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Acute oral Toxicity Study of *Sharpunkhadi* granules on Swiss Albino Rats - A novel herbal formulation for Sickle Cell Anemia

Research Article

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Abstract

Background: Sickle cell disease (SCD) is one of the most common global monogenic disorders with an autosomal recessive inheritance. It has been estimated that, between 2010 and 2050, about 14.2 million babies will be born with sickle cell anemia (SCA). Hydroxyurea, an essential anticancer drug is one of the most promising drugs for this condition used in present. However, decreased production of platelets, red blood cells, and white blood cells are the major known side effects of Hydroxyurea. Objectives: To determine median lethal dose of *Sharpunkhadi* granules when administered a single dose to rats, followed by an observation period of 14 days. Material and method*: Sharpunkhadi* granules is a novel self-designed formulation comprised of 8 herbs which possess Anticancer, Anti-inflammatory, Anti-oxidant, Immunomodulator, Analgesic and Hepatoprotective activities. The acute toxicity study of *Sharpunkhadi* granules was conducted as per the OECD Guideline for the testing of Acute Toxic Class Method No.423. Results: No mortality was observed in all treated rats at the dose level of 2000mg/kg B.wt. Based on the results, single oral administration of *sharpunkhadi* granules in female wistar rats at a dose level 2000 mg/kg b.wt. did not result in any mortality under the conditions and procedures followed in the study. Hence the LD50 cut off value for the test item was 5000 mg/kg b.wt. or infinitive. Conclusion: It was concluded that the novel herbal *Sharpunkhadi* granules are safe and might be a potential management option for Sickle Cell Anemia.

Keywords: *Ayurved,* Experimental, Sickle Cell Anaemia, Sickle Cell Disease*, Sharpunkhadi granules*.

Introduction

Sickle cell disease (SCD) is the most common monogenic disorders globally with an autosomal recessive inheritance (1). Numerous subgroups exist within the umbrella of sickle cell anaemia (SCA), haemoglobin SC disease (HbSC), and haemoglobin sickle-beta-thalassemia (beta-thalassemia positive or beta-thalassemia negative). Several other minor variants within the group of SCDs also, but not as common as the forenamed varieties. Sickle cell trait (HbAS), a heterozygous mutation rarely has clinical signs and symptoms. SCA is the most common form of SCD with a lifelong affliction for haemolytic anaemia requiring blood transfusions, pain crises, and organ damage (2). Not only in India but globally Sickle cell diseases (SCDs) is a global health burden. It has been estimated that, between 2010 and 2050, about 14.2 million babies will be born with Sickle Cell Anemia (SCA) (3). Three quarters of the total SCD cases of the world occur in Africa, where in few parts, the prevalence of sickle cell trait (SCT) is as high as 30%(4)

The prevalence of sickle cell carriers among different tribal groups varies from 1 to 40 per cent (5). Madhya Pradesh has the highest load with an estimated number of 9, 61,492 sickle heterozygotes and 67,861 sickle homozygote (6). *Gonds* and *Bhils* constitute the largest tribal groups in central India while *Dhodia*, *Dubla*, *Gamit*, and *Naika* tribes in Gujarat reported 13-31 % of high prevalence. Recently a very extensive population surveys have been done by the Indian Red Cross Society, Gujarat State Branch where 1,68,498 tribals from 22 districts were screened and the overall prevalence of sickle cell carriers was 11.37 per cent they reach adulthood (7). As per a survey conducted by ICMR (Indian Council of Medical Research), about 20% of children with SCD expired by age of two and 30% of children with SCD among the tribal community

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die before they reach adulthood (14 years) and the remaining 70% die by the age of 50 (8,9).

The conventional therapy with Hydroxyurea, L-glutamine, Crizanlizumab have the potential to improve survival and quality of life of patients may result in organ dysfunction and painful episodes. The only curative approach for this disease till date are Stem cell and Gene therapy (10).

