A STUDY TO EVALUATE ACUTE DERMAL TOXICITY WITH AGNIJITH (HERBAL WOUND HEALING OINTMENT)IN WISTAR RATS

Project No :.TRC 107/04

Sponsor

M/s. Padanjali Ayurvedic (P) Ltd.,
Kuttippuram,
Malappuram District,
Kerala.

EXPERIMENT GUIDELINE

OECD GUIDELINES FOR TESTING OF CHEMICALS
(Sec. 4, No.402, Adopted 24th February, 1987)

TEST FACILITY

Department of Pharmacology,
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Pallavaram,
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India.

Date: 11.10.2004

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CERTIFICATE

It is certified that this study report entitled, "A Study to Evaluate Acute Dermal Toxicity with Agnijith (Herbal Wound Healing Ointment) in Wistar Rats" is based on the study conducted at the Department of Pharmacology, Vel's College of Pharmacy, Pallavaram, Chennai, Tamil Nadu, India and truly reflects the raw data.

Dr. G. Srinivasa Rao, M.Pharm., Ph.D.
STUDY DIRECTOR.

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QUALITY ASSURANCE STATEMENT

This is to certify that the final report of the study entitled "A Study to Evaluate Acute Dermal Toxicity with Agnijith (Herbal Wound Healing Ointment)in Wistar Rats" has been examined with respect to the OECD Guidelines for Testing of Chemicals and raw data. It could be stated that the study has been conducted as per the guideline and the report has truly reflected the raw data.

Date: 11/10/2004

Dr. D. Satish Kumar, M. Tech. (Biotechnology),
Quality Assurance Unit.

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SUMMARY

The study was performed by to assess the acute dermal toxicity study (limit test) of Agnijith (Herbal Wound Healing Ointment) supplied by M/s. Padanjali Ayurvedