

Acute Dermal Toxicity study of *Rasapushpadi Malhara* & It's Modified Dosage forms

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Abstract:

Introduction

Rasapushpadi Malahara is one such preparation mentioned in *Rasa tarangini* containing ingredients like *parada*, *saindhava*, *kasisa* and *siktha taila* having indications like *phiranga*, *vicharchika*, *vrana* etc. In the present study The *Rasapushpadi Malhara* prepared by classical method and its modified dosage forms cream and lotion prepared by using chemicals as per modern text.

Material and methods:

Acute dermal toxicity study was carried out as per OECD 402. Wistar albino rats of female sex were used in the experimentation. The rats were obtained from animal house of DMIMS Wardha (MH), The experimental protocols were approved by Institutional Animal Ethics Committee (DMIMS (DU) /IAEC/2018-19/08).

Results and discussion:

Dermal toxicity study results showed weight of all animals of control & test groups was slightly increased or decreased. All animals were found active and healthy no any changes in behavior and activity was observed. All Hematological parameters, Biochemical parameters and Histopathological parameters was normal in range

Conclusion

The Results of this study shows Nontoxic effects of *Rasapushpadi Malahara* and its modified dosage forms (Cream & Lotion). All observed parameters like body weight , cage side observations , DDISS, Hematological parameters, Biochemical parameters and Histopathological parameters was normal in range.

Key words :Rasapushpadi Malahara , Dermal Toxicity, OECD 402 .

Introduction:

The use of metals and minerals in therapeutics in the form of *Rasayoga* has been started from the period of classical texts. However their use has flourished only after the development of *Rasashastra*. Scholars of *Rasashastra* have preferred *Rasayoga* over herbal preparations because of their supremacy in providing quick relief and even treating the incurable diseases.

*Rasapushpadimalhara*¹ is one such preparation mentioned in *Rasa tarangini* containing ingredients like *parada*, *saindhava*, *kasisa* and *sikthataila* having indications like *phiranga*, *vicharchika*, *vrana* etc. In the present study The *Rasapushpadi Malhara* prepared by

classical method and its modified dosage forms cream and lotion prepared by using chemicals as per modern text. After preparation of samples they were evaluated for Acute dermal toxicity .

Rasapushpa and other Rasashatriya preparation are very toxic if they are not prepared properly or given in higher dose level. All the scholars of Rasashastra know about the adverse effect of metal and mineral preparations, if which are prepared improperly. Acharya Sushruta has described the ways to judge the toxicity of the food and drugs before human use and he has used small animals and birds for this purpose. Now a day, drug is allowed to be marketed only after a detailed toxicity and pharmacological evaluation to be assured about safety and efficacy aspects.

Background for selection of Rasapushpadimalaharaandits modified Dosage forms as test drugs for dermal toxicity evaluation:

Mercury and its compounds have a long history in medicine. It is well established fact that exposure to mercury either internally or externally leads to several toxic and adverse effects. Yet since time immemorial mercury has been used in various systems of medicines like Ayurveda after proper purification, incineration and other related processes. Rasapushpa is a mercurial preparation. At the present day scientific requirement a detailed comparative dermal toxicological evaluation was carried out to find safety margin and toxic effect of RasapushpadiMalahara& its modified Dosage forms in experimental animals

Materials and methods:

Animal profile: Wistar albino rats of female sex were used in the experimentation. The rats were obtained from animal house of DMIMS Wardha (MH), The experimental protocols were approved by Institutional Animal Ethics Committee (DMIMS (DU) /IAEC/2018-19/08) in accordance with the guidelines formulated by CPCSEA, India.

Husbandry conditions: The animals were exposed to 12 hours light and 12 hours dark cycle with relative humidity of 50 to 70% and ambient temperature of $22 \pm 3^{\circ}\text{C}$. All animals were kept on same environmental conditions.

Acclimatization period: All the selected animals were kept under acclimatization for 7 days before experimentation.