An essential anticancer drug, Hydroxyurea is one of the available promising drugs presently used for this condition. Painful crises and acute chest syndrome in adults can be treated with hydroxyurea and to lessen the need for blood transfusions. Hydroxyurea works by increasing the foetal haemoglobin in the RBCs. Being a potential anticancer drug it has its own side effects like decreased production of platelets, red blood cells and white blood cells etc. The effect of long-term hydroxyurea is yet to be established (11).

Modern medical science started from the quest for its genesis in the genes, aided with the recent advances in the molecular biology and genetics, to know the causative genes etc (12,13).

However, these are at present still in experimental stages, till they become available, approachable, and affordable reality, the palliation of this courage of mankind named Sickle cell disorder, lies in the basic amenities of body ache & Painful Crisis, Avascular necrosis, Recurrent infections and Blood transfusion, so the need to accept and prevent appropriately or manage the associated complications of this disease.

World Health Organization have emphasized role of various herbal medicines in the management of diseases along with standard guidelines of their usage (14). This study was conducted to find the safety parameters in search of formulate a novel herbal formulation for Sickle Cell Anemia. *Sharpunkhadi* granules are novel herbal granules made of eight plants which are *Sharpunkha (Tephrosia purpurea Linn), Vikankat (Gymnosporia montana Roth.) Benth), Punarnava (Boerhaavia diffusa Linn), Guduchi (Tinosphora cordifolia Willd), Amalaki (Phyllanthus embelica L), Bumyamlaki (Phyllanthus niruri Linn), Ikshu (Saccharum officinarum Linn), Gorakhmundi (Spharanthus indica Linn.).*

Materials and Methods

The raw drugs were procured from local vendor at Vadodara. Identification and authentication of raw drugs was done by Raw Drug Identification Committee (RDIC) of Parul Institute of Ayurveda, Vadodara and *Sharpunkhadi granules* were prepared at GMP certified Parul Ayurved Pharmacy, Vadodara. Standardization of granules was carried at Institutional Quality Control Laboratory as per the guidelines of Ayurveda Pharmacopeia of India (API).

Table 1: Ingredients of Sharpunkhadi granules

Sr. No.	Drug	Latin Name	Part Used	Quantity
1	*Sharpunka*	*Tephrosea purpurea Linn.*	whole plant	1 Part
2	*Vikankat*	*Gymnosporia montana (Roth.) Benth*	whole plant	1 Part
3	*Punarnava*	*Boerhavia diffusa Linn.*	whole plant	1 Part
4	*Guduchi*	*Tinosphora cordifolia Willd.*	Stem	1 Part
5	*Amalaki*	*Emblica officinalis Gaertn.*	Fruit	1 Part
6	*Bhumyamlaki*	*Phyllanthus niruri Linn.*	whole plant	1 Part
7	*Ikshu*	*Saccharum officinarum Linn.*	Stem	1 Part
8	*Gorakhmundi*	*Spharanthus indica Linn.*	whole plant	1 Part
9	*Ghrita*	Cow's Ghee	-	Q.S.
10	*Sharkara*	Sugar	-	4 Part

Preparation of Sharpunkhadi granules

Preparation of *Sharpunkhadi* Granules was carried out at GMP certified Pharmacy of Parul Institute of Ayurved, Vadodara, Gujarat. Freshly prepared juices of Amalaki and Ikshu was poured in a vessel. Powdered sugar was added into it and heated till it attains one stranded consistency. Fine powders of all the other drugs were mixed into it and stirred continuously. The mixture was added in granulating machine to form granules. The granules were sealed in airtight container and labelled.

Study Guidelines

The study was conducted in compliance with the OECD Guideline for the testing of chemicals. Acute Oral Toxicity: Acute Toxic Class Method No.423, Adopted 17 December 2001 (15).

The Limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. As the ingredients of Sharpunkhadi granules are non-toxic. No any individual drug toxicity of granules has been reported till date. The present Limit test was carried out to find out the acute oral toxicity of self-formulated combination Sharpunkhadi granules.

