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RESEARCH ARTICLE

Safety Profiling of the *Ayurvedic* Formulation *Niruryadi Gulika* in Wistar Rats

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Abstract

Background: *Niruryadi Gulika* is a classical *Ayurvedic* formulation conventionally used for the treatment of diabetes mellitus. It is also used for the treatment of fever, respiratory ailments, along digestive ailments. This herbal medicine consists of a blend of potent herbs known for their antidiabetic, anti-inflammatory, antipyretic, and digestive properties. Niruri (*Phyllanthus niruri*) is one of its key ingredients, renowned for its hepatoprotective and antiviral effects. Along with other herbs, this formulation works to balance aggravated doshas, particularly Pitta and enhances overall vitality. **Aim:** In the present study, we have studied the acute and subacute safety profiling of *Niruryadi Gulika* in Wistar rats. **Methods:** During acute toxicity tests (2000 mg/kg b.w.) followed by monitoring for 2 weeks, *Niruryadi Gulika* showed no adverse effects on the overall health parameters of the rats. In subacute toxicity tests, *Niruryadi Gulika* was administered at 200 mg/kg body weight for 28 consecutive days. After the administration, animals were observed for the first 48 h, starting from half an hour for any toxic effect or altered behaviour, and the observation continued for a period of 24-48 h. **Results:** In post-treatment, serum haematological parameters and lipid profiling showed no significant differences with a transient rise in High-Density Lipoprotein (HDL), albumin, and chloride levels. No significant change is detected in any of the parameters when compared to the untreated animals. **Conclusion:** These results show that the *Ayurvedic* formulation is relatively nontoxic in Wistar rats and provides basic information on the toxicological profile of *Niruryadi Gulika* used in *Ayurvedic* medicine.

Major Findings: *Niruryadi Gulika* showed no signs of acute or subacute toxicity in Wistar rats, confirming its safety and non-toxic nature. It also supported normal physiological and biochemical functions throughout the treatment period.

Keywords: *Ayurvedic* Medicine, Acute Toxicity, *Niruryadi Gulika*, Herbal Formulation, Subacute Toxicity

1. Introduction

Ayurveda is an old traditional medical system from India that provides a comprehensive methodology for disease management. *Ayurvedic* practitioners, using centuries of knowledge and experience, prioritise specific treatment approaches customised to individual constitutions, or doshas, which include *Vata, Pitta,* and *Kapha*[1,2]. Many traditional plants and their extracts are used as treatment strategies in *Ayurveda*. Over the past few years, there has been a crucial rise in the popularity of alternative therapy, which includes dietary treatments and ancient herbal medicines from systems including *Ayurveda*[3].

Several *Ayurvedic* medicines are believed to have a potential role in curing major ailments associated with metabolism, such as diabetes, based on the herbs and ingredients they contain. However, there has been

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limited data exhibiting the potency and harmlessness of *Ayurvedic* medicines in treating many disorders[4]. In line with this, *Niruryadi Gulika* is an *Ayurvedic* herbal formulation that has been traditionally used in *Ayurveda* and typically contains various herbs and minerals extracted from plant-based sources[5]. *Niruryadi Gulika* contains certain herbs, such as *Gymnema sylvestre* (*Gurmar*), that have been widely studied for their potential to reduce blood sugar levels[6,7]. *Niruryadi Gulika* consists of a combination of medicinal plants meticulously chosen for their synergistic properties and has been employed for many years to treat several conditions, including digestive, respiratory, and neurological disorders[2].

Niruryadi Gulika is widely recognised for its effectiveness in enhancing digestive health by mitigating indicators of indigestion, along with abdominal discomfort and gas. Additionally, it exhibits anti-inflammatory and analgesic characteristics, rendering it beneficial for treating ailments like arthritis and joint pain[8]. In *Ayurveda*, *Niruryadi Gulika* is mainly used in the management of diabetes, which is associated with an imbalance in the *Vata dosha*[8]. Moreover, *Ayurvedic* remedies like *Niruryadi Gulika* are thought to help balance the doshas and may be used as part of an *Ayurvedic* approach to managing diabetes[9,10]. This study's primary strength is its comprehensive methodology, merging ancient *Ayurvedic* wisdom with modern scientific frameworks. *Niruryadi Gulika* is a polyherbal and mineral formulation, distinguishing it from prior studies that often concentrate on individual herb extracts, thereby reflecting authentic *Ayurvedic* practice. This study provides novel insights by assessing the safety profile of the formulation in its entirety, rather than examining its components.

