groups II, III and IV) received the test drug (Ayush SL) in TD (225 mg/kg), 5 times of TD (1125 mg/kg), and 10 times of TD (2250 mg/kg), respectively, once daily for 28 days. The animals were observed for morbidity, mortality and clinical signs of toxicity during this period. Clinical chemistry and hematological investigations have been carried out. This is followed by euthanization, organ necropsy, and histopathological examination of various organs, including the liver, spleen, lungs, kidneys, testis/ovary, stomach, and intestine.

Sub-chronic oral toxicity study

The experiment was performed as per CCRAS based on OECD Guideline 408 and the provisions of Schedule Y of the Drugs and Cosmetics Act, 1940¹². A total of 40 Wistar rats (20 males and 20 females) were randomly allocated into four equal groups. Healthy male and female rats weighing 110-150 g and aged 5-6 weeks were selected for the 90-day sub-chronic toxicity assessment. Treated animals were housed in appropriately labeled cages to ensure proper identification. The control group (Group I) received distilled water in volumes adjusted according to body

weight. Animals in groups II, III, and IV were administered Ayush SL orally at doses equivalent to the therapeutic dose (225 mg/kg), five times the therapeutic dose (1125 mg/kg), and ten times the therapeutic dose (2250 mg/kg), respectively, once daily for a period of 90 consecutive days. Throughout the study, animals were monitored for signs of morbidity, mortality, and any clinical indications of toxicity. At the end of the treatment period, blood samples were collected for clinical biochemical and hematological analyses. Subsequently, animals were euthanized for gross necropsy, and major organs-including the liver, spleen, heart, lungs, kidneys, reproductive organs (testis/ovary), stomach, and intestine-were harvested for detailed histopathological evaluation.

Statistical analysis

The data generated during the experiment were analysed using GraphPad Prism Software. All values are presented as the Mean \pm SEM. The weekly body weight data from the acute toxicity study were analyzed using a Student's t-test. For the 90-day repeated-dose oral toxicity study, data analysis was performed using one-way ANOVA followed by Dunnett's test. A significance level of $p < 0.05$ was deemed statistically significant.

Results

Acute toxicity study

Clinical signs and mortality

Cage-side monitoring did not reveal any abnormal behavioral changes in mice during the observation period. No mortality was observed during the study period. These findings were comparable to the vehicle control group upon single-dose administration of test drug Ayush SL.

Sub-acute toxicity study

Pre-terminal deaths were not recorded in animals receiving the test drug in various doses, *viz.*, therapeutic, average, and high doses. The results of the following parameters in animals belonging to all groups are provided.

Effect on food and water intake

No significant changes were observed with respect to the food intake between test groups and vehicle control. The water intake was found to be adequate. All the animals in all the groups were active before and after the exposure to the test drug.

Effect on faeces colour and consistency

Faeces showed semisolid consistency in the 1st week of the study, but was normalized later.

Physical and neurological observations

During the 28-day toxicity evaluation, animals were monitored for general physical signs, condition of the fur, presence of piloerection, lacrimation, salivation, respiration pattern, eye prominence, and eyelid status. Neurological assessment, such as convulsions, biting, tremors, locomotor activity, rearing activity, tail posture, limb position, gait pattern, head position, and pinna touch response, was also recorded. All observed parameters in the sub-acute toxicity study were found normal.

Effect on body weights during 28-day toxicity study

During the 28-day toxicity study, the rats' weekly body weights remained consistent throughout the dosing period. No significant differences were observed when compared to the vehicle control group (Table 1).

Effect on biochemical and haematological parameters during 28-day toxicity study

In the 28-day toxicity study, no significant differences were observed in biochemical and haematological parameters across all treated groups (Table 2 & 3).

Effect on gross morphological changes during necropsy

No gross morphological changes were observed in the vital organs of animals that received the test drug and vehicle during the 28-day toxicity study.

