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An experimental study to evaluate acute dermal toxicity of Rasakriya in Wistar rats

Research Article

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Abstract

Background: *Rasakriya* is a herbo-mineral preparation explained under the heading of *Shashti upakrama* by *Acharya Sushruta* in the management of *Dushtavrana*. Safety of drugs prior to application to see its efficacy is very important as Chronic Wound is more prone for infection. Objective: To evaluate Acute Dermal toxicity of *Rasakriya* on Female Wistar rats. Materials and Methods: The study was conducted as per the OECD guidelines 402. The study was conducted under two Headings i.e. Sighting study and Limit test study. In Sighting study the prepared *Rasakriya* at the dose of 2000 mg/kg body weight was applied on the depilated area and observed for the signs of Toxicity for 24 days, later limit test group animals were similarly treated and observed for 14 days. Observation was done for body weight changes, clinical signs of toxicity and mortality. Results: Dermal application of *Rasakriya* did not exhibit any signs toxicity with respect to Dermal, Circulatory, Respiratory, Central Nervous System and Behavioural pattern. There were no unscheduled deaths reported in animals during the study period, so, the animals were rehabilitated after the completion of the study. Conclusion: *Rasakriya* is safe for application as mortality and signs of toxicity were not observed in the study.

Key Words: *Rasakriya,* Acute dermal toxicity, Sighting Study, Limit test study.

Introduction

Acharya Sushruta in the context of *Vrana* explained *Rasakriya* which is one among the topically used methods. *Rasakriya* means a substance which is in a thick, concentrated, wet form like collyrium is indicated in wounds where taila *upakramas* fail to provide relief and in *sthiramamsa* (1,2). *Rasakriya* are of 2 types, *Shodhana* and *Ropana Rasakriya* which are indicated in the management of *Dushtavrana* (3). *Dushtavrana* is long standing ulcer with slough and profuse discharge. Removal of debris constitutes vital part of the treatment so that applied drug reaches the healthy tissue and promotes granulation tissue. Chronic ulcer creates severe emotional and physical stress and causes a significant financial burden on patients and the whole healthcare system (4). Assessment of Acute dermal dose toxicity is considered as an important part of drug development program to ensure the protection of patients against the possible adverse reaction (5). The OECD guideline 402 defines acute dermal toxicity as adverse effects observed within a short period after dermal application of a single-dose of a test substance. Studies reported that certain herbal medicine prepared from eucalyptus, henna, yohimbine, camphor, aloevera have induce allergic dermatitis and granulomatous dermatitis on application (6). It is essential to undertake toxicological approach of *Rasakriya* for ensuring possible adverse reaction before to consider it as safe. Hence, the present study was conducted to evaluate acute dermal toxicity of *Rasakriya* in Wister rats, in an attempt to establish a safe classical Ayurveda medicine to treat *Dushtavrana* (Chronic ulcers).

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Materials and methods

Experimentation: The Animal study was carried out with the Institutional Animal Ethical Committee Clearance (Ref: BMK/IAEC/Res-20/2020-02). In view of ascertaining the Dermal Toxic characteristics of *Rasakriya*, Acute Dermal Toxicity study was conducted.

Method of Preparation of the *Rasakriya*:
Pre-operative

The required drugs of *Rasakriya* were procured from GMP certified Ayurveda pharmacy. Identification and Authentication of the drugs were done at AYUSH approved testing laboratory. The required materials for the preparation were collected and kept ready.

Materials

- *Paatra* (A large vessel)
- *Koshti* (Stove)
- Match box
- *Darvi* (Spoon)
- *Vastra* (Cloth for filtration)
- *Jala* (Water)
- *Dravya* (Drugs) - Coarse powder of the drugs viz, *Salasaraadi gana dravyas*, *Patola*, *Triphala* in equal quantity
- *Udukhala yantra* (Pounding machine)

- Weighing machine
- Prakshepaka dravyas – Shodhita Sphatika, Kasisa, Manahshila, Haratala
- Madhu and Matulunga swarasa

Operative

One part of the coarse powder *(kwatha churna)* of the drugs, of which *Rasakriya* has to be prepared, was taken in a large vessel and 16 parts of the water is added to it. Then it was kept on the stove *(koshti)* and boiled on a low flame *(Mandagni),* till it is reduced to 1/8th. Then it was filtered and the filtrate was taken in a fresh vessel and again boiled in a water bath till it became semisolid *(Ghanatwam)*.