The primary objectives of this study are to evaluate the effect on haematological, biochemical parameters, and histopathological examination in the subacute toxicity study and to evaluate the behavioural and morphological alterations of the rats in the acute toxicity study.

2. Materials and Methods

2.1 *Niruryadi Gulika* Formulation

Niruryadi Gulika is a conventional *Ayurvedic* medicine and is composed of a mixture of traditional herbs and plant products carefully chosen for their medicinal nature. This particular composition

Table 1. Constituents of *Niruryadi Gulika* along with quantity present in one tablet

S. No.	Sanskrit Name	Botanical Name	Component	Quantity
1	Niruri	*Phyllanthus reticulatus*	Root	0.030 g
2	Vairi	*Salacia reticulata*	Root	0.030 g
3	Teli	*Strychnos potatorum*	Seed	0.030 g
4	Suka	*Mucuna pruriens*	Seed	0.030 g
5	Pulimkuruttol	*Tamarindus indica*	Seed Coat	0.030 g
6	Haritaki	*Terminalia chebula*	Fruit	0.030 g
7	Amalaki	*Phyllanthus emblica*	Fruit	0.030 g
8	Vibhitaki	*Terminalia bellirica*	Fruit	0.030 g
9	Vilampasa	*Limonia accidissima*	Fruit	0.030 g
10	Amballari	*Nymphaea alba*	Flowers	0.030 g
11	Kantam	Iron	Iron	0.030 g
12	Chemman	Red ochre	Red ochre	0.030 g
13	Nisa	*Curcuma longa*	Rhizome	0.030 g
14	Daruharidra	*Berberis aristata*	Bark	0.030 g
15	Himam	*Santalum album*	Leaves	0.030 g
16	Sita	*Saccharum officinarum*	Processed from Sugarcane	0.030 g
17	Attinir	*Ficus racemosa*	Bark	q. s.

includes herbs such as *Niruri*, *Triphala*, *Haridra*, and *Daruharidra*, which are used for their distinct effect on health, ranging from suppressing inflammation and neutralising free radicals to digestive support and immune modulation[11-14]. The plant parts used in this study are mentioned in Table 1. All the herbal ingredients of *Niruryadi Gulika* were procured from Arya Vaidya Sala, Kottakkal (Kerala, India). The plant materials were processed using a Soxhlet extraction apparatus, and the resulting crude extract was concentrated under a vacuum using a rotary evaporator. The final extract was stored in a refrigerator at 2-8°C until further use.

2.2 Animals

Wistar rats present in the weight range of 180-250g were utilised for the experimentation. Animals were housed in a habituated environment, in the temperature range under 25°C and following a 12-hour day-length cycle. The animals were provided with water and commercial rat feed freely for two weeks before the commencement of the research. The entire study was conducted in accordance with the Institutional Animal Ethics Committee guidelines, as approved by Sri Jayendra Saraswathi Ayurveda College and Hospital (Approval No. IAEC/190/2022).

2.3 Acute Toxicity Tests

An acute toxicity study for *Niruryadi Gulika* was conducted as per OECD guideline 423, wherein a higher concentration - 2000 mg/kg body weight was used. Experimental rats were fasted overnight (10 h) before the commencement of each experiment, with no restriction to water. 2 study groups, including three healthy rats of either gender, were used. 1st group, with three healthy rats of either gender, considered an untreated group, received a calculated amount of water, and group 2, three healthy rats of either gender, received a *Niruryadi Gulika* at 2000 mg/kg body mass. According to OECD procedures, the initial dose for animals is in the range of 2000 mg/kg body weight. And was mixed with 500 mg/mL of Carboxy Methyl Cellulose (CMC) suspension. After oral administration of the drug, animals were observed for the first 48 hours, starting from half an hour for any toxic effect or altered behaviour, and the observation continued for a period of 24-48 h.

The observations for this study were carried out based on OECD guidelines 423. It consists of examining the wellness parameters of six healthy rats of either gender were used in two groups, starting from 30 min till the next 24 h (with additional care specifically during the initial 4 h). Later, the rats were noted for 14 days. Each

Table 2. Behavioural aspects of rats in *Niruryadi Gulika*-treated (2000 mg/kg BW) and control animals

Parameters	Observations of vehicle control and Niruryadi Gulika treated groups											
	30 minutes		4 h		24 h		48 h		7 days		14 days	
	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG
Fur and skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	↑	N	↑	N	N	N	N	N	N	N	N
Respiration	N	↑	N	N	N	N	N	N	N	N	N	N
Urination(color)	N	N	N	N	N	N	N	N	N	N	N	N
Faeces consistency	N	N	N	N	N	N	N	N	N	N	N	N
Somatomotor activity	↑	↑	N	↑	N	N	N	N	N	N	N	N
Sleep	N	↑	↑	↑	N	N	N	N	N	N	N	N
Mucous membrane	↑	N	N	↑	N	N	N	N	N	N	N	N
Convulsions and tremors	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Itching	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Coma	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Mortality	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F

observation was noted and documented for individual animals. The observations focused on alterations in the colour of coating and hair, eye, and nasal pores, along with differences in behaviour. Mortality, weight of the animal, intake of feed, and uptake of water were also monitored daily for 14 days, along with shaking, spasms, secretion of saliva, loose stools, exhaustion, slumber, and loss of consciousness. The differences in these parameters were correlated with the pattern observed in control animals. Various health aspects observed were related between the treated animal group and the control group. The changes observed in various wellness parameters are represented in Table 2.

2.4 Subacute Toxicity Study

The Subacute oral toxicity of *Niruryadi Gulika* was estimated in compliance with OECD guideline 407 on Wistar rats comprising both males and females. Rats were separated into 2 sets of n = 6 (3 male and 3 female rats). The Control animals were administered orally with the vehicle, and the test group animals were administered orally with the *Niruryadi Gulika* formulation of 200mg/kg b.w. continuously for 28 days. The compound treatment was performed every day through an oral gavage needle. During the experiment, all animals were provided with water and feed *ad libitum* throughout. After the test period, experimental animals were fasted overnight (8 h) priorly with no restriction to water, and later the animals were anaesthetised under ether, and their blood was collected from the cardiac region into appropriate blood collection tubes for haematological and biochemical analysis. Blood was collected into sterile non-heparinised tubes, and after clotting, it was centrifuged, and serum was separated. The separated serum was stored in a clean bottle for biochemical analyses.

2.4.1 Haematological Assays

The blood samples containing anticoagulants were then utilised for estimating haematological parameters. Haematological analysis was measured with the unit KX-21 (Sysmex). The haematological parameters were completely analysed and compared between the control and the test groups.

2.4.2 Biochemical Analyses

Circulatory parameters such as Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Triglycerides (TG), Total Cholesterol (TC), High-density lipoprotein, Low-Density Lipoprotein (LDL), total bilirubin, and protein were estimated from the collected blood. Serum electrolytes include sodium, potassium, chloride, calcium, and phosphate. Biochemical parameters were assessed with a fully automated clinical chemistry analyser (EM 360, Transasia, Germany) using Erba diagnostics kits (ERBA Diagnostics Mannheim GmbH, Germany). Biochemical analysis was performed with the separated serum, and it was compared with the control and test groups.

2.4.3 Necropsy and Histopathology

The rats were euthanised, and a necropsy was carried out on animals of each group, including male and female animals, to study the macroscopic characteristics of each organ after the end of the experiments. Once the fixation was completed, the tissues were sliced, evaporated using a higher concentration of absolute alcohol, diaphanized in xylene, and fixed in paraffin. The tissues were cleaved at 5µm thickness, stained with H & E stain, and kept on glass slides and visualised under a light microscope.

2.4.4 Statistical Analysis

Figures are presented as Mean ± SEM (n = 3 per group for male and female rats). The variation between the control and test groups was analysed using Student's t-test. GraphPad Prism version 8.0.1 for Windows (GraphPad Software, La Jolla, California, USA) was used for analysis. The value of significance was $p<0.05$.

