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ACUTE ORAL TOXICITY STUDY OF *SHIGRUPUNARNAVADI YOGA* – A UNIQUE POLY-HERBAL FORMULATION

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ABSTRACT

Shigrupunarnavadi yoga has been extensively used for decades as a *lepa* (external application) among Keraliya visha vaidyas (Traditional practicers of Kerala) to treat *Vishaja Shopha* (swelling due to toxin). Despite of its effectiveness as an external application, there have been no studies conducted to assess its potency when administered internally as a *kwatha* (decoction). Therefore, this study aims to evaluate the acute oral toxicity of *Shigrupunarnavadi Kwatha* according to Organisation for Economic Co-operation and Development 425 Guidelines, ensuring its safety for clinical use. During the acute toxicity studies (over 72hrs), no clinical signs of toxicity or mortality were observed in any of the wistar strain albino rats at different dose levels tested (50 mg to 2000 mg/kg body weight). Considering the outcome, it was inferred that *Shigrupunarnavadi Kwatha* is safe and non-toxic for internal administration.

KEYWORDS: *Shigrupunarnavadi yoga, Kwatha, Vishaja shopha,* Acute oral toxicity.

INTRODUCTION

Shigrupunarnavadi Yoga is a unique poly herbal formulation revealed in well-regarded ayurvedic toxicology text books of Kerala such as "*Prayoga Samuchayyam*," authored by Sri Kochunni Thampuran and "*Visha Jyotsnika",* authored by Karattu namboothiri in the context

of *Mandali visha chikitsa* as a *lepa* (external application) to reduce the swelling caused by *Visha*.[1],[2] This particular *yoga* contains ten ingredients which are readily accessible and economical. Inspite of its antiphlogistic action when applied externally, an analysis of the pharmacodynamics of the individual drugs in the formulation revealed significant antioxidant, anti-inflammatory, and diuretic effects. Consequently, to explore its potential nephroprotective benefits, the *Lepa Choorna* was converted into *Kwatha Choorna*, from which *kwatha* was prepared and feeded to experimental animals.

The purpose of acute oral toxicity testing is to assess the biologic activity of a combination and gain insight into its mechanism of action.[3] So far, no studies has been conducted on the toxicity profile of *Shigrupunarnavadi Yoga* as *Kwatha* for internal administration. In the present communication, the acute oral toxicity of *Shigrupunarnavadi Kwatha* was assessed according to OECD 425 guidelines to evaluate its long-term safety and tolerance profile.

MATERIALS AND METHODS

Selection of Plant materials

All the ten drugs such as *Shigru (Moringa oleifera Linn.), Punarnava (Boerhaavia diffusa), Haridra (Curcuma longa), Vacha (Acorus calamus Linn.), chandana (Santalum album Linn.), Patha (Cissampelos pareira), Ishwari mula (Aristolochia indica), Yashtimadhu (Glyzyrrhiza glabra), Shirisha (Albizia lebbek)* and *Gokshura (Tribulus terrestris)* were collected in equal quantity, authenticated and *Kwatha Choorna* (Coarse Powder) were prepared as per the general protocol from G.M.P. certified S.D.M. Ayurveda Pharmacy, Kuthpady, Udupi, Karnataka, India and the same formulation was standardised in the form of *Kwatha choorna* and *Kwatha*[4] from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.

Approval from Animal ethics committee: The study was performed after getting approval from IAEC of S.D.M Ayurveda college in its meeting held on 11/09/2022 - Ref. No. SDMCRA/IAEC/SD-A-04.

Selection of Animal species

- Animal species - Wistar strain albino rats
- Source - Animal house attached to SDM Research centre
- Selection - A total of 5 healthy either sex of body weight 160-200g and rats were selected according to AOT software.

- Acclimatization period - All the selected animals were kept under acclimatization for 7 days before dosing.
- Numbering and identification - The animals were marked with saturated picric acid solution in water for proper identification (Figure No1)

Table No. 1: Identification mark and numbering of individual rats within the cage.

Animal number	Marking
1	Head
2	Neck
3	Middle of the back
4	Base of the tail
5	No mark

Husbandry condition[5]

1. **Housing:** The rats were housed in polypropylene cages with stainless steel top grills. Dry husk was used as bedding material and was replaced every morning.
2. **Environment:** The animals were exposed to a 12-hour light and 12-hour dark cycle, with a relative humidity of 50% to 70% and an ambient temperature of 22 ± 3^0C.
3. **Diet:** Rat pellet feed, supplied by Sai Durga Feed, Bangalore, was provided throughout the study period, except on the night before dosing, during which the animals were fasted overnight. Drinking water was provided ad libitum in polypropylene bottles with stainless steel sipper tubes.

Dose Preparation and Schedule[5]

Test Drug: *Shigrupunarnavadi Kwatha*

Dose fixation: According to the AOT Software.