To this the following *prakshepaka dravyas* were added after *shodhana*,

1. *Haritala* - Purification was done by *swedana* in *churnodaka* for 3 hours
2. *Manahshila* - *Bhavana* in *Ardraka swarasa* for 7 times
3. *Sphatika* - *Bharjana* in *Sharava*
4. *Kasisa* - *Bhavana* in *Bhringaraja swarasa* 1 time

Then this semisolid substance was taken out of the flame and allowed for drying in hot air oven at 60 to 70°C. After the substance was dried properly, the mass was grinded and it was made into powder form and collected and stored in an air tight container.

Post–Operative

The formulation was then made in paste form prior to application on *Vrana* by mixing with *Madhu* and *Matulunga swarasa*.

Acute dermal toxicity study

The study was conducted according to the OECD guidelines 434 (7).

Preparation of Animals

Experimental animals were procured from Animal House, KLEU JNMC, Belagavi. All animals were acclimatized to the laboratory conditions for seven days prior to the start of the study. All animals were housed in colony cages at an ambient temperature of 22±3ºC and relative humidity at least 30% and preferably not exceed 70% with artificial lighting, the sequence being 12 hours light, 12 hours dark. They were provided with free excess of standard pellet diet (Amrut feeds, VRK scientists choice Laboratory animals feed, Baramati, Supplied by Sai durga feeds and foods, Bangalore) and fresh water *ad libitum*. So as to maintain hygienic condition, the floor bed was replaced every day. Five female rats were selected for the study. 10 % of the total body surface area was shaved from the dorsal area of the trunk, 24 hours before the application of test drug. Each animal was properly numbered and identified by marking with saturated picric acid.

Study Design

a) Number of Animals : 5 (female rats)
b) Number of groups : 2
c) Weight : 150-200 gm
d) Route of application : External
e) Duration of study : 15 days
f) Identification of Animal: Each animal cage was properly numbered and identified by marking

Table No.1: Study Groups with number of Animals

Sl.No.	Test Study	No. of Animals	Test Drug	Observation
1	Sighting study	1 female rat	*Rasakriya*	For 24 hours
2	Limit test study	4 female rats	*Rasakriya*	For 14 days

Sighting Study

- **Dose Selection:** The test drug was selected 2000 mg/ kg body weight of rats.
- **Method of Application:** Test drug was applied to exposed skin with spatula and it was maintained in contact with the skin with a porous gauze dressing and non-irritating tape throughout 24 hour exposure period. After 24 hours skin was washed with Luke warm water and wiped with gauze.

Observations

Animal was observed immediately after application once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and observed for any signs of toxicity. Animal was observed for 24 hours in sighting dermal toxicity study, no signs of toxicity were observed; hence, remaining four rats were selected for limit dermal toxicity study.

Limit Test Study

The test substance was taken 2000 mg/kg body and it was applied to exposed skin of next four female rats with spatula and it was maintained in contact with the skin with a porous gauze dressing and non-irritating tape and observed for every 24 hours up to 14 days for any signs of toxicity. All observations were systematically recorded, with individual records being maintained for each animal.

Parameters selected for observation (8)

- Behavioural pattern.
- Body weight - Individual weight of animals was determined on the day of application of the test substance and weekly thereafter and record was maintained for each animal.
- Food and water intake
- Urine and faecal matter
- Changes in skin (rashes, secretions, inflammation etc.) fur, eyes.
- Tremors, Convulsions, Salivation, Diarrhoea, Lethargy, Sleep and Coma.

Observations

The Study was started with application of test substance and observed for signs of toxicity, food and water intake, behavioural pattern, body weight for a

period of 14 days. The following parameters were assessed.

Behavioural pattern

- Immediately after application of test drug animals were observed frightened as depilation was done prior to study and after application animals were found silent.
- Animals were found silent with minimal physical activity with normal food and water intake during first 24 hrs.
- No changes in behavioural pattern were observed in all experimental animals throughout the study.

Food and water intake

- Reduction in intake of food and water was observed immediately after application.
- No change in food and water intake was observed during first 24 hrs. Food and water intake was found normal throughout the study.

Body weight

- Prior to Administration - Animals weighing within 150-200 gm were chosen for study.
- No abnormal change in the body weight was seen. Normal increase in animal body weight was observed. Animals gained 5-7 gm weight during this study period.