3. Results

3.1 Acute Toxicity Study

An acute toxicity study of *Niruryadi Gulika* in Wistar rats with a dose of 2000 mg/kg body weight with CMC as a vehicle was conducted. After the oral dose administration, no mortality was observed. During the 2-week observation period, there were no significant alterations in the wellness parameters in comparison to the control animals. Following this, the LD50 dose of *Niruryadi Gulika* composition in Wistar rats is assessed to be more than 2000 mg/kg b.w. of each animal.

Histopathology of the liver samples of animals treated with 2000 mg/kg B.W. of *Niruryadi Gulika* showed no

modifications in the tissue architecture for the tissues obtained from control animals. Similarly, the histology results also revealed the non-toxic effects of *Niruryadi Gulika* (Figure 1). There were no toxic signs observed in the organ tissues, such as fibrosis, infiltration of leukocytes, tissue congestion, degeneration, necrosis, apoptosis, and blood extravasation.

3.1.1 Behavioural and Morphological Changes

In acute toxicity studies, we noted the changes in physiological parameters and compared these effects with the control group animals (Table 2). There was no adverse sign of toxicity in the animals administered with *Niruryadi Gulika*. Moreover, the wellness parameters of the animals, such as alterations in the colour of coat and hair, eyes, and nasal pores, along with differences in behaviour, were unaltered. We also observed symptoms of sleep, coma, tremors, convulsions, salivation, and mortality in the 14 days.

3.2 Subacute Toxicity Study

3.2.1 Effect of Niruryadi Gulika on Serum Haematological Parameters

The effect of *Niruryadi Gulika* on serum and haematological parameters in male and female Wistar rats dosed with the extract is given in Table 3. In male rats, haematological parameters such as haemoglobin, Red Blood Cells (RBC), Mean corpuscular haemoglobin concentration (MCHC), Red cell Distribution Width (RDW), and platelets showed mild variation compared to the untreated animals. In female rats, parameters such as haemoglobin, Packed cell volume (PCV), Mean Corpuscular Volume (MCV), RDW, White Blood Cells (WBC), Lymphocytes, and platelets showed less variation in the *Niruryadi Gulika*-treated rat groups. No significant change is detected in any of the parameters when compared to the untreated animals.

3.2.2 Effect of Niruryadi Gulika on Serum Electrolyte Parameters

The effect of *Niruryadi Gulika* on serum electrolyte parameters in rats dosed with the extract is given in Table 4. In male rats treated with *Niruryadi Gulika*, electrolytes such as sodium, potassium, and chloride showed mild variation when evaluated with the control group. In female rats dosed with *Niruryadi Gulika*, plasma, except for chloride levels, all other parameters showed a mild decrease among the treatment groups when compared with the untreated group. There was a crucial variation in chloride levels in female rats dosed with the *Niruryadi Gulika* formulation when evaluated with the control group.

3.2.3 Effect of Niruryadi Gulika on Biochemical Parameters

The effect of *Niruryadi Gulika* on biochemical parameters in rats dosed with the extract is represented in Table 5. In male rats treated with *Niruryadi Gulika*, circulatory parameters such as albumin, globulin, and bilirubin were assessed. No notable variation in the biochemical parameters in dosed animals in comparison with the control group animals was

