ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

TOXICOLOGICAL STUDY OF *SHASHILEKHA VATI* WITH SPECIAL REFERENCE TO ITS CHRONIC TOXICITY STUDY IN ALBINO MICE

Patil Sachin[1], Patil Kavita[2], Narode Sagar[3]*

[1]Associate Professor, *[3]Assistant Professor, P.G. Dept. of Agadtantra, Shree Saptashrungi Ayurveda College, Nashik, Maharashtra, India.

[2]Assistant Professor, Dept. of Kayachikitsa, Shree Saptashrungi Ayurveda College, Nashik, Maharashtra, India.

Received on: 26/07/2015 Revised on: 12/08/2015 Accepted on: 19/08/2015

ABSTRACT

Safety is the major concern today. It mainly depends on the method of preparation. To assess the quality of a finished product, there should be some basic standards as well as methods of preparation. *Shashilekha Vati* is one such Herbo-mineral compound used in the treatment of Vitiligo. A chronic Toxicity Study for *Shashilekha Vati* carried out in the National Toxicology Centre (N.T.C.) Pune to establish the tolerability and to evaluate the effective and non-toxic human dose of *Shashilekha Vati* by assessing the histopathological and subjective changes in animals. Young (6-8 wks old) male and female Swiss albino mice (28-32 gms of weight), bred at the N.T.C. were used for the study. The duration of the chronic toxicity study was of 3 months (90 days). *Shashilekha Vati* was prepared according to the method mentioned in the classical text of *Yogaratnakara*. During the course of the study, all the animal belonging to the entire different groups were observed for food intake, signs of toxicity, histo-pathological study. Clinical parameters (food intake, body weight, sings of Toxicity) indicate safety of *Shashilekha Vati* for Human Dose Group. Histological findings for day 90; for Ten Times Human Dose Group showed degenerative changes in the liver and kidneys. While it shows mild to moderate degenerative changes in Ten Times Human Dose. The study showed positive results as the *Shashilekha Vati* is safe and non toxic when given in human dose.

KEYWORDS: Safety, National Toxicology Centre, Chronic Toxicity, *Shashilekha Vati.*

INTRODUCTION

Ayurvedic practice involves the use of medications that typically contain herbs, metals, minerals or other materials. Health officials in India and other countries have taken steps to address some concerns about these medications. Concerns relate to toxicity, formulations, interactions, and scientific evidence. Ayurvedic medications have the potential to be toxic. Many materials used in them have not been thoroughly studied in either Western or Indian research. Most clinical trials (i.e., studies in people) of *Ayurvedic* approaches have been small, had problems with research designs, lacked appropriate control groups, or had other issues that affected how meaningful the results were.[2] Therefore, scientific evidence for the effectiveness of Ayurvedic practices varies, and more rigorous research is needed to determine which practices are safe and effective. These are the challenges which you have to face or questions you should have in mind before planning any clinical trial for *Ayurvedic* and traditional medicines. However, improper processing/manufacturing of *Ayurvedic* medicines may result into severe toxicity[4]. Recently, heavy metal contamination was also reported in some *Ayurvedic* medicines sold in USA.[5] This raised concerns regarding safety of such products for human use as medicines.[6] A chronic Toxicity Study for *Shashilekha Vati* carried out in the National Toxicology Centre (N.T.C.), Pune.

A) Need for Toxicity study

- To establish the tolerability of *Shashilekha Vati.*
- To evaluate the effective and non-toxic human dose of *Shashilekha Vati.*
- To assess the histopathological and subjective changes in animals.
- To establish the safety of human subjects as per OECD (Organization for economic and commercial Development).

B) Methodology

A) Preparation of study Drug (*Shashilekha Vati*)[1]

- *Shashilekha Vati* was prepared according to the method mentioned in the classical text of *Yogartnakara*.