Diet: Rat & Mice pellets, feed supplied by VRK Nutritional solutions Ltd. was provided throughout the study period. Drinking water was given *ad libitum* in polypropylene bottles with stainless steel sipper tube. The animals were fasted overnight before experimentation.

Route of administration: Dermal application

Dose calculation²: Dose selected as 2000 mg/kg body weight of rats (Limit dose- OECD 402).

Frequency and duration of administration: Single dermal application.

Experimental protocol:

Female Wistar albino rats were divided into four groups containing five rats of each as follows:

- Group I Normal control group (Vehicle Sesame oil)
- Group II RasapushpadiMalahara (2000 mg/kg, dermal)
- Group III Rasapushpadi Cream (2000 mg/kg, dermal)
- Group IV Rasapushpadi Lotion (2000 mg/kg, dermal)

24 hours before the test, furs were removed from the dorsal area of the trunk of the rats by hair clipper and trimmer machines. Care was taken to avoid abrading the skin, which could alter its permeability. Approximately 10 per cent of the body surface area was cleared for the

application of the test drugs. The weight of the animal was taken in to account while deciding the area to be cleared (Derelenko and Hollinger, 1995).

The study was carried out as per limit test of OECD 402. A limit test at dose level of 2000 mg/kg bodyweight was carried out in a group II, III, and IV consisting of 5 female animals each. The test drugs were applied uniformly over an area which is approximately 10 per cent of the total body surface area. Test drugs were held in contact with the skin by dressing with porous gauze and non-irritating tape throughout a 24-hour exposure period. At the end of the exposure period, residual test drugs were removed.

Parameters observed

Following application of the test substance, the rats were observed frequently during the first day and then a careful clinical examination was made once in each day for 14 days on the under mentioned parameters,

- Cage side observations, changes in fur, eyes and mucous membranes, respiratory and circulatory, autonomic, central nervous system and behaviour pattern of rats.
- Observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.
- The time of death if any.
- Individual weight of an animal was taken before the test drugs were applied and on the last day. Changes in weight was calculated and recorded.

Haematological and serum biochemical parameters:

To estimate haematological parameters 0.08 ml blood was mixed with 0.02 ml of EDTA (33.33 mg/ml) and fed to the auto analyzer (ERBA CHEM-5, Trans Asia). The parameters measured were WBC, Haemoglobin, RBC, Platelet count, ESR. For estimation of biochemical parameters, serum was separated from collected blood and requisite quantity of serum was fed to the auto analyzer which was automatically drawn in to the instrument for estimating different parameters. Biochemical parameters like Serum creatinine³, Serum glutamic oxaloacetic transaminase (SGOT)⁴, serum glutamic pyruvic transaminase (SGPT)⁵ were estimated.

Observations & Results:

1. Weight:

Table 1 Showing the effect on body weights in control and test groups :

Group No.	Sex	Body Weight Initial	Body Weight Day 7	Body Weight Day 14
1.	F	220.4 ±3.12	228 ±3.74↑	231.6±3.48↑
2.	F	240.8±16.59	246.8±17.87↑	247.6±17.61↑
3.	F	259.2±15.86	262.8±16.82↑	263.6±20.42↑
4.	F	224.4±9.74	223.2±7.63↓	222.8±9.39↓

Data: Mean+SEM, ↑- increase, ↓- decrease ,

2. Cage side observation:

Table 2 Showing the effect on cage side observations in control and test groups :

Group No.	Sex	Findings		
		Initial	Day 7	Day 14
1	F	Active & Healthy	Active & Healthy	Active & Healthy
2	F	Active & Healthy	Active & Healthy	Active & Healthy
3	F	Active & Healthy	Active & Healthy	Active & Healthy
4	F	Active & Healthy	Active & Healthy	Active & Healthy

3. Draize dermal irritation scoring system (DDISS)⁶

Erythema and Eschar Formation	Score	Edema Formation	Score
No erythema	0	No edema	0
Very slight erythema	1	Very slight erythema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 mm)	3
Severe erythema to slight, eschar formation (injuries in depth)	4	Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Table 3 Showing the effect on DDISS in control and test groups :