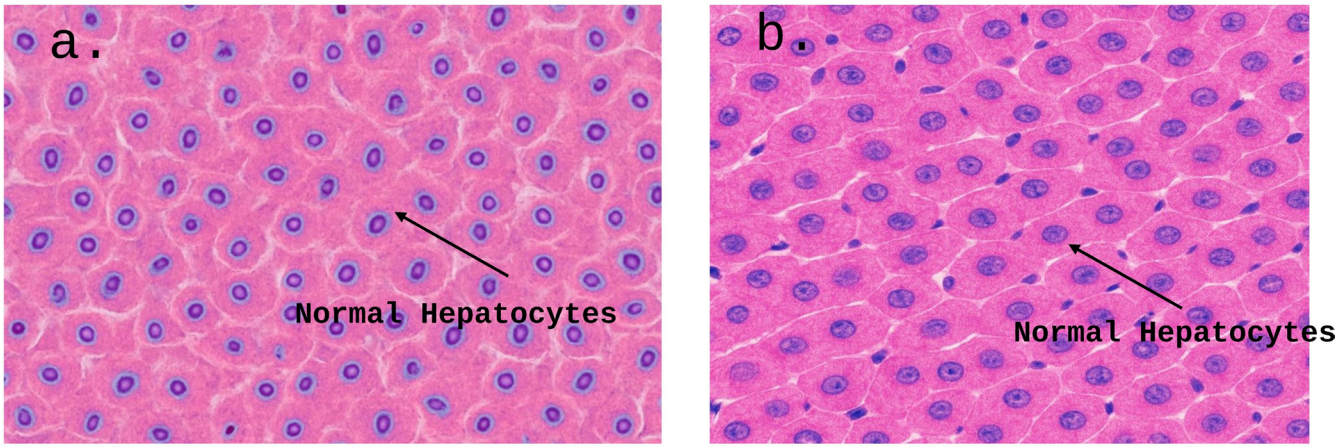


Figure 1. Histopathological examination of liver tissue from Wistar rats. **(a)** Liver tissue from a control animal, **(b)** Liver tissue from rats treated with *Niruryadi Gulika* at an acute dose of 2000mg/kg BW.

Table 3. Serum haematological parameters of Wistar rats administered with *Niruryadi Gulika* suspension

Parameters	Control	Niruryadi Gulika (200 mg/kg)
Male rats (n=3)		
Hb (g/L)	151.34 ± 7.3	157.67 ± 1.86
RBC (x10^{12}/L)	7.54 ± 0.04	8.06 ± 0.03
PCV (L/L)	0.46 ± 0.03	0.45 ± 0.01
MCV (fL)	50 ± 1.52	51 ± 0.78
MCH (pg)	17 ± 0.07	17.3 ± 0.04
MCHC (g/L)	332 ± 3.4	339 ± 3.5
RDW (%)	18.42 ± 0.8	19.17 ± 0.27
WBC (x10^3/mm3)	11.4 ± 1.02	11.37 ± 1.42
NEUTROPHIL (x10^9/L)	2.2 ± 0.65	2.00 ± 0.43
LYMPHOCYTE (x10^9/L)	6.4 ± 0.55	6.7 ± 0.7
MONOCYTE (x10^9/L)	0.27 ± 0.15	0.3 ± 0.25
EOSINOPHIL (x10^9/L)	0.1 ± 0.01	0.09 ± 0.06
PLATELET (x10^9/L)	909 ± 45	919 ± 57
Female rats (n=3)		
Hb (g/L)	130.71 ± 1.7	137.0 ± 1.8
RBC (x10^{12}/L)	7.78 ± 7.0	7.80 ± 18.8
PCV (L/L)	0.35 ± 0.02	0.38 ± 0.01
MCV (fL)	55.57 ± 0.36	60.07 ± 1.41
MCH (pg)	17.29 ± 0.21	17.67 ± 0.23
MCHC (g/L)	317.29 ± 6.84	315.17 ± 4.4
RDW (%)	16.27 ± 0.51	17.45 ± 0.86
WBC (x10^3/mm^3)	51.43 ± 11.81	54.0 ± 12.72
NEUTROPHIL (x10^9/L)	12.14 ± 2.59	12.0 ± 2.21
LYMPHOCYTE (x10^9/L)	37.00 ± 8.20	38.17 ± 10.07
MONOCYTE (x10^9/L)	1.71 ± 0.61	1.83 ± 0.40
EOSINOPHIL (x10^9/L)	1.67 ± 0.42	2.00 ± 1.55
PLATELET (x10^9/L)	913 ± 40	902 ± 98

Results were expressed as the mean±SEM of 3 rats in each gender. n=3 in male rats, n=3 in female rats.