- *Shuddha Parada* (Mercury) and *Shuddha Gandhaka* (Sulphur) in equal preparations were thoroughly mixed and made into *Kajjali*.
- *Kajjali* was mixed with *Shuddha Tamra Bhasma* taken in equal proportion.
- *Bakuchi Kvatha* (decoction of *Psoralia corylifolia*) was also mixed with the above mentioned ingredients and grinding for about 24 hours.
- This mixture was then dried in a heater.
- This dried mixture was then passed through a sieve to get granules.
- These granules were then loaded in the tablet-making machine to make tables of 1 *Gunja* (125 mg) each. (Table 1)

Table 1: Ingredients of *Shashilekha Vati*

Shashilekha Vati			
Ingredients	***Shuddha Parada***	***Shuddha Gandhaka***	***Tamra Bhasma***
Proportion	1 part	1 part	1 part
Mardana with *Bakuchi kvatha*			

Preparation of Study animals

- Young (6-8 wks old) male and female Swiss albino mice (28-32 grams of weight), bred at the N.T.C. were used for the Study.
- The animals were housed in polypropylene cages with paddy husk as feeding at a temperature of 24-26^{0}C and a relative humidity of 30-70%. A 12: 12 light dark circle was followed.
- All animals had free access to filtered water and a standard polluted laboratory animal diet was given as food to the animals.

All the experimental procedure / Protocols used in this study were in accordance with the guidelines of the Committee for the purpose of control and supervision of experiments on Animals (CPCSEA).

Experimental protocol

- The numbers of animals used in the study were 18 (9 males and 9 females)
- These animals were divided into three different groups namely :
  1. Control Group
  2. Human dose group
  3. Ten times human dose group
- Each group contained 3 male and 3 Female animals
- The duration of the chronic toxicity study was of 3 months (90 days).

(This was because any long-term treatment in man is to be preceded by 3-12 months of chronic toxicity studies in animals as per the prescribed guidelines).

- Calculations for doses administered were based on standard conversion constant (0.0026 times Human Dose) related to Swiss albino mice.

Dose in mice = 0.0026 x 425 mg/day.

= 1.105 mg/day

Thus, each animal received the following dose everyday through a gastric tube. (2 ml syringe with a 14 no. needle curved at an angle of 130). (Table 2)

Table 2: Drug dose animal received everyday through a gastric tube

Groups	**Dose per animal per day**
a) Control group	0.2 ml distilled water
b) Human dose group	1.105 mg *Shashilekha Vati* dissolved in *Bakuchi taila* and *Madhu*
c) Ten times human dose group	11.05 mg *Shashilekha Vati* dissolved in *Bakuchi taila* and *Madhu*

During the course of the study, body weight and food Intake were measured at regular intervals of every seven days.

- Also after dosing all the animals were observed for toxicity signs and mortality.
- On the 90th day of the study, all animals were sacrificed by cervical dislocation.
- Autopsy was performed on these animals with their vital organs like liver and kidney being examined for gross changes as well as for any histological changes

Observation

- During the course of the study, all the animal belonging to the entire different group were observed for the following parameters.

i) Food Intake

- 50 grams of standard polluted laboratory animal diet was kept as food every day, in one cage containing 3 animals.
- The food intake per animal was calculated every 7 days in the following way:

Food given (in grams) - Food remaining (in grams) x 3 = Food consumed.

ii) Body Weight

- Body weight (in grams) for each and every animal was taken at an interval of every seven days.
- The animals were individually weighed on a standard weighing scale.

iii) Signs of Toxicity

All the animals belonging to the different groups were observed every day for the following signs of toxicity:-

- Ataxia
- Excessive Lacrimation
- Convulsions
- Tremors
- Diarrhoea
- Abnormal micturition
- Excessive salivation

iv) Histo Patholgical study

- The histo pathological study of the vital organs like, the liver and the kidneys of all the animals (No.1 to N.18) were done at Pradhan Surgical Pathology Laboratory, Pune.