Dose: 175mg/kg, 550mg/kg, 2000mg/kg test substance (Figure No:2)

Schedule: Single dose per animal

Administration: Oral route at different dose levels to respective animal through oral feeding needle (Figure No.3)

Table No 2: AOT Dose calcultaion according to individual body weight.

Sl. No	Identification of animals	Desired dose (according to AOT)	Bodyweight (grams)	Calculated dose (ml)
1	Head	175mg/kg	216	2.16
2	Neck	550mg/kg	252	2.52
3	Back	2000mg/kg	145	1.45
4	Base of the tail	2000mg/kg	217	2.17
5	No mark	2000mg/kg	182	1.82

Procedure of acute toxicity assay[6]

Staircase method - Single animals are dosed in sequence, typically at 48-hour intervals. However, the exact time intervals between doses are adjusted based on the onset, duration, and severity of any toxic signs observed. The next dose is only administered when there is confidence in the survival of the previously dosed animal. To select the starting dose, all available information is considered, including data on structurally related substances and results from any other toxicity tests on the test material, to approximate the LD50 and the slope of the dose-response curve.

The first animal is dosed at a level one step below the best preliminary estimate of the LD50. If the animal survives, the next one receives a higher dose. If the first animal dies or shows signs of morbidity, the second animal is given a lower dose. If no estimate of the substance's lethality is available, dosing starts at 175 mg/kg. Dosing continues based on the outcomes observed at the fixed time intervals (e.g., 48 hours) for all animals up to that point. Testing is stopped once the first stopping criterion is met - either three consecutive animals survive at the upper dose limit, or another predefined stopping criterion is reached. The estimated LD_{50} was then calculated from the maximum likely-hood calculation.

Figure No 1: Identification staining with picric acid.

Figure No 2: Dose preparation of *Shigrupunarnavadi kwatha (2000, 175,550mg/kg)*.

Figure No 3: Oral dose feeding.

OBSERVATION

Examination of Physical and Behavioural changes

The animals were observed continuously for 4 hours after dosing. Careful cage-side observations were conducted without disturbing the animals, and at the end of each hour, each animal was individually placed in an open arena to record any behavioral changes.

These included increased or decreased motor activity, convulsions, Straub's reaction, muscle spasms, catatonia, spasticity, opisthotonus, hyperesthesia, muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation, diarrhea, writhing, respiratory patterns, changes in skin color, and signs of CNS depression such as hypoactivity, passivity, relaxation, ataxia, and narcosis and other relevant symptoms.[7]

Mortality: All the animals were monitored at ½, 1, 2, 3, 4, 24, and 48-hours post-dosing, and then once daily for any signs of morbidity and mortality over the entire 14-day study period.

RESULT

Table No 3: Results of Acute oral toxicity – Short term.

Sequence No	ID No.	Dosage(mg/kg)	Result	
			Short term	Long term
1	1	175	O	O
2	2	550	O	O
3	3	2000	O	O
4	4	2000	O	O
5	5	2000	O	O

Note: ***X = Died, O = Survived***

Dose Recommendation: The main test is complete

Stopping Criteria : 3 at limit test

Table No 4: Summary of long-term results.

Dose	O	X	Total
175	1	0	1
550	1	0	1
2000	3	0	3
All Doses	5	0	5

Stastical Estimate based on long term outcomes: The LD50 is greater than 2000mg /kg.

DISCUSSION

Phytotherapy is becoming increasingly popular as the WHO supports the proper use of ethnomedicinal practices and highlights the need for safety evaluations of herbal medicines. The FDA and WHO both underscore the necessity of scientific research to confirm the effectiveness and safety of herbal treatments.[7] Therefore, carrying out preliminary toxicological assessments is crucial for ensuring the safety of herbal medications.

Physical and behavioural examination: There were no physical and behavioural changes (except mild increase in motor activity and piloerection seen in 2 rats in the group of 2000mg/kg) in all the treated animals on day one at ½, 1,2,3,4 hours intervals after dosing and there after once daily for 14 consecutive days. Thus, the data obtained from the study on single oral dose administration of *Shigrupunarnavadi Kwatha* up to 14 days of observation period does not result in any physical and behavioural changes.

Mortality: All the animals in the treated group remained alive throughout the 14-day observation period after dosing.

CONCLUSION

The test drug *Shigrupunarnavadi kwatha* did not produce any mortality even up to the dose of 2000mg/kg per oral which is equivalent to 22.4g total dose for a human being weighing 70 kg man. At the dose level studied, the drug also did not produce any observable toxic effect except mild increase in motor activity and piloerection seen in 2 rats in the group of 2000mg/kg. In light of these findings, it could be concluded that the test drug *Shigrupunarnavadi kwatha* was found to be safe and non- toxic for internal use. Additionally, this study provides preliminary data that can support further research to establish safe and effective doses for preclinical evaluation.

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