Study Duration and Protocol Number

The study was performed in 2021 and the protocol number "ARL/PT/175/2021"

Experimental Procedure

Justification of Selection System

The Wistar rat (*Ratus norvegicus*) was selected as the test system because it is a readily available rodent species. It has been historically shown to be a suitable model for acute oral toxicity assessment and is recommended by OECD and other regulatory authorities (16).

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Test system details

- Species: Wistar Rat (*Rattus norvegicus*)
- Sex: Female (nulliparous and non-pregnant)
- Age: 8 to 9 weeks
- Body weight range: 250gm -300gm
- No. of animals: Three [3] animal /step (total 6 animal)
- Source: Rodent Research India Private Limited, Haryana.
- Dose: 2000 mg/kg body weight (Limit test dose for toxicity study as per the guidelines of OECD 423
- IAEC Protocol no ARL/PT/175/2021)

Grouping

Table 1: Grouping of Animals

Group	Sex	Number of animals	Dose
Step 1	Female	3	2000 mg/kg body weight.
Step 2	Female	3	2000 mg/kg body weight.

Housing of Animal

Animals were housed in groups (3 animal per cage) in clean, sterilized Poly-carbonate cages having provision for holding pelleted food and drinking water in bottle with stainless steel nozzle throughout the study period. The quantity and quality of feed and water is regularly monitored. Cages and water bottles were cleaned as per the Standard Operating Procedure (SOP) of Good Laboratory Practice (GLP) (17).

Environmental Conditions

Animals were maintained under the following environmental conditions:

- Temperature: 19.1 – 23.1 ˚C
- Relative humidity: 38-55%
- Light/dark cycle (photoperiod): 12 hour light and 12 hour dark cycle

Experimental Procedure

The study was carried out in 2 steps. In each step 3 female rats were dosed. The gap of approximately 48 hours was kept between step 1 and step 2. Dose volume was calculated based on overnight-fasted body weight on the day of dosing for each animal. The test formulation was administered by oral gavage. As per the Limit test, dose was decided as 2000 mg/kg body weight.

Observations and Results

The rats were observed for Mortality/Morbidity twice daily throughout the study period, General clinical signs at 30 minutes, 1 hour, 2 hour and 4 hour post administration on day 0 and thereafter once daily until end of observation period. Observation also includes changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems and somato-motor activity and behaviour pattern. Attention was directed to observation of tremors, salivation, diarrhoea, lethargy, sleep, convulsion and coma. Body weight was recorded on day 0 prior treatment and on day 7 and day 14 after treatment.

Haematology was performed for all animals at prior to treatment and on day 14 post dose.

Blood was collected from overnight fasted animals through orbital plexus method. Blood collected in tubes containing EDTA for CBC and in tubes without anticoagulant for Renal and Hepatic function test. Urine was collected for Urine routine and micro test.

At the end of the observation period Gross Pathology was done. All surviving animals were euthanized by CO2 asphyxiation followed by exsanguination and subjected to gross necropsy, including examination of external surfaces, orifices and thoracic/abdominal cavities.

Results

Mortality/Morbidity

No mortality was observed in all treated rats at the dose level of 2000mg/kg body weight

Table 2: Individual animal Mortality and Morbidity Observation

Animal No.	Group	Sex	Dose (mg/kg	Day (At Morning/Evening)														
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Step 1	F	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2		F	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3		F	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Step 2	F	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5		F	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6		F	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

F= Female, - No mortality/morbidity

Clinical Signs Observation -

All animal treated with *sharpunkhadi* granules were found normal throughout the study period.