Table 3: Food Consumption Data (In grams) for Control Group Mice

Mice No.	Sex	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
		0	7	14	21	28	35	42	49	56	63	70	77	84	90
1	M	2.66	16.66	12	10	9.33	6.66	6.66	6.66	7.33	7.33	9.33	9.33	8.66	9.33
2	M	2.66	16.66	12	10	9.33	6.66	6.66	6.66	7.33	7.33	9.33	9.33	8.66	9.33
3	M	2.66	16.66	12	10	9.33	6.66	6.66	6.66	7.33	7.33	9.33	9.33	8.66	9.33
4	F	2.66	11.33	10.66	8.66	7.33	4.66	4.66	4.66	5.33	4.66	7.33	6.66	7	7.33
5	F	2.66	11.33	10.66	8.66	7.33	4.66	4.66	4.66	5.33	4.66	7.33	6.66	7	7.33
6	F	2.66	11.33	10.66	8.66	7.33	4.66	4.66	4.66	5.33	4.66	7.33	6.66	7	7.33

Table 4: Food Consumption Data (In grams) for Human Dose Group Mice

Mice No.	Sex	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
		0	7	14	21	28	35	42	49	56	63	70	77	84	90
7	M	2.66	14	8	7.33	7.33	5.33	7.33	7.33	6.66	6.66	7.33	7.33	6.66	5.33
8	M	2.66	14	8	7.33	7.33	5.33	7.33	7.33	6.66	6.66	7.33	7.33	6.66	5.33
9	M	2.66	14	8	7.33	7.33	5.33	7.33	7.33	6.66	6.66	7.33	7.33	6.66	5.33
10	F	1.33	11.33	8	8.66	7.33	4	4.66	4.66	4.66	4.66	5.33	5.33	4.66	4.66
11	F	1.33	11.33	8	8.66	7.33	4	4.66	4.66	4.66	4.66	5.33	5.33	4.66	4.66
12	F	1.33	11.33	8	8.66	7.33	4	4.66	4.66	4.66	4.66	5.33	5.33	4.66	4.66

Table 5: Food Consumption Data (In grams) for Ten Times Human Dose Group Mice

Mice No.	Sex	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
		0	7	14	21	28	35	42	49	56	63	70	77	84	90
13	M	0.66	8	6	5.33	4.66	3.33	7	7	8	7	6.66	7	6.66	5.33
14	M	0.66	8	6	5.33	4.66	3.33	7	7	8	7	6.66	7	6.66	5.33
15	M	0.66	8	6	5.33	4.66	FD	FD	FD	FD	FD	FD	FD	FD	FD
16	F	0	4.66	7.33	6.66	4.66	3.33	4	4.66	4.66	4.66	5.33	4.66	5.33	5.33
17	F	0	4.66	7.33	6.66	4.66	3.33	4	4.66	4.66	4.66	5.33	4.66	5.33	5.33
18	F	0	4.66	7.33	6.66	4.66	3.33	4	4.66	4.66	4.66	5.33	4.66	5.33	5.33

FD = Found dead; **Note:** Animal no. 15 was found dead on day35.

Table 6: Body Weight Data (In grams) for Control Group Mice

Mice no	Sex	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
		0	7	14	21	28	35	42	49	56	63	70	77	84	90
1	M	36	40	44	46	48	50	50	50	50	50	50	50	50	50
2	M	34	36	40	42	44	46	46	46	48	50	50	50	50	50
3	M	36	38	40	44	46	48	48	50	50	52	52	52	52	54
4	F	32	34	36	38	38	40	40	40	40	42	42	42	42	44
5	F	28	28	30	34	34	34	36	38	36	40	40	40	40	42
6	F	32	34	38	40	40	40	42	44	42	44	44	44	44	44

Table 7: Body Weight Data (In grams) for Human Dose Group Mice

Mice No.	Sex	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
		0	7	14	21	28	35	42	49	56	63	70	77	84	90
7	M	36	38	38	40	40	40	44	46	44	46	48	50	50	52
8	M	30	32	32	34	34	34	36	38	38	40	42	44	46	48
9	M	30	32	32	34	34	36	36	34	36	38	40	44	46	48
10	F	28	30	30	32	34	34	34	34	34	34	34	34	34	40
11	F	28	26	30	32	34	36	34	34	36	38	38	40	40	42
12	F	28	30	30	30	30	32	36	36	32	36	36	36	36	38

Table 8: Body Weight Data (In grams) For Ten Times Human Dose Group Mice

Mice no	Sex	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 90
13	M	36	32	30	34	34	36	36	34	36	34	34	34	34	36
14	M	34	28	26	28	28	30	30	30	30	30	30	30	30	40
15	M	30	26	24	26	24	22	FD	FD	FD	FD	FD	FD	FD	FD
16	F	32	26	24	26	28	30	30	34	30	34	34	36	36	40
17	F	28	30	28	32	32	34	34	36	34	36	36	38	38	42
18	F	28	28	30	34	36	36	36	38	36	38	38	40	40	40

FD = Found dead **Note**: Animal no. 15 was found dead on day 35.

Sings of Toxicity for Control Group Mice

After administering the prescribed doses the animals (No.1 to No 6) appeared normal and showed no clinical signs of toxicity such as ataxia, convulsions, diarrhoea, excessive salivation, lacrimation, tremors, abnormal micturation, death etc. till the end of the study (90 days).

Sings of Toxicity for Control Group Mice

After administering the prescribed doses the animals (No.7 to No 12) appeared normal and showed no clinical signs of toxicity such as ataxia, convulsions, diarrhoea, excessive salivation, lacrimation, tremors, abnormal micturation, death etc. till the end of the study (90 days). The only observation made was that the animals belonging to this group passed black coloured stool during the course of the study.

Sings of Toxicity for Ten Times Human Dose Group Mice

After administering the prescribed doses the animals (No.13 to No 18) appeared normal and showed no clinical signs of toxicity such as ataxia, convulsions, diarrhoea, excessive salivation, lacrimation, tremors, abnormal micturation.

The observation made was that the animals belonging to this group passed black coloured Stool during the course of the study, (90 days) Also animal No 15, was found dead on Day 35.

Table 9: Histopathological Report of Animals Sacrificed On

S. No.	Group	Animal no.	Sex	Liver
1	Control	1	M	NAD
2	Control	2	M	NAD
3	Control	3	M	NAD
4	Control	4	F	NAD
5	Control	5	F	NAD
6	Control	6	F	NAD
7	Human dose	7	M	Mild regenerative activity
8	Human dose	8	M	Mild regenerative activity vacuolated nuclei
9	Human dose	9	M	Sinusoidal congestion
10	Human dose	10	F	Sparse sinusoidal lymphocytes vacuolated nuclei
11	Human dose	11	F	Sinusoidal congestion and mononuclear cells. Few collections of mononuclear cells in lobules
12	Human dose	12	F	Sinusoidal congestion and mononuclear cells and few collections of mononuclear cells in lobules and chuffer's cell hyperplasia
13	Ten times human dose	13	M	Sinusoidal congestion and mononuclear cells and few collections of mononuclear cells in lobules and chuffer's cell hyperplasia
14	Ten times human dose	14	M	Sinusoidal congestion and mononuclear cells chuffer's cell hyperplasia
15	Ten times human dose	16	F	Congestion, sinusoidal mononuclear cells, vacuolated cells, pan lobular necrosis
16	Ten times human dose	17	F	Focal mononuclear cells. Focal liver cell necrosis
17	Ten times human dose	18	F	Focal liver cells necrosis and congestion

NAD:-No abnormality detected.