Table 4. Serum electrolyte parameters of Wistar rats administered with *Niruryadi Gulika* suspension

Parameters	Control	Niruryadi Gulika (200 mg/kg)
Male rats (n=3)		
Sodium (mmol/L)	139.47 ± 1.47	140.82 ± 1.21
Potassium (mmol/L)	5.40 ± 0.35	5.14 ± 0.17
Chloride (mmol/L)	97.57 ± 0.68	102 ± 0.38
Calcium (mmol/L)	2.45 ± 0.23	2.45 ± 0.05
Phosphate (mmol/L)	2.92 ± 0.05	2.94 ± 0.49
Female rats (n=3)		
Sodium (mmol/L)	140.73 ± 1.30	140.97 ± 0.77
Potassium (mmol/L)	5.20 ± 0.34	5.26 ± 0.31
Chloride (mmol/L)	107.65 ± 0.64	91 ± 0.48*
Calcium (mmol/L)	2.48 ± 0.04	2.61 ± 0.42
Phosphate (mmol/L)	3.22 ± 0.13	2.99 ± 0.26

Results are expressed as the mean±SEM of 3 rats in each gender. n=3 in male rats, n=3 in female rats. *Comparison with control, $p<0.05$

Table 5. Biochemical parameters of Wistar rats administered with *Niruryadi Gulika* suspension

Parameters	Control	Niruryadi Gulika (200 mg/kg)
Male Rats (n=3)		
TP (g/L)	66.21 ± 3.34	65.34 ± 4.5
Albumin (g/L)	27.40 ± 1.21	30.42 ± 0.46
Globulin (g/L)	38.24 ± 1.09	41.00 ± 3.03
ALP (IU/L)	113.3 ± 4.5	104.7 ± 4.7
Bilirubin (mol/L)	2.13 ± 0.59	2.09 ± 0.63
AST (IU/L)	221.32 ± 12.4	216.5 ± 21.3
ALT (IU/L)	59.0 ± 3.61	51.33 ± 1.86
Female Rats (n=3)		
TP (g/L)	68.0 ± 1.43	73.7 ± 1.45
Albumin (g/L)	30.01 ± 1.00	40.07 ± 0.23*
Globulin (g/L)	44.0 ± 0.93	44.00 ± 1.63
ALP (IU/L)	200.3 ± 11.8	204.7 ± 30.7
Bilirubin (mol/L)	2.0 ± 0.67	2.00 ± 0.67
AST (IU/L)	212.7 ± 3.33	206.67 ± 25.40
ALT (IU/L)	40.31 ± 2.46	38.24 ± 2.32

Results were expressed as the mean±SEM of 3 rats in each gender. n=3 in male rats, n=3 in female rats.*Comparison with control, p<0.05

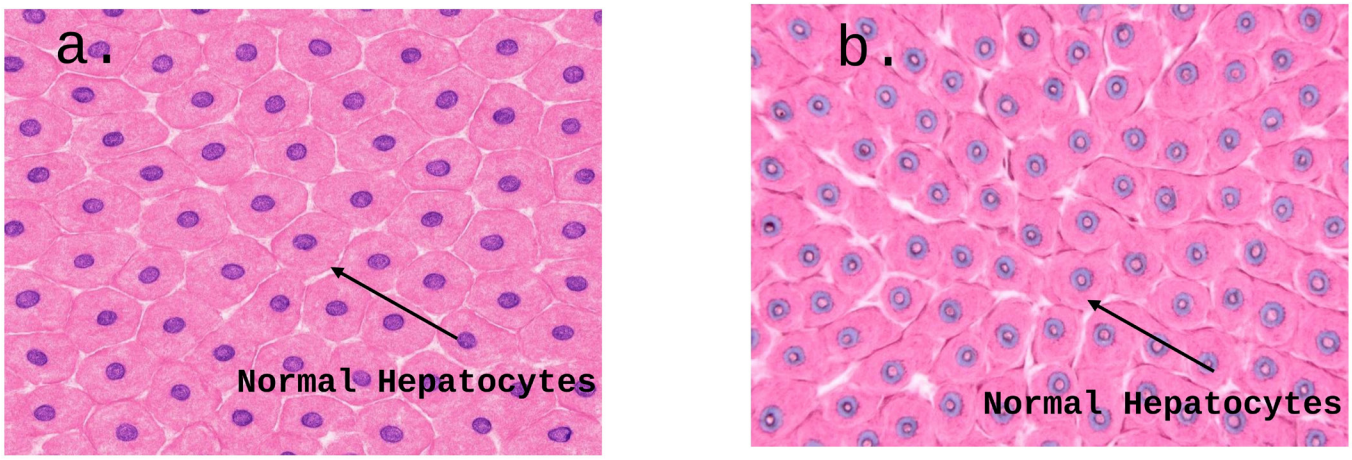


Figure 2. Histopathological examination of liver tissue from Wistar rats. **(a)** Liver tissue from a control animal; **(b)** Liver tissue from rats administered with *Niruryadi Gulika* at a sub-acute dose of 200mg/kg body weight for 28 days.

observed. In female rats treated with *Niruryadi Gulika*, albumin levels showed a significant increase (*p<0.05). Other characteristics showed no significant variation.

