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Research Article

AN EXPERIMENTAL STUDY TO EVALUATE ACUTE DERMAL TOXICITY OF *DHATAKYADI* YOGA IN WISTER RATS

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ABSTRACT

Dhatakyadi yoga is a herbal preparation indicated in Agnidagda Vrana (Burns) which reduces daha (burning sensation), sphota (Blisters), vedana (pain) and has *Vranaropana* (wound healing) action. Prior evaluating its topical effectiveness, the dermal safety has to be established as burn wound is more prone for infection. The present study focuses on establishing the acute dermal safety of *Dhatakyadi yoga* (Mixture of *Woodfordia fruticosa* powder and Linseed oil) on wistar rats. To determine the acute dermal toxicity of the *Dhatakyadi yoga* (DY) in female wister rats. The test substance was applied to depilated area of sighting group animal at the dose of 2000 mg/kg body. Limit test group animals were similarly treated for 14 days. Following dosing, the rats were observed for mortality and clinical signs of toxicity. Body weight was noted weekly. At the end of 14 days observation period all animals were subjected for necropsy and sent for histopathological study. No visible signs of toxicity, such as changes in the respiratory, circulatory, central nervous system, behavioral pattern were observed in the study. Gross pathological examination did not reveal any lesion that could attribute to the toxicity of the substance. Since no mortality was observed in the study, under the condition of this test, it is concluded that dermal LD50 of *Dhatakyadi yoga* (Herbal wound healing formulation) for Wister rats was ≥ 2000 mg/kg body weight.

Keywords – *Dhatakyadi yoga*, Acute dermal toxicity, sighting study, Limit test study.

INTRODUCTION

Dhatakyadi yoga is wound healing preparation which mainly contain *Dhataki pushpa churna* (*Woodfordia fruticosa* powder) and *Atasi taila* (*Lusitissimum oil*) as ingredient. *Dhataki* Flowers are proved to have Anti bacterial, Antihelmithic, Anti tumor activity, Styptic, Anti pyretic¹. *Lusitissimum oil* has been reported to exhibit significant anti-inflammatory, anti arthritic, antiulcer properties².

Adverse effects of plants on skin reviewed include: irritant contact dermatitis or by irritant chemicals in plant sap; phytophotodermatitis resulting from plants containing furocoumarins, immediate (type I) or delayed hypersensitivity contact reactions mediated by the immune system in individuals sensitized to plants or plant products (e.g. peanut allergy, poison ivy (*Toxicodendron*) poisoning)³. Dermal toxicity is important initial test done before assessing topical effectiveness. The dermal risk is considered to be related to the amounts of penetration and toxicity and the state of the test substance (Mattie *et. al.*, 1994)⁴. It is timely and appropriate to endeavour toxicological approach to *Dhatakyadi yoga* for the possible adverse effects with the intent of using this yoga as potential external application in burns

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Hence study is taken to evaluate dermal toxicity in wister rats.

MATERIALS AND METHODS

Experimentation:

The animal studies were carried out with the institutional animal ethical committee clearance (Ref: BMK/IAEC/Res-07/2012). In view of ascertaining the dermal toxic characteristics of *Dhatakyadi yoga*, acute dermal toxicity study was conducted.

Preparation of Dhatakyadi yoga⁵

DY was prepared by mixing one part of drug (*Woodfordia fruticosa* flower) and sufficient

quantity of *Atasi taila* to form paste of thin consistency and applied over dermal surface.

Preparation of animals

The animals were acclimatized to the laboratory conditions for at least five days prior to the start of the study with room temperature of $22\pm 3^{\circ}\text{C}$ and relative humidity at least 30% and preferably not exceed 70% with artificial lighting, the sequence being 12 hours light, 12 hours dark. Approximately 24 hours before the study, 10 % of the total body surface area was made clear for the application by depilating fur from the dorsal area of the trunk⁶.