Table 3 , 4: Individual Animal Clinical signs

Animal No.	Group	Sex	Dose (mg/kg body weight)	Day 0				Day 1	Day 2	Day 3	Day 4
				30 min.	1 hr.	2 hr.	4 hr.				
1	Step 1	F	2000	1	1	1	1	1	1	1	1
2		F	2000	1	1	1	1	1	1	1	1

3		F	2000	1	1	1	1	1	1	1	1
4		F	2000	1	1	1	1	1	1	1	1
5	Step 2	F	2000	1	1	1	1	1	1	1	1
6		F	2000	1	1	1	1	1	1	1	1

1= Normal, F=Female

Animal No.	Group	Sex	Dose (mg/kg body weight)	Days									
				5	6	7	8	9	10	11	12	13	14
1		F	2000	1	1	1	1	1	1	1	1	1	1
2	Step 1	F	2000	1	1	1	1	1	1	1	1	1	1
3		F	2000	1	1	1	1	1	1	1	1	1	1
4		F	2000	1	1	1	1	1	1	1	1	1	1
5	Step 2	F	2000	1	1	1	1	1	1	1	1	1	1
6		F	2000	1	1	1	1	1	1	1	1	1	1

1= Normal, F=Female

Body weight:

Compared to day 0, normal gain in body weight was recorded on day 7 and day 14.

Table 5: Individual Animal Body weight (gram)

Group	Animal No	Dose (mg/kg body	Sex	Body Weight(g)		
				Day 0	Day 7	Day 14
Step 1 (N=3)	1	2000 mg/kg body weight	F	257.0	265.0	272.0
	2		F	231.0	238.0	245.0
	3		F	245.0	249.0	254.0
Mean				244.33	250.67	257.00
SD				13.01	13.58	13.75
Step 2 (N=3)	4	2000 mg/kg body weight	F	230.0	242.0	255.0
	5		F	242.5	249.5	259.5
	6		F	237.0	247.5	260.5
Mean				236.50	246.33	**258.33↑↑**
SD				6.26	3.88	2.93

F=Female, SD=Standard Deviation, ↑↑=Significant increase at 95% confidence interval.

Haematology

There was statistically significant increase observed in White blood cells and absolute count of neutrophils and significant decrease observed in relative count of basophils in female animals of step 2 in comparisons of pre-treatments haematological parameters in all the female animals. The ranges obtained for haematology parameters are within the normal biological range.

Table 6: Haematology Data

Parameters	**Step 1(N=3)**									
	Pre-Treatment					**Post treatment**				
Animal No.	**1**	**2**	**3**	**Mean**	**SD**	**1**	**2**	**3**	**Mean**	**SD**
Red blood corpuscles (RBC) (10^12/L)	6.95	7.12	7.88	7.32	0.50	7.34	8.23	5.32	6.96	1.49
White blood corpuscles (WBC) (10^9/L)	4.25	4.27	10.81	6.44	3.78	3.81	5.20	4.49	4.50	0.70
Hemoglobin (HGB) (g/dL)	15.1	14.8	16.0	15.30	0.62	15.0	18.2	10.6	14.60	3.82
Hematocrit (HCT) (%)	40.3	41.0	44.2	41.83	2.08	44.0	52.1	29.8	41.97	11.29
Platelates (PLT) (10^9/L)	770	767	844	793.67	43.62	800	525	749	691.33	146.29
Polymorphocytes %	26.20	24.88	27.01	26.03	1.08	38.65	26.23	22.14	29.01	8.60
Lymphocytes %	53.77	55.45	46.32	51.85	4.86	44.23	45.09	52.56	47.29	4.58
Eonsinophills %	1.85	2.23	3.95	2.68	1.12	3.74	5.65	6.20	5.20	1.29
Monocytes %	18.07	17.08	22.48	19.21	2.87	13.10	22.65	18.95	18.23	4.82
Basophills %	0.11	0.36	0.24	0.24	0.13	0.28	0.38	0.15	0.27	0.12
Neutrophills(10^9/L)	1.116	1.064	2.922	1.70	1.06	1.474	1.367	0.997	1.28	0.25
Lymphocytes (10^9/L)	2.285	2.367	5.007	3.22	1.55	1.685	2.344	2.359	2.13	0.38
Eonsinophills (10^9/L)	0.078	0.095	0.426	0.20	0.20	0.142	0.293	0.278	0.24	0.08
Monocytes (10^9/L)	0.767	0.729	2.430	1.31	0.97	0.499	1.177	0.850	0.84	0.34
Basophills (10^9/L)	0.004	0.015	0.025	0.01	0.01	0.010	0.019	0.006	0.01	0.01
Red blood corpuscles (10^12/L)	6.95	7.12	7.88	7.32	0.50	7.34	8.23	5.32	6.96	1.49