Table 1 — Body weight gain in grams during the 28-day toxicity study

	I Week	II Week	III Week	IV Week
Vehicle Control	8.75±2.14	12.08±1.56	13.33±2.70	14.16±1.82
Therapeutic Dose	8.75±2.14	12.91±3.04	14.58±1.78	15.41±1.78
Average Dose	9.16±1.93	12.08±2.34	11.25±2.69	12.50±2.26
High Dose	12.08±1.99	13.75±1.52	10.83±2.02	11.66±1.42

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Table 2 — Biochemical parameters during the 28-day toxicity study

	Glucose (mg/dL)	Creatinine (mg/dL)	SGOT (U/L)	SGPT (U/L)
Vehicle control	76.5±8.852	0.75±0.005	213.5±41.13	78.25±5.15
Therapeutic dose	68.75±9.63	0.77±0.0047	183.75±26.22	73.75±17.7
Average dose (TD X 5)	67.00±7.92	0.75±0.0028	138.25±5.31	64.50±3.01
High dose (TD X 10)	70.50±10.55	0.80±0.004	174.25±16.48	71.00±5.24

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Effect on weights of the organ during 28-day toxicity study

Ayush SL administration for 28 days did not show any significant change in mean organ weights of rats compared to the vehicle control group (Table 4).

Sub-chronic toxicity study

No deaths were observed in animals receiving the test drug in various dose levels, viz., therapeutic dose, average dose and high dose. The results of various parameters assessed in all groups are summarized below.

Effect on food and water intake

No significant changes were observed with respect to the food intake between the test groups and the vehicle control. The water intake was found to be adequate. All animals in all groups were active before and after exposure to the test drug.

Effect on faeces colour and consistency

Faeces showed semisolid consistency between the 2nd and 3rd weeks of the study, but were normalized later.

Physical and neurological observations

Observations for physical parameters such as hair coat condition, piloerection, lacrimation, salivation, respiration rate, eye prominence and eyelid closure and neurological observations like convulsions, biting, tremors, locomotor activity, rearing activity, tail elevation, static limb position, gait, head position, and pinna touch response made during the sub-Chronic toxicity study were found to be normal.

Effect on body weights during 90-day toxicity study

During the 90-day toxicity study, no significant change in weekly body weight was observed in rats treated with the test drug compared with vehicle-treated controls (Table 5).

Table 3 — Haematological parameters during 28-day toxicity study

	Vehicle control	Therapeutic dose	Average dose	High dose
Haemoglobin (Hb) (g/dL)	11.4±0.24	11.8±0.44	10.9±0.28	10.9±0.51
Packed Cell volume (%)	37.2±4.47	37.2±4.51	37.2±3.32	34.2±1.43
RBC count (10 ⁶ /cu.mm)	4.42±0.17	4.1±0.23	4.3±0.18	3.7±0.31
Total Leucocyte count (10 ³ /cu.mm)	4.2±0.01	3.7±0.26	3.97±0.27	4.22±0.14
Polymorph count (%)	23.7±3.22	22.5±1.19	26.0±1.68	27.0±4.94
Lymphocyte count (%)	76.2±0.322	77.5±1.19	74.0±1.68	73.0±4.94
Prothrombin time (Sec.)	19.87±3.89	19.12±1.37	15.0±3.27	13.37±1.14

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Table 4 — Organ weight (% Body weight) during 28-day toxicity study

	Liver		Spleen		Kidney		Heart		Testes	Ovary
	Male	Female	Male	Female	Male	Female	Male	Female		
Vehicle control	3.86 ± 0.11	3.85 ± 0.24	0.30 ± 0.0008	0.32 ± 0.0024	0.9 ± 0.0083	0.85 ± 0.0041	0.39 ± 0.0033	0.44 ± 0.0032	1.37 ± 0.0023	0.098 ± 0.007
Therapeutic dose	4.59 ± 0.16	4.05 ± 0.34	0.39 ± 0.0025	0.51 ± 0.11	1.07 ± 0.0024	0.91 ± 0.0091	0.43 ± 0.0015	0.43 ± 0.0031	1.55 ± 0.008	0.13 ± 0.015
Average dose (TD X 5)	3.91 ± 0.11	4.21 ± 0.22	0.45 ± 0.0059	0.28 ± 0.0015	1.32 ± 0.0077	0.88 ± 0.0042	0.45 ± 0.0026	0.45 ± 0.001	1.39 ± 0.003	0.075 ± 0.004
High dose (TD X 10)	4.43 ± 0.15	4.15 ± 0.17	0.36 ± 0.0006	0.27 ± 0.0021	1.22 ± 0.0065	0.92 ± 0.0023	0.47 ± 0.003	0.52 ± 0.0099	1.37 ± 0.006	0.1 ± 0.006

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Table 5 — Body weight gain in grams during 90-day toxicity study

Groups	Vehicle Control	Therapeutic Dose	Average Dose	High Dose
I Week	2±1.33	5.5±1.57	6.5±1.30	5.5±1.57
II Week	9.5±2.16	12.5±2.5	12.5±2.38	12.5±2.81
III Week	17±3.09	23.5±3.65	20±3.65	21±4.2
IV Week	26.5±4.01	36±4.98	30±5.42	31±5.85
V Week	34±5.71	48±5.97	42±6.92	43.5±7.81
VI Week	45±7.71	57.5±7.08	51.0±8.87	55±9.06
VII Week	54.5±9.47	65.5±8.11	61±10	65±10.61
VIII Week	64±10.53	72.5±8.47	69±10.89	72±11.47
IX Week	73.5±11.62	79.5±9.38	76.5±12.24	81.5±12.29
X Week	81.5±12.29	85±9.91	83±12.93	90±13.14
XI Week	89±13.34	91.5±10.77	88.5±13.66	96±13.65
XII Week	95±14.08	94±10.77	92±13.8	98.5±14.02

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Urine examination (Qualitative) during 90-day toxicity study

No abnormalities in the samples of urine tested qualitatively for the presence of leukocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, glucose, etc., before and after exposure to the test drug at all dose levels as compared to the vehicle control group.

Effect on hematological and biochemical parameters during 90-day toxicity study

No significant differences were observed between the treated groups and the vehicle control group for hematological and biochemical parameters, and the values were within physiological limits (Table 6 & 7).

Effect on gross morphological changes during necropsy

No gross morphological changes were observed in the vital organs of animals that received the test drug and vehicle during the 90-day toxicity study.

Table 6 — Biochemical parameters during 90-day toxicity study

Groups	Vehicle Control	Therapeutic Dose	Average Dose	High Dose
Blood Glucose (mg/dL)	75.6±8.4	80.6±6.01	87±4.83	103.9±8.38
Serum Creatinine (mg/dL)	0.8±0.04	0.79±0.04	0.81±0.02	0.82±0.03
SGOT (U/L)	138.3±6.34	167.6±8.41	154±6.37	149.1±11.27
SGPT (U/L)	37.7±2.17	44.6±2.76	50.5±3.92	45.9±2.95

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Table 7 — Haematological parameters during 90-day toxicity study

Groups	Vehicle control	Therapeutic dose	Average dose	High dose
Haemoglobin (g/dL)	12.83±0.44	12.04±0.43	12.78±0.34	12.8±0.41
Packed Cell volume (%)	42.4±1.69	40.8±1.49	44.5±1.41	44.1±1.65
RBC count (10 ⁶ /cu.mm)	3.52±0.09	3.58±0.07	3.81±0.13	3.84±0.22
Total Leucocyte count (10 ³ /cu.mm)	3.94±0.34	4.26±0.28	4.23±0.22	4.19±0.36
Polymorph count (%)	37.9±1.31	28.3±0.87	30.3±1.37	23.7±1.45
Lymphocyte count (%)	72.1±1.31	72.7±0.84	66.7±3.83	76.3±1.45
Prothrombin time (PT) (Sec.)	12.6±0.5	11.5±0.84	10.4±0.3	10.2±0.44

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Effect on organ weights and histopathology of organs during 90-day toxicity study

In the sub-chronic toxicity study, Ayush SL administration for 90 days did not show a significant change in mean organ weights of rats compared to the vehicle control group (Table 8). No significant histopathological changes were observed between the vehicle control and the test groups (Fig. 1 & 2)

Discussion

Ayush SL is a polyherbal formulation developed for Lymphatic filariasis (LF) or Shlipada. This study aimed to assess the safety of Ayush SL through acute, 28-day and 90-day toxicity studies.

Table 8 — Organ weight (% body weight) during 90-day toxicity study

Groups	Vehicle Control	Therapeutic Dose	Average Dose	High Dose
Liver	2.69±0.13	2.96±0.09	2.75±0.12	2.85±0.11
Spleen	0.28±0.01	0.30±0.01	0.32±0.03	0.33±0.008
Heart	0.31±0.01	0.32±0.01	0.32±0.01	0.33±0.008
Lung	0.56±0.02	0.61±0.02	0.53±0.03	0.62±0.02
Kidney	0.69±0.02	0.72±0.01	0.71±0.02	0.72±0.03
Testes	1.17±0.05	1.09±0.03	1.05±0.05	1.11±0.07
Ovary	0.04±0.002	0.05±0.004	0.05±0.005	0.5±0.006

Values are given as Mean± SEM and p-value< 0.05 was considered significant

Administration of Ayush SL at a single dose of 3250 mg/kg in the acute oral toxicity study did not produce any signs of toxicity, mortality, or moribund condition during the observation period. Clinical signs were normal, and gross necropsy did not reveal any treatment-related morphological alterations in comparison with the vehicle-treated control group. This indicates that the maximum tolerated dose of Ayush SL was 3250 mg/kg.

Repeated-dose toxicity studies (28-day and 90-day) are designed to generate information on dose regimens and target organ toxicity, and to identify possible adverse effects that could affect the average lifespan of experimental animals¹³. In the present investigation, daily oral administration of Ayush SL for 28 or 90 days did not result in any mortality or moribund stage. No