Study Design

Table No.1

No.	Test Study	No. of animals	Weight	Test drug	Observation
1)	Sighting study	1 female rat	180-200 gm	Dhatakyadi yoga lepa	For 24 hrs
2)	Limit test study	4 female rat	180-200 gm	Dhatakyadi yoga lepa	For 14 days

SIGHTING STUDY:

The test substance was taken 2000 mg/kg body weight of rats and applied to exposed skin and held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period, after completion of 24 hours skin was washed with luke warm water and wiped away with gauze.

Observations:

Animals were observed immediately after dosing at least 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 were observed for 24 hours there was no signs of toxicity so study was proceeded with limit test.

Table No.2- Evaluation of skin reactions⁷

Sl no.	Evaluation of skin reactions ⁷	Value
	Erythema and Eschar Formation	
1.	No erythema	0
2.	Very slight edema(barely perceptible)	1
3.	Well defined erythema	2
4.	Moderate to severe erythema	3
5.	Severe erythema(Beef redness) to slight eschar formation(injuries to depth)	4

Sl no.	Oedema Formation	Value
1.	No edema	0
2.	Very slight oedema(Barely perceptible)	1
3.	Slight oedema(edges of area well defined by definite raising)	2
4.	Moderate oedema(raised approximately 1 millimetre)	3
5.	Severe oedema(raised more than 1 mmm and extending beyond the area of exposure)	4

Observations for Signs of Toxicity⁸

- Changes in Fur- Falling of fur, Discoloration, Piloerection
- Changes in Eyes-Ptosis, Exophthalmus, Lacrimation, Redness, Pupil constricted, Pupil dilated
- Salivation – Viscid, Watery
- Respiration- Depression, Stimulation, Failure
- Behavioral pattern- Restlessness, Grooming Lying flat on belly, lying flat on side, Lying flat on back, Sleeping
- CNS- Defecation, Urination, Squatting, Ataxic gait, Timidity, Writhing, Tremors, Paresis of hind limbs, Paresis of forepaws, Twitches, Convulsions (Clonic, Tonic)

Limit test study:

The test substance was taken 2000 mg/kg body and applied to next four female rats and observed for every 24 hours up to 14 days for any signs of toxicity. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for signs of toxicity to be delayed. All observations were

systematically recorded, with individual records being maintained for each animal.

Body weight:

Individual weights of animals was determined on the day of administration of the test substance, weekly thereafter and record was maintained for each animal.

Pathology

All test animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal.

Statistical Analysis:

Paired 't' test– To know the pre treatment and post treatment effect of skin reactions in both the groups.

DATA AND REPORTING

Individual animal data was summarized in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity and necropsy findings.

RESULTS

Table No.3- Mortality Data

Sl no.	Group	Total no. of animals	Dose	Percent mortality (upto 15 days)
1)	Sighting study	1 female	2000 mg/kg	0
2)	Limit test	4 female	2000 mg/kg	0

Table No.4 - Signs of Toxicity

Sl no	Group	Days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Sighting	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	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

Table No.5 - Skin reactions

Sl no.	Dose	Animals	Observations	Initiation of exposure(hrs)				Effects noted after initiation of exposure (days)		
				1/2	1	2	4	1	7	14
1	2000 mg/ kg	Sighting test (n=1)	Erythema	0	0	0	0	0	0	0
			Oedema	0	0	0	0	0	0	0
2		Limit test (n=4)	Erythema	0	0	0	0	0	0	0
			Oedema	0	0	0	0	0	0	0
'0' – No reactions										

Table No.6 - Body Weight

Sl no.	BODY WEIGHT		
	Day 0	Day 7	Day 14
1	176 gm	180 gm	184gm
2	178gm	181 gm	184 gm
3	179 gm	180 gm	185 gm
4	179 gm	181 gm	186 gm
5	179 gm	181 gm	185 gm