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Parameters	Step 2(N=3)									
	Pre-Treatment					Post treatment				
Animal No.	**4**	**5**	**6**	**Means**	**SD**	**4**	**5**	**6**	**Mean**	**SD**
Red blood corpuscles (RBC) (10^12/L)	7.17	8.06	6.97	7.40	0.58	7.25	7.88	7.12	7.42	0.41
White blood corpuscles (WBC) (10^9/L)	2.85	3.59	2.77	3.07	0.45	4.00	6.00	5.96	**5.32**↑	1/14
Hemoglobin(HGB) (g/dL)	15.3	15.6	15.4	15.43	0.15	14.8	15.9	15.0	15.23	0.59
Hematocrit(HCT) (%)	42.7	45.7	41.9	43.43	2.00	43.0	45.8	43.9	44.23	1.43
Platelates(PLT) (10^9/L)	750	568	813	710.33	127.23	846	707	1044	865.67	169.36
Polymorphocytes %	22.53	25.13	35.13	27.60	6.65	32.90	29.70	38.25	33.62	4.32
Lymphocytes %	57.57	50.05	43.16	50.26	7.21	47.56	51.52	42.27	47.12	4.64
Eonsinophills %	8.11	5.13	3.26	5.50	2.45	5.66	4.23	4.65	4.85	0.74
Monocytes %	11.40	19.15	18.10	16.22	4.20	13.74	14.49	14.74	14.32	0.520.14
Basophills %	0.39	0.54	0.35	0.43	0.10	0.14	0.06	0.09	**0.10**↓↓	0.04
Neutrophills(10^9/L)	0.644	0.904	0.975	0.84	0.17	1.318	1.784	2.281	**1.79**↑	0.48
Lymphocytes (10^9/L)	1.640	1.796	1.195	1.54	0.31	1.902	3.091	2.519	2.50	0.59
Eonsinophills (10^9/L)	0.231	0.184	0.090	0.17	0.07	0.226	0.253	0.277	0.25	0.03
Monocytes (10^9/L)	0.324	0.687	0.501	0.50	0.18	0.549	0.869	0.878	0.77	0.19
Basophills (10^9/L)	0.011	0.019	0.009	0.01	0.01	0.005	0.003	0.005	0.00	0.00
Red blood corpuscles (10^12/L)	7.17	8.06	6.97	7.40	0.58	7.25	7.88	7.12	7.42	0.41

N=Total No. of animals in the group, SD= standard deviation, ↑- significant increase at 95% confidence interval, ↓↓- significant decrease at 95% confidence interval

Renal Function Test and Hepatic Function Test

There was statistical increase observed in Alanine Amino Transferase, Aspartate Amino Transferase, Potassium whereas statistically significant decrease observed in creatinine and calcium in female of step 1 in comparison of pre-treatments biochemistry parameters. Statistically significant decrease observed in Aspartate Aminotransferase and Calcium in female animals of step 2 in comparison of pre-treatment. There was no statistically significant difference observed in other biochemistry parameters in all female animals. The range obtained for biochemistry parameters are within the normal biological range.