Fig No.1: Fur removed from the dorsal area of the trunk

Fig No.2: Instrument Tray

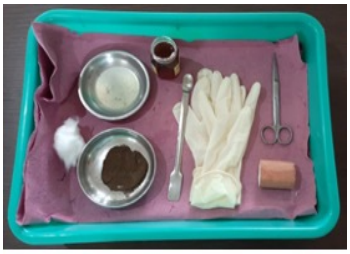

Fig No.3: Drug application

Fig No.4: Covering with porous gauze

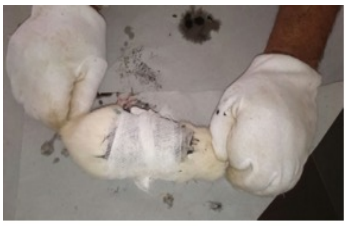

Table No.2: Observations for Signs of Toxicity in relation to Skin, Fur, Eye

Sl. No.	Changes in Skin	1st half hour	2nd half hour	2nd hr–24hr	2nd day – 14th day
1	Blanching	Absent	Absent	Absent	Absent
2	Cyanosis				
3	Erythema				
4	Itching				
5	Falling of fur	Absent	Absent	Absent	Absent
6	Discoloration				
7	Piloerection				
8	Ptosis	Absent	Absent	Absent	Absent
9	Exophthalmos				
10	Lacrimation				
11	Redness				
12	Pupil constricted				
13	Pupil dilated				
14	Viscid	Absent	Absent	Absent	Absent
15	Watery				
16	Depression	Absent	Absent	Absent	Absent
17	Stimulation				
18	Failure				

Table No.3: Observations for Signs of Toxicity in relation to Behavioural factor, Central Nervous System

Sl. No.	Behavioural pattern	1st half hour	2nd half hour	2nd hr – 24hr	2nd day – 14th day
1	Restlessness	Absent	Absent	Absent	Absent
2	Grooming				
3	Lying flat on belly				
4	Lying flat on side				
5	Lying flat on back				
6	Sleeping				
7	Defecation	Absent	Absent	Absent	Absent
8	Urination				
9	Squatting				
10	Ataxic gait				
11	Timidity				
12	Writhing				
13	Tremors				
14	Paresis of hind limbs				
15	Paresis of forepaws				
16	Twitches				
17	Convulsions • Clonic • Tonic • Rolling and jumping convulsions				

Results

Table No.4: Showing Mortality Data, Body weight and Signs of Toxicity

Mortality Data																
Sl. No.	**Group**	**Total no. of Animals**			**Dose**			**Percent mortality (up to 15 days)**								
1	Sighting Study	1 Female Rat			2000 mg/kg			0								
2	Limit Test	4 Female Rats			2000 mg/kg			0								
Average Body Weight																
Sl. No.	**Group**	**Dose (kg/body weight)**			**Body weight**											
1	Sighting Test	2000 mg			**Day 0**			**Day 7**						**Day 14**		
					171 gm			176 gm						180 gm		
2	Limit Test	2000 mg			170 gm			175 gm						181gm		
Signs of Toxicity																
Sl. No.	**Group**	**Days**														
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	Sighting Test	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
2	Limit Test	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/1 – Number of Animals exhibited signs / Number of Animals dosed																

Discussion

Rasakriya is a treatment modality mentioned under *Shasthi Upakrama* in the management of *Dushtavrana (9)*. There is a general consideration in the society that all medicinal plants are safe for consumption and application is risky, as researchers have discovered compounds of certain plants could result in toxicity (10). Nowadays Dermal care products are getting high interest and acceptance among individuals considering it as safe for application, but scientific evidence particularly on dermal toxicity study is very essential to claim its safety (11). Apart from that, toxicity study offers data on the doses or concentrations range for concocting of safe products (12). The management of *"Vrana"* was given utmost importance in classics and *Rasakriya* is one such preparation indicated for the *shodhana* and *ropana* of *Dushtavrana (13)*. Non-healing ulcers are often challenging due to infection so, before application its dermal toxicity is essential to prove whether it is safe or not, later its effectiveness can be emphasized. The study showed that there was normal gain of body weights, feed and water intake, behavioural pattern. There were no unscheduled deaths reported in animals during the study. *Rasakriya* is non-toxic in nature so the signs of toxicity were not observed until the end of the study. Pednekar et al. reported that no changes in the feed intake, water consumption, body weight on topical application of essential oil on rat's skin once daily for 28 consecutive days (14).

Conclusion

Acute dermal toxicity study of *Rasakriya* concludes that, it did not exhibit any allergic skin and systemic toxic reactions so, it is safe for external application.

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