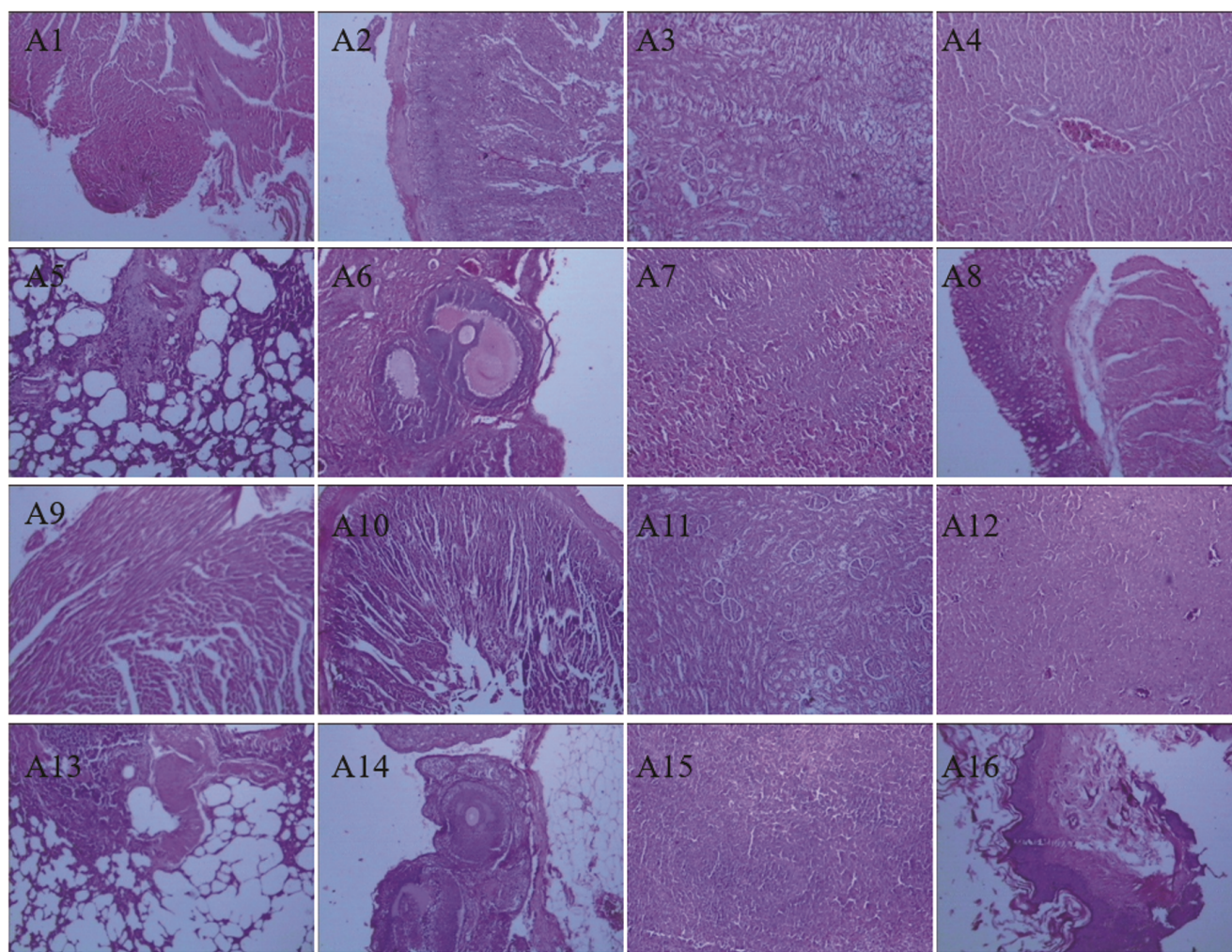


Fig. 1 — Photomicrograph of histopathology of organs/tissues of vehicle control female (A1-A8) and Ayush SL-treated female (High dose) (A9-A16) rats in sub-chronic toxicity study. No treatment-related pathological changes were found in Heart (A1, A9), Intestine (A2, A10), Kidney (A3, A11), Liver (A4, A12), Lung (A5, A13), Ovary (A6, A14), Spleen (A7, A15) and Stomach (A8, A16). The histopathology of organs of Ayush SL treated group was compared with vehicle control group