Table 7: Renal Function Test and Hepatic Function Test

Parameters	Step 1(N=3)									
	Pre-Treatment (Day 0)					Post treatment (Day 14)				
Animal No	1	2	3	Mean	SD	1	2	3	Mean	Sd
ALT(SGPT)(u/l)	16.61	19.63	21.72	19.32	2.57	80.43	33.62	88.11	**67.39**↑	29.49
AST(SGOT)(u/l)	48.43	54.25	58.32	53.67	4.97	68.85	80.73	84.27	**77.95**↑	8.08
UREA (u/l)	57.74	35.92	33.27	42.31	13.43	23.65	31.09	38.251	31.00	7.30
BUN (u/l)	26.96	16.77	15.54	19.76	6.27	11.04	14.52	17.86	14.48	3.41
Creatinine	0.62	0.65	0.56	0.61	0.05	0.43	0.22	0.36	**0.34**↓	0.11
Sodium (mmol/l)	154.78	139.72	111.09	137.20	18.97	144.99	146.92	148.03	146.65	1.54
Calcium (mg/dl)	11.67	11.72	11.76	11.72	0.05	4.51	10.34	10.73	**8.53**↓	3.48
Potassium	3.91	4.45	3.53	3.96	0.46	7.80	7.45	8.47	**7.91**↑↑↑	0.52

Parameters	**Step 2(N=3)**									
	Pre-Treatment (Day 0)					**Post treatment (Day 14)**				
Animal No	**4**	**5**	**6**	**Mean**	**SD**	**4**	**5**	**6**	**Mean**	**SD**
ALT(SGPT)(u/l)	35.75	24.49	43.66	34.63	9.63	39.95	65.89	40.93	48.93	14.70
AST(SGOT)(u/l)	90.62	92.44	95.21	92.76	2.31	67.34	36.88	38.32	**47.51**↓	17.19
UREA (u/l)	14.78	32.77	29.83	25.79	9.65	17.34	22.85	31.43	23.87	7.10
BUN (u/l)	6.90	15.30	13.93	12.05	4.51	8.09	10.67	14.67	11.15	3.32
Creatinine (mmol/l)	0.39	0.65	0.55	0.53	0.13	0.32	0.13	0.4	0.28	0.14
Sodium (mmol/l)	117.05	147.90	139.99	134.98	16.02	159.56	149.19	113.33	140.69	24.26
Calcium (mg/dl)	12.14	12.21	12.73	12.36	0.32	7.7	7.73	8.06	**7.83**↓↓↓↓	0.20
Potassium (mmol/l)	4.80	6.11	3.73	4.88	1.19	6.63	3.95	6.13	5.57	1.43

N=Total no of animal in the group, Sd=standard deviation, ↓↓↓↓- significant decrease at 95% confidence interval, ↓- significant decrease at 95% confidence interval.

Urine Routine and Micro Test

No drastic changes were observed in any of the urinalysis parameter when compared with pre-treatment. The ranges obtained for urine analysis were within normal biological range.

Table 8: Urine Routine and Micro test

Parameters	Step 1(N=3)					
	Pre-Treatment (Day 0)			Post treatment (Day 14)		
Animal No.	**1**	**2**	**3**	**1**	**2**	**3**
Urobilinogen (µ mol/L)	Normal	Normal	Normal17	17	17	17
Bilirubin (µ mol/L)	Negative	Negative	Negative	16	17	17
Ketone (m mol/L)	Negative	Negative	Negative	7.8	7.8	16
Blood	Negative	Negative	Negative	Negative	Negative	Negative
Protein(g/L)	1/0++	0.3	3.0+++	0.3	1	3
Nitrate	Negative	Negative	Negative	Negative	Negative	Negative
Leucocyte (µ mol/L)	Negative	Negative	Negative	Negative	Negative	Negative
Glucose	Negative	Negative	Negative	Negative	Negative	Negative
Specific Gravity	1.015	1.02	1.01	1.005	1.01	1.01
pH	8	7.5	8.5	6.5	6.5	6.5
Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Clarity	Clear	Clear	Clear	Clear	Clear	Clear