3.2.4 Effect of Niruryadi Gulika on Lipid Profile

The effect of *Niruryadi Gulika* on lipid profile parameters in male and female Wistar rats administered with the extract is represented in Table 6. In male rats administered with *Niruryadi Gulika*, there was an elevation in the levels of High-density lipoprotein (p<0.05). Other parameters showed mild alteration in comparison with the untreated animal group. In female rats administered with *Niruryadi Gulika*, the lipid profile parameters are observed. There were no notable alterations that were observed in various parameters in comparison with the untreated group of rats.

Histopathology of the rat liver tissues after 28 days of *Niruryadi Gulika* oral dosage at a concentration of

Table 6. Lipid profile parameters of Wistar rats administered with *Niruryadi Gulika* suspension

Parameters	Control	Niruryadi Gulika (200 mg/kg)
Male rats (n=3)		
TC (mmol/L)	1.76±0.42	1.68±0.27
TG (mmol/L)	0.66±0.02	0.51±0.04
HDL (mmol/L)	0.83±0.13	0.95±0.07*
LDL (mmol/L)	0.59±0.16	0.54±0.04
Female rats (n=3)		
TC (mmol/L)	1.72±0.20	1.67±0.12
TG (mmol/L)	0.53±0.04	0.44±0.03
HDL (mmol/L)	0.90±0.11	0.91±0.05
LDL (mmol/L)	0.55±0.11	0.54±0.08

Results were expressed as the mean±SEM of 3 rats in each gender. n=3 in male rats, n=3 in female rats. *Comparison with control, $p<0.05$

200 mg/kg body weight showed no morphological changes related to liver tissue architecture (Figure 2). However, there was mild congestion in the liver tissues of *Niruryadi Gulika*-treated rats in comparison with the liver morphology of control animals.

4. Discussion

The *Ayurvedic* approach to disease management is primarily preventive, focusing on lifestyle modifications, dietary adjustments, and mental well-being to promote overall health and prevent illness. *Ayurveda* emphasises personalised treatment plans, as each individual's constitution is unique. The system incorporates therapies such as Panchakarma, which detoxifies the body, and herbal remedies, which use medicinal plants to treat specific conditions[15]. *Ayurvedic* treatments are particularly renowned for managing chronic conditions, such as diabetes, arthritis, digestive disorders, and skin issues. Moreover, lifestyle modifications such as *yoga*, meditation, and breathing exercises complement *Ayurvedic* medicine by promoting mental clarity and stress reduction, which are essential for maintaining overall well-being[16].

In recent years, *Ayurvedic* medicine has gained global recognition, particularly for its integrative approach to health care. It complements modern medicine by addressing not only physical symptoms but also the root causes of illness. However, despite its widespread use, *Ayurvedic* medicine faces challenges, including concerns about mechanism, standardisation, dosage, and the lack of large-scale clinical trials to validate traditional claims. To address these gaps, many research investigations are focusing on the mode of action and safety profiling of various *Ayurvedic* and herbal formulations[17].

In line with this, our study focuses on the healing properties of *Ayurvedic* extract, *Niruryadi Gulika* formulation, which encompasses various herbs and mineral extracts[18]. Some of these ingredients possess properties that are traditionally associated with managing diabetes. This study offers a unique investigation into the safety and efficacy of the *Ayurvedic* formulation *Niruryadi Gulika*, supplying critical preclinical evidence to substantiate its conventional application in the treatment of metabolic disorders. Although the therapeutic potential of *Ayurvedic* formulations has been acknowledged for a long time, scientific validation is still scarce. Our work addresses this lacuna by providing systematic acute and sub-acute toxicity profiling in Wistar rats, thereby establishing the foundation for future clinical translation.