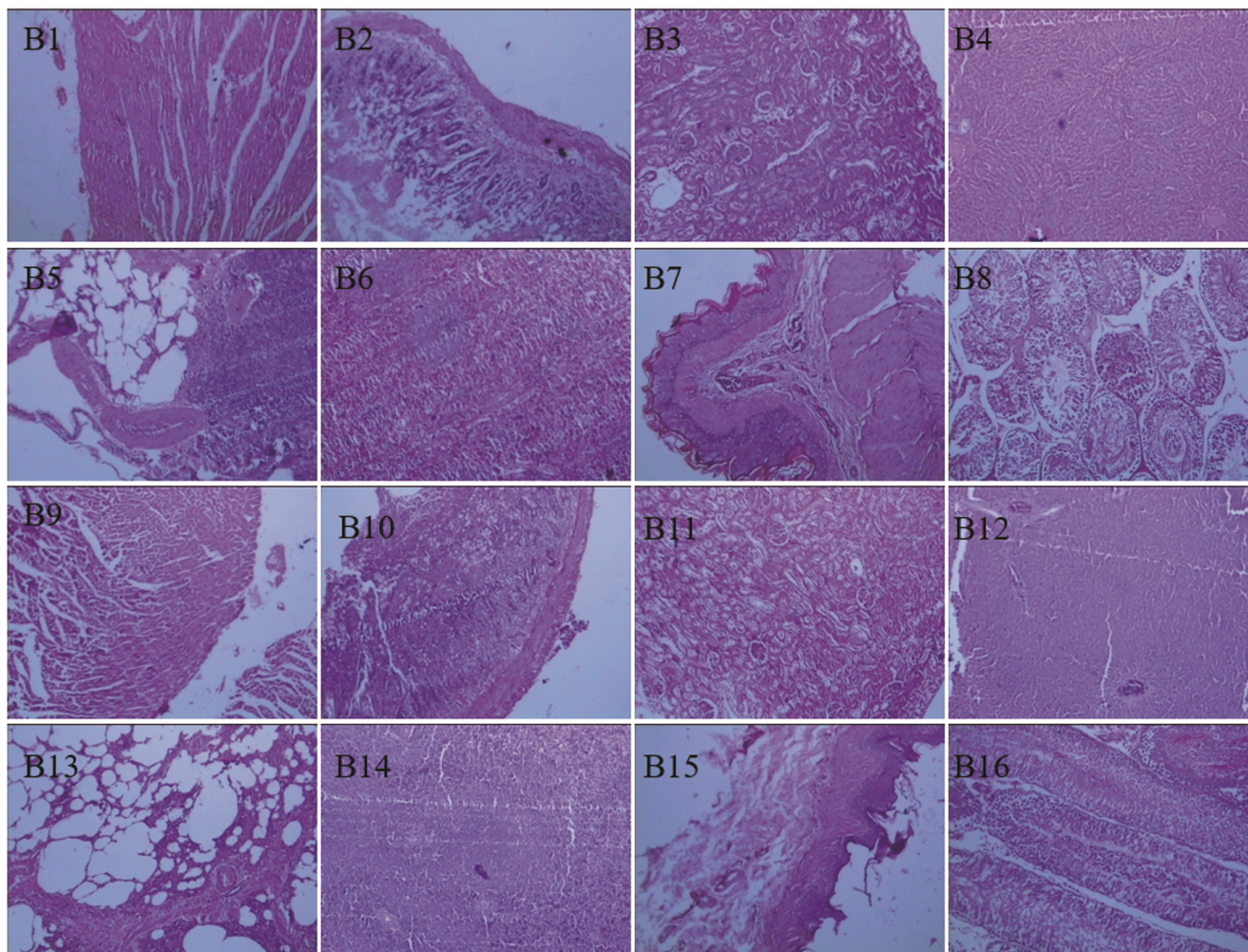


Fig. 2 — Photomicrograph of histopathology of organs/tissues of vehicle control male (B1–B8) and Ayush SL-treated male (High dose) (B9–B16) rats in sub-chronic (90 days repeated dose) oral toxicity study. No treatment-related pathological changes were found in Heart (B1, B9), Intestine (B2, B10), Kidney (B3, B11), Liver (B4, B12), Lung (B5, B13), Spleen (B6, B14), Stomach (B7, B15) and Testis (B8, B16). The histopathology of organs of Ayush SL treated group was compared with vehicle control group

abnormal clinical manifestations were observed, and Ayush SL did not produce any significant alterations in neurological function. Functional Observational Battery assessments for locomotor activity, behaviour, and reflexes revealed no untoward effects on the sensory, motor and cognitive functions attributable to the test drug.

The body weight gain, feed intake, and water intake of the animals were not significantly different from those of vehicle-treated control rats. It indicates that the test substance does not substantially increase energy metabolism or disrupt normal metabolic or nutrient-utilization processes that could affect body weight and food intake. Preclinical toxicology studies commonly utilize easily obtainable biofluids such as urine, serum, and plasma and this approach is

essential for monitoring multiple-dose toxicity studies and determining NOAELs. Qualitative urinalysis showed no evidence of proteinuria or altered specific gravity, suggesting no nephrotoxicity. The absence of glycosuria and haemoglobinuria further indicated the absence of metabolic disturbance or red blood cell damage. Assessment of clinical biochemical and haematological indices is a critical measure of systemic health, as deviations in these parameters often reflect pathological conditions or toxic effects. In the present study, no statistically significant changes were observed in glucose, SGOT, SGPT levels and liver histopathology remained comparable to controls, indicating that Ayush SL did not disrupt essential metabolic functions in the experimental animals. Furthermore, repeated oral administration of

Ayush SL for 28 and 90 days did not produce any significant alterations in haematological profiles, suggesting that the formulation had no detrimental impact on the haemopoietic system.

Twenty-eight and Ninety-day administration of the test drug produced no significant changes in mean organ weights compared with the vehicle control group, suggesting no treatment-related hypertrophy or atrophy of vital organs.

In this preclinical evaluation, histopathological examination revealed no treatment-related abnormalities in the lungs, liver, heart, spleen, or kidneys of rats receiving the test formulations, and the findings were comparable to those observed in the control group. The microscopic architecture of all examined organs in the animals that received the test drug remained normal and comparable to those in the vehicle control group. These findings suggest that the drug, at the tested dose and duration, does not exert toxic effects on major vital organs, supporting the drug's preliminary safety profile. Thus, Ayush SL, when given by oral route to the Wistar rats of both sexes, exhibited no toxicity of any nature and was found safe on oral administration up to the highest dose level of 2250 mg/kg/day for 28 and 90 consecutive days during the sub-acute and sub-chronic toxicity study. Hence, the NOAEL of Ayush SL is 2250 mg/kg/day in the sub-acute and sub-chronic toxicity studies.

Accordingly, oral administration of Ayush SL to Wistar rats did not yield evidence of systemic or organ-specific toxicity. The formulation was well tolerated at doses up to 2250 mg/kg when administered daily for 28 and 90 consecutive days in the sub-acute and sub-chronic studies. Based on these findings, the NOAEL for Ayush SL was established at 2250 mg/kg under the conditions of the repeated dose toxicity evaluations.

Conclusion

In conclusion, acute, 28-day, and 90-day oral administration of Ayush SL did not show any treatment-related adverse findings. The test drug was found to be safe up to 3250 mg/kg when administered orally in an acute toxicity study in mice. The oral administration of the test drug for 28 days (sub-acute toxicity study) and 90 days (sub-chronic toxicity study) did not cause any abnormalities in physical, behavioural, biochemical, hematological, or histopathological parameters at three doses in rats under the experimental conditions. Ayush SL was

found safe on repeated dose administration with NOAEL of 2250 mg/kg for 28-day and 90-day toxicity study.