Group No.	Erythema and Eschar Formation			Edema Formation		
	Initial	Day 7	Day 14	Initial	Day 7	Day 14
1.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2.	0 ± 0	0.4 ± 0.24 ↑	0.4 ± 0.24 ↑	0 ± 0	0 ± 0	0 ± 0
3.	0 ± 0	0.6 ± 0.24 ↑	0.8 ± 0.2 ↑	0 ± 0	0.4 ± 0.24 ↑	0.6 ± 0.24 ↑
4	0 ± 0	0.4 ± 0.24 ↑	0.4 ± 0.24 ↑	0 ± 0	0 ± 0	0 ± 0

Data: Mean ± SEM, ↑ - increase, ↓ - decrease

4. Hematological Parameters:

Table 4 Showing the effect on Hematological parameters in control and test groups

Group no.	Hb (gm/dl)	WBC (10 ³ /mm ³)	RBC (10 ⁶ /mm ³)	PLT. (10 ³ /mm ³)	ESR
1.	13.72 ± 0.67	2.72 ± 0.20	5.75 ± 0.41	758.8 ± 51.45	4.6 ± 1.53
2.	13.28 ± 0.34	2.74 ± 0.23	5.76 ± 0.16	748.8 ± 86.45	4.6 ± 1.02
3.	12.72 ± 0.17	2.84 ± 0.22	4.15 ± 0.91	606.6 ± 64.19	5.2 ± 1.85
4	12.58 ± 0.14	2.98 ± 0.23	4.93 ± 0.65	725 ± 56.07	3.8 ± 0.66

Data: Mean+SEM

5. Biochemical Parameters:

Table 5 Showing the effect on Biochemical parameters in control and test groups :

Group no.	SGOT(U/L)	SGPT(U/L)	Serum Creatinine (mg/dl)
1.	145.52±11.72	68.10±8.09	0.65±0.06
2.	154.16±18.92	80.37±7.66	0.63±0.05
3.	153.28±14.90	65.27±4.66	0.63±0.03
4	174.67±11.60	57.96±4.30	0.54±0.04

Data: Mean+SEM

6. Histopathological study:

Procedure followed for preparation of histopathological slide⁷:

The skin fixed in 10% formalin solution were later on processed for histopathological studies following the standard procedure described by NIN manual.

Tissue processing:

Tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. The usual dehydrating agent is ethyl alcohol; acetone and isopropyl alcohol can also be used. Following dehydration the tissue was transferred to a paraffin solvent. This is miscible with the dehydrating agent as well.

Tissue were thoroughly washed by placing them under running tap water and then conveyed through a series of following solvents as per schedule for dehydration, clearing and paraffin infiltration. Next the tissues were embedded in paraffin wax to prepare tissue blocks, which are oriented in such a manner that the sections are cut in desired plane of the tissue. Tissues are fixed to metal object holder after trimming them to suitable size.

Section Cutting:

A smear of 5% Mayer's egg albumin was prepared and smeared on to the slide and dried. The tissue sections of the 4-6 μ m thickness were cut with the help of Spencer type rotating microtome. The tissue sections were put on slide and were floated in water on slide between 55-60°C; water drained off and slide dried on hot plate at about 50°C for 30 min. This section is ready for staining.

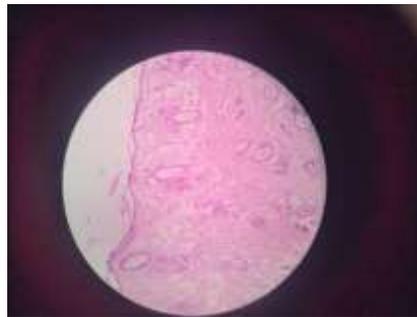
Dermal Histopathological Examination :

Section from skin of Wistar rats stained with H&E showed normal histomorphology in control and all test groups. No evidence of dermal toxicity (Inflammation, Necrosis) seen.(fig.1)

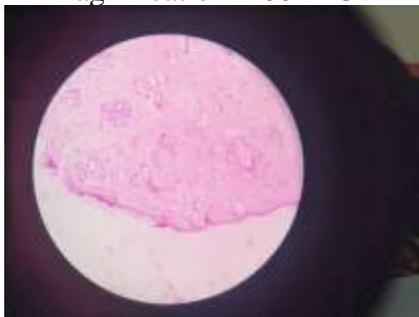
Fig.1 Effects on Dermal Histopathological examination:



Magnification- 100x G1-I



Magnification- 100xG1-II



Magnification- 100x G2-I



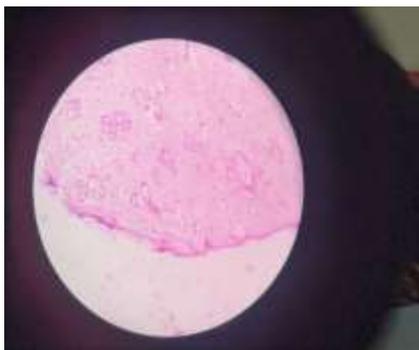
Magnification- 100x G2-II



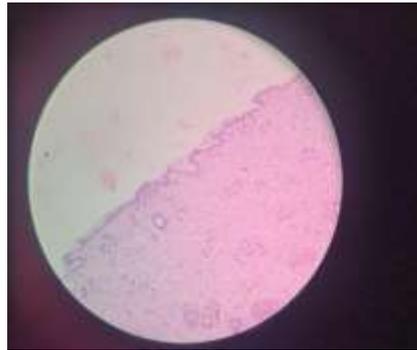
Magnification- 100xG3-I



Magnification- 100xG3-II



Magnification- 100xG4-I



Magnification- 100x G4-II

Discussion:

Dermal toxicity study results showed weight of all animals of control & test groups was slightly increased or decreased after 14 days . It may be due to feeding habits and individual body constitution . All animals were found active and healthy no any changes in behavior and activity was observed . In DDISS two parameters were observed and scored . Erythema & Eschar formation and Edema formation . No significant changes were found in both parameters. In hematological and biochemical parameters Hb, WBC, RBC , ESR , SGOT , SGPT , Sr.Creatinine was observed after 14 days . All parameters were normal in range, no significant changes were found. Histopathological study of dermal section was found No evidence of dermal toxicity like Inflammation and Necrosis of tissue .

Conclusion:

The Results of this study shows Non-toxic effects of *Rasapushpadi Malahara* and its modified dosage forms (Cream & Lotion). All observed parameters like body weight ,cage side observations , DDISS, Hematological parameters, Biochemical parameters and Histopathological parameters was normal in range. To support this conclusion chronic and subchronic dermal toxicity are suggested to be carried out .

References:

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- ¹ Sharma Sadanand, Rasa Tarangini, Hindi Commentary By Pt. KashinathShastri, Edition 11th, MotilalBanarasidas, Delhi, Page no. 113 ,Verse – 6 / 54-55
 - ² OECD Guideline for testing of Chemicals 402 , Adopted 24 feb.1987 .
 - ³ Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method.Scand J Clin Lab Invest 1965; 17:381-87.
 - ⁴Tietz N.W., ed. 1995, Clinical guide to laboratory tests, 3rd ed. Philadelphia, PA: WB Saunders, 76.
 - ⁵Burtis, C.A. and Ashwood, E.R. (Ed).1999, Tietz textbook of Clinical Chemistry, 3rd edition, Philadelphia, P.A. Moss D.W., Henderson A.R.; pp. 652.
 - ⁶Investigation of acute dermal irritation/corrosion, acute inhalation toxicity and cytotoxicity tests for Nanobiocide,M. Hemmati et al.
 - ⁷Raghuramulu N, Nair K.M, Kalyanasundaram S (Eds).A manual of laboratory techniques. National Institute of Nutrition (NIN), Hyderabad India 1983; 246-253.