Table 10: Histopathological Report of Animals Sacrificed on Day 90

S.No	Group	Animal no.	Sex	Kidneys
1	Control	1	M	NAD
2	Control	2	M	NAD
3	Control	3	M	NAD
4	Control	4	F	NAD
5	Control	5	F	NAD
6	Control	6	F	NAD
7	Human dose	7	M	NAD
8	Human dose	8	M	NAD
9	Human dose	9	M	NAD
10	Human dose	10	F	NAD
11	Human dose	11	F	NAD
12	Human dose	12	F	NAD
13	Ten times human dose	13	M	NAD
14	Ten times human dose	14	M	NAD
15	Ten times human dose	16	F	Cloudy focal changes and tubular necrosis
16	Ten times human dose	17	F	Cloudy focal changes and tubular necrosis
17	Ten times human dose	18	F	Cloudy focal change and tubular necrosis casts intubules

NAD:-No abnormality detected.

Table 11: Blood Chemistry Data of Control Group Mice Sacrificed On Day 90

S. No.	Animal No.	SEX	BSL	SGPT	AP	BUN	TP
1	1	M	98	36	28	20	5.8
2	2	M	96	38	26	24	5.6
3	3	M	102	37	24	22	5.9
4	4	F	104	38	26	20	5.6
5	5	F	98	37	28	20	5.4
6	6	F	102	35	22	22	5.2
7	7	M	98	38	26	22	5.9
8	8	M	104	35	26	20	5.6
9	9	M	100	36	24	24	5.4
10	10	F	96	38	24	24	5.4
11	11	F	98	35	22	20	5.0
12	12	F	104	36	26	22	5.2
13	13	M	98	42	27	29	5.6
14	14	M	94	40	24	29	5.8
15	16	F	98	39	26	29	5.0
16	17	F	102	42	24	27	5.2
17	18	F	100	40	26	27	5.0

BSL: Blood sugar level in mg\100ml.
SGPT: Serum glutei pyretic transaminase in u\l
AP: Alkaline phosphate in U\l.
BUN: Blood Urea Nitrogen in mg\100ml.
TP: Total protein in g\100ml.

Result

i) Food Intake

- The food intake in the control group had gradually increased upto 90 days.
- The food intake in the Human Dose Group and Ten Times Human Dose Group had significantly reduced during the course of 90 days.

ii) Body Weight

- Statistically significant changes in weight were seen in the Animals belonging to Human Dose Group and Ten Times Human Dose Group.
- Thus, the groups that received *Shashilekha Vati* at Human Dose and Ten Times Human Dose showed less increase in the weight than the animals belonging to Control Group.

Histopathology study

- The results of the histopathology study was that the animalsNo.1 to 6 (Control Group) did not showed any abnormality in liver and no changes in kidneys.
- Animals No.7 to 13 (Human Dose Group) showed mild degenerative activity, sinusoidal congestion and collection of mononuclear cells in lobules of liver and no changes in kidneys.
- Animals No.14 to 18 (Ten Times Human Dose Group) showed sinusoidal congestion, focal liver

cell necrosis in liver and cloudy focal changes and tubular necrosis in kidneys.

CONCLUSION

Clinical parameters (food intake, body weight, sings of Toxicity) indicate safety of *Shashilekha Vati* for Human Dose Group.

Histological findings for day 90; for Ten Times Human Dose Group showed degenerative changes in the liver and kidneys. Thus, overall *Shashilekha Vati* is safe at Human Dose.