Parameters	Step 2(N=3)					
	Pre Treatment (Day 0)			Post treatment (Day 14)		
Animal No.	**4**	**5**	**6**	**4**	**5**	**6**
Urobilinogen (µ mol/L)	Normal	Normal	Normal	16	16	15
Bilirubin (µ mol/L)	Negative	Negative	Negative	17	16	17
Ketone (m mol/L)	Negative	Negative	Negative	16	7.8	7.8
Blood	Negative	Negative	Negative	Negative	Negative	Negative
Protein(g/L)	1.0++	1.0++	>20.0++++	1	1	3
Nitrate	Negative	Negative	Negative	Negative	Negative	Negative
Leucocyte (µ mol/L)	Negative	Negative	Negative	Negative	Negative	Negative
Glucose	Negative	Negative	Negative	Negative	Negative	Negative
Specific Gravity	1.025	1.015	1.015	1.005	1.005	1.01
pH	7	7.5	8	7.5	7.5	7.5
Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Clarity	Clear	Clear	Clear	Clear	Clear	Clear

Gross Pathology:

No gross pathological changes were found in any of the treated animals, performed on day 14.

Table 9: Individual Animal Gross Pathology Data

Group	Dose (mg.kg body weight)	Sex	Animal No.	Sacrifice	Gross (Macroscopic) Observation
Step 1	2000	F	1	Terminal	No Abnormality Detected
		F	2	Terminal	No Abnormality Detected
		F	3	Terminal	No Abnormality Detected
Step 2	2000	F	4	Terminal	No Abnormality Detected
		F	5	Terminal	No Abnormality Detected
		F	6	Terminal	No Abnormality Detected

Discussion

Based on the results, single oral administration of *sharpunkhadi* granules in female wistar rats at a dose level 2000 mg/kg b.wt. did not result in any mortality under the conditions and procedures followed in the study. Hence the LD50 cut off value for the test item was 5000 mg/kg b.wt. or infinitive. Additionally, considering globally harmonized system (GHS) for the classification of chemicals, the test item was classified under category 5 or unclassified

The present study aimed to evaluate the acute oral toxicity of *Sharpunkhadi* granules, in search of formulate a novel herbal formulation designed for managing Sickle Cell Anemia (SCA). The results

indicated that the granules were well tolerated at the highest dose of 2000 mg/kg body weight, with no observed mortality or morbidity in the tested animals. Clinical signs, including skin and fur condition, respiratory and circulatory functions, and behavioural patterns, remained normal throughout the study period. Additionally, body weight gain was observed in all animals, suggesting no adverse impact on general health. The absence of toxic effects in acute oral toxicity testing supports the safety of *Sharpunkhadi* granules for further pharmacological evaluation.

Haematological parameters revealed a significant increase in white blood cell count and neutrophil levels, which could indicate an immunomodulatory effect of the formulation. The relative decrease in basophils, while statistically significant, remained within normal biological limits, suggesting no adverse haematological impact. Furthermore, no major deviations in liver and kidney function parameters were observed, implying that the formulation does not cause significant systemic toxicity. These findings align with previous studies on individual herbal ingredients, such as *Tephrosia purpurea* and *Tinosphora cordifolia* (18), *Phyllanthus niruri Linn.* (19) which are known for their hepatoprotective and immunomodulatory properties. A recent study on *Boerhavia diffusa Linn* showed for the very first-time anti-sickling effect (20).

Based on these findings, *Sharpunkhadi* granules exhibit a promising safety profile and could be explored further for their therapeutic potential in SCA management. However, chronic toxicity studies, along with detailed pharmacodynamic and pharmacokinetic evaluations, are necessary to establish long-term safety and efficacy. Future research should also focus on clinical trials to assess the effectiveness of this herbal formulation in human subjects. The study underscores the potential of traditional herbal medicine in addressing global health challenges such as Sickle Cell Disease, emphasizing the need for integrative approaches in modern healthcare.

Conclusion

In this study observations of the mortality-morbidity rate, clinical signs, body weight, Haematology (CBC), Renal function test, Hepatic function test, Urine routine and micro, Gross pathology it was concluded that the novel herbal *Sharpunkhadi* granules are safe and it might be a potential management option for Sickle Cell Anemia.

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