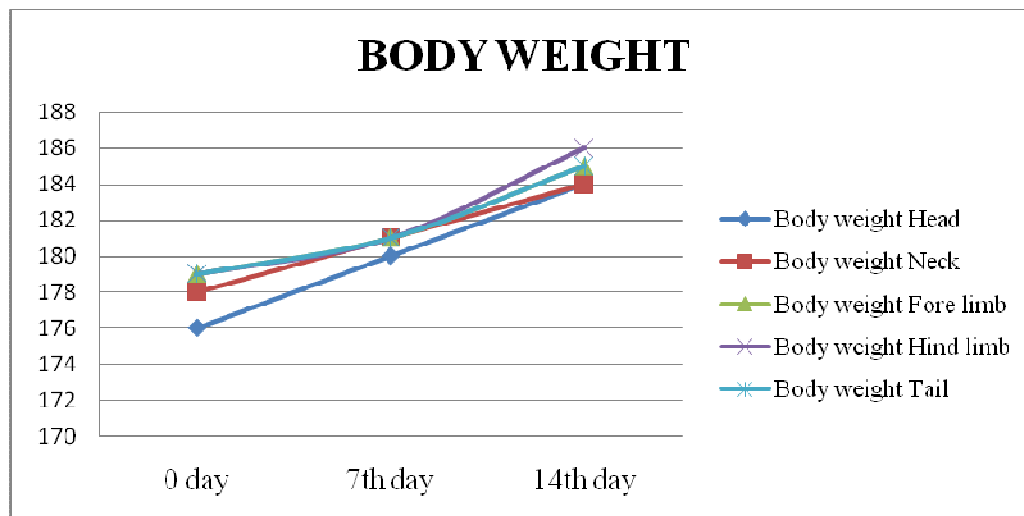


Table No.7 - Histopathological findings-

	Organ					
	Skin	Spleen	Lungs	Heart	Liver	Kidney
Microscopic Findings	Re-epithelization	Red pulp	Congestion	Necrosis	Vein congestion	Tubular congestion
	Dermal Edema	White	Interstitial edema	Congestion	Sinus congestion	Loss of brush border
	Dermal congestion	Congestion	Interstitial Pneumonia	Interstitial edema	Focal Haemorrhage	Tubular cell swelling
	Dermal inflammatory infiltration	Lymphoid follicles	Pulmonary Haemorrhage	Inflammatory cells	Portal triaditis	Tubular cytoplasmic vacuoles
	Giant cells	Capsule	Pulmonary edema	Intravascular haemolysis	Inflammation	Tubular Desquamation
	Neutrophilic infiltration	Fibrous septae	Exudates in alveoli	Myofibrils Fragmentation	Centrilobular degeneration	Tubular Degeneration
	Lymphocytic infiltration	Vessels	Perialveolar Lymphocytes	Focal Haemorrhage	Spotty necrosis	Peritubular inflammation
	Macrophages		Emphysema	Cytoplasmic vacuolation	Centrilobular necrosis	Tubular necrosis
	Dermal granulation tissue			Over all damage	Perivenular fibrosis	Glomerular Congestion
	Dermal fibroblasts				Acidophilic Bodies	Glomerular Atrophy
	Dermal collagen/ Fibrosis				Kupffer cell Hyperplasia	Interstitial edema
Inference	Normal	Normal	Normal	Normal	Normal	Normal

DISCUSSION

Lepa (Topical application) is a treatment modality practiced by ancient *Acharya* in management of *Vrana(Wound)*, *Vidradhi(Abscess)*, *Dagdavrana(Burns)*⁹.

The concept of “Vrana” was given prime importance in ancient *samhitas* and *Dhatakyadi yoga* is preparation explained in context of *Agni dagdavrana* (burns)⁵. Burn injuries are very much sensitive and more prone for infection so before application its dermal toxicity is essential¹⁰. A skin sensitizer is a substance that will induce an allergic response following skin contact or after positive results from an appropriate animal test (Chaudhry *et al.*, 2010)¹¹. The results of the study showed normal gain of body weights, food and water intake, behavioural pattern. No mortality, signs of toxicity, changes in skin was observed until end of study and gross necropsy findings were normal probable reason may be the nontoxic and nonirritant nature of formulation.

CONCLUSION

A single dermal dose to *Dhatakyadi yoga* had no toxic effects on mortality, signs of toxicity, body weight changes and gross necropsy findings in both groups at dose of 2000 mg/kg body weight. Therefore, the approximate lethal dose of test item might be higher than 2000mg/kg in both groups of rats.

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