In acute toxicity studies, we noted the changes in physiological parameters and compared these effects with the control group animals. There was no adverse sign of toxicity in the animals administered with *Niruryadi Gulika*. Moreover, the wellness parameters of the animals, along with differences in behaviour, were unaltered. Our observations revealed unaltered wellness and behavioural parameters of the rats administered with *Niruryadi Gulika* formulation. In addition to this, our histopathology data revealed that there was no deleterious effect of the ayurvedic formulation in the liver tissues of rats, which confirms the non-toxic effect of the *Niruryadi Gulika* formulation. Collectively, these data can confirm the safety of *Niruryadi Gulika* acute dosage in rats and state that the lethal dose of *Niruryadi Gulika* can be above 2000mg/kg b.w. in rats.

In sub-acute toxicity tests, we treated rats of either gender with oral administration of *Niruryadi Gulika* at a dosage concentration of 200 mg/kg b.w. for twenty-eight consecutive days. After the treatment period, serum haematology, electrolyte, biochemical, and lipid profiles of the dosed rats were studied and compared with the untreated animal group rats. In the haematology test, all the serum parameters were

normal, while the blood haemoglobin level was elevated non-significantly in the test group rats, irrespective of gender. This can be attributed to the effect of *Salacia reticulata* (*Vairi*) present in *Niruryadi Gulika* on increasing red blood corpuscle volume in the rats, as demonstrated in a previous study on mice[19].

Our results are consistent with the observed safety profiles of *Ayurvedic* formulations containing similar herbal constituents, as compared to previous studies. However, they provide a further contribution by assessing multi-component preparation under standardized conditions. For example, *Salacia reticulata* has been demonstrated to possess erythropoietic potential in rodents[19], and our investigation corroborates this finding in an alternative model. In the same vein, the HDL-elevating effect that was observed is consistent with previous research on tannin-rich plant extracts[20].

The treatment showed no significant difference in the levels of various biochemical parameters, while there was a robust elevation in albumin levels of female rats administered with Niruryadi Gulika. We also studied the lipid profile markers of the control group and test group rats and found that there were no marked alterations in the lipid profile, but a significant elevation in the HDL levels of female rats was noticed in comparison to the control group animals. In relevance to this, an earlier study has reported a marked elevation in HDL levels in rats treated with tannins from *Ficus racemosa*, which is present in the *Niruryadi Gulika* formulation as Attinir (20). Along with this, we also studied the impact of our *Ayurvedic* formulation on the liver tissues of rats treated with the sub-acute dose. In line with the blood parameters, our histopathology data showed no impairment of liver tissue composition after the 28-day dosage regimen. Interestingly, we found mild congestion in the liver morphology of *Niruryadi Gulika*-administered rats in comparison with the control group. This may be due to the pathological changes caused by the formulation in the tissues and highlights the need to monitor liver health during long-term treatment with repetitive dosing.

5. Conclusion

Taken together in conclusion, our study analysed the safety and toxicity profile of *Niruryadi Gulika* on adult Wistar rats and revealed that *Niruryadi Gulika* is essentially safe below the dose range of 2000mg/kg body weight and is found to be non-toxic in rats for a specific periodic exposure. Our data also suggests that *Niruryadi Gulika* does not alter any vital parameters in rats and does not cause any adverse effect on the metabolism. Thus, further exploration is required to investigate the function of *Niruryadi Gulika* in various disorders and to elucidate its molecular-level mechanisms.

6. Ethical Approval Statement

The entire protocol was conducted in accordance with the Institutional Animal Ethics Committee guidelines, as approved by Sri Jayendra Saraswathi Ayurveda College and Hospital (Approval No. IAEC/190/2022). This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

7. Author Contribution

Satish Kumar RC: conceptualisation, investigation, data curation, formal analysis, methodology, validation, writing – original draft, visualisation. Das C: conceptualisation, data curation, resources, project administration, funding acquisition, supervision. Mothishwaran B: Data validation, Review and editing. Ramkumar KM: Data validation, Review and editing. All authors have read and agreed to the published version of the manuscript.

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