REFERENCES

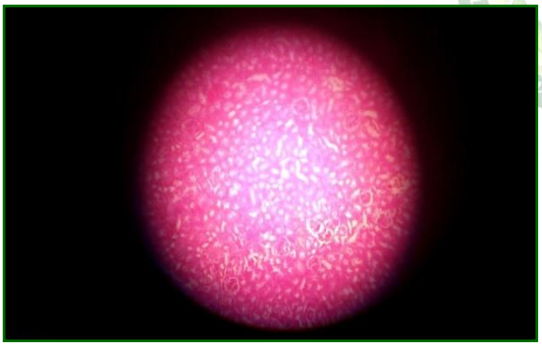
1. Bhikshakratna Shastri, Shribhramhashankar, Yogaratnakar, Chaukhambha Sanskrit Sansthan, 8th Edition, 2004, Kushthachikitsa, Shwitrachikitsa 1, Pg. No. -234.
2. Narayana A, Subhose V. Bull Indian Inst Hist Med Hyderabad. 2005 Jan-Jun;35(1):21-32. Standardization of Ayurvedic formulations: a scientific review.
3. Acharya Jadavji Trikramji. Siddha Yogya Sangrah [Hindi]. Jhansi, India: Baidyanath Bhawan, XIIIth edition, 1935.
4. Saper RB, Kales SN, Paquin J, Burns M J, et.al. (2004) Heavy Metal Content of Ayurvedic Herbal Medicine Products. JAMA 292: 2868 - 2873.
5. Saper RB, Phillips RS, Sehgal A, et.al. (2008) Lead, Mercury, and Arsenic in US - and Indian-Manufactured Ayurvedic Medicines Sold via the Internet. JAMA 300: 915 - 923.
6. Prakash Vaidya Balendu et al Acute and Sub acute Toxicity Study of Ayurvedic Formulation (AYFs) Used for Migraine Treatment International Journal of Toxicological and Pharmacological Research 2010; 2(2).

Cite this article as:
Patil Sachin, Patil Kavita, Narode Sagar. Toxicological Study of Shashilekha Vati with Special Reference to its Chronic Toxicity Study in Albino Mice. International Journal of Ayurveda and Pharma Research. 2015;3(8):95-100.

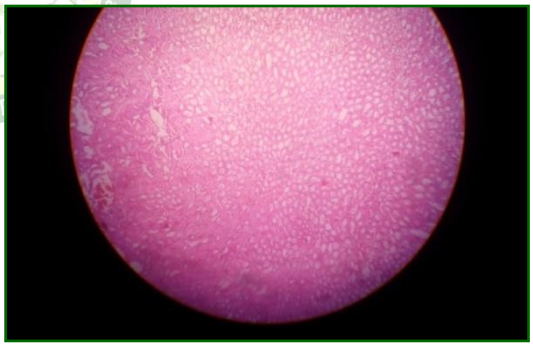
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Sagar Narode
Flat No.2, Siddhivinayak apartment, Tilakwadi, Nashik 422002, Maharashtra, India.
Email: sagarnarode@gmail.com
Mob no. 09028100121.

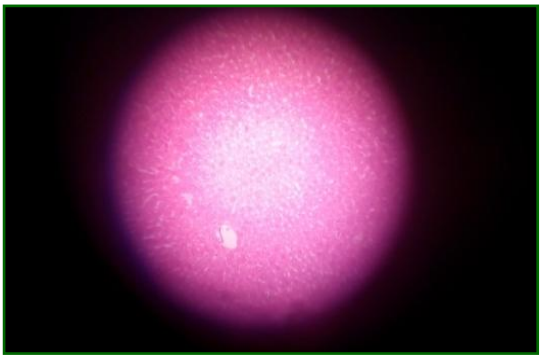
HISTOPATHOLOGICAL SLIDES



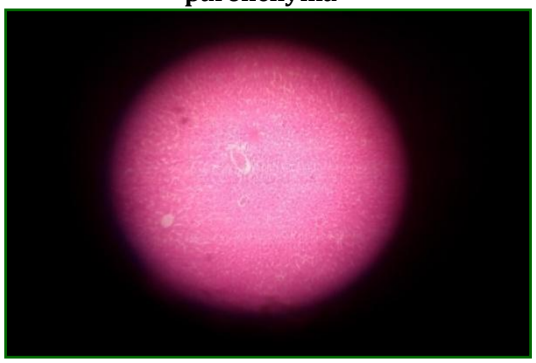
Renal Parenchyma Human Dose – NAD



10 Times human dose Cloudy Focal Change and Tubular Necrosis Renal parenchyma



Hepatic Cells Human Dose Mild Regenerative Activity



10 times human dose Focal Liver cells necrosis and congestion