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Author Contributions

Sanjaya Kumar YR and Thamizh Selvam N. were involved in the investigation, methodology, and formal analysis. Sanjaya Kumar YR and Deepak Lamba wrote the original draft as well as reviewed and edited the manuscript. Sudesh N Gaidhani, Arjun Singh, MM Padhi, GS Lavekar, and Srikanth N contributed to the conceptualization, funding acquisition, project administration, and supervision of the study.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

The permission for the present study was obtained from the Institute Animal Ethics Committee (IAEC) to conduct the experiments.

Data Availability

Data will be made available by the corresponding author upon request.

References

- 1 WHO, Lymphatic filariasis, Available online: <https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis> (access date 21 November 2024)
- 2 Ghadage P, Sahani G, Sundar B, Raj A S, Prakash A, *et al.*, Ayurvedic insights into lymphatic filariasis and holistic approaches towards its management, *Int J Mosq Res*,

- 10 (6) (2023) 173-176. DOI: <https://doi.org/10.22271/23487941.2023.v10.i6c.731>
- 3 Murthy K R S & Madhava N, *Sushruta Samhita*, (Chaukhamba Orientalia, Varanasi), (2009) 135.
- 4 Tripathi S K, Singh R, Bhuyan G, Mitra A, Sharma L, *et al.*, A multi-center collaborative double-blind study on clinical evaluation of AYUSH-SL in patients receiving mass drug administration for management of chronic filarial lymphedema – study protocol, *J Res Ayurvedic Sci*, 5 (3) (2021) 139-147. https://doi.org/10.4103/jras.jras_46_21
- 5 Ala S, Gaidhani S N, Reddy S V, Sanjaya Kumar Y R & Jamadagni S B, Safety assessment of AYUSH-64 through acute and 90-days repeated dose oral toxicity, *Int J Ayurveda Res*, 4 (4) (2023) 231-241. DOI: 10.4103/ijar.ijar_90_23
- 6 Suvarna R, Suryakanth V B, Bakthavatchalam P, Kalthur G, Deepak Nayak M, *et al.*, Acute and sub-chronic toxicity of Liberin, an anti-diabetic polyherbal formulation in rats, *J Ayurveda Integr Med*, 14 (6) (2023) 100804, <https://doi.org/10.1016/j.jaim.2023.100804>
- 7 Gaidhani S N, Reddy G D, Ganesan R, Tiwari P, Srikanth N, *et al.*, Pre clinical safety assessment of AYUSH Carctol S through acute and sub chronic oral toxicity study: safety assessment of AYUSH Carctol S, *Toxicol Int*, 31 (4) (2024) 539-549. <https://doi.org/10.18311/ti/2024/v31i4/36421>.
- 8 Mitra A, Bhuyan G, Muralikrishna C, Naser S M, Ratha K K, *et al.*, Clinical evaluation of AYUSH-SL in patients receiving mass drug administration for the treatment of chronic inflammatory lymphedema: A double-blind placebo-controlled multicentric study, *J Vector Borne Dis*, 62 (2) (2025) 202-210. DOI: 10.4103/JVBD.JVBD_61_24.
- 9 Paget G E & Barnes J M, Toxicity tests, In: *Evaluation of drug activities: Pharmacometrics*, Vol. 1, 1st ed., Laurence D R, Bacharach A L, (Eds), (Academic Press, London, UK), (1964) 135-166.
- 10 OECD guideline for Testing of Chemicals, Test No. 423, *Acute Oral Toxicity - Acute Toxic Class Method*, OECD Publishing Paris, (2001) 1-14.
- 11 OECD guideline for Testing of Chemicals, Test No. 407, *Repeated Dose 28-day Oral Toxicity Study in Rodents*, OECD Publishing Paris, (1998) 1-13.
- 12 OECD guideline for Testing of Chemicals, Test No. 408, *Repeated Dose 90-Day Oral Toxicity Study in Rodents*, OECD Publishing Paris, (1998) 1-14
- 13 Porwal M, Khan N A & Maheshwari K K, Evaluation of acute and subacute oral toxicity induced by ethanolic extract of *Marsdenia tenacissima* leaves in experimental rats, *Sci Pharm*, 85 (3) (2017) 29. doi: 10.3390/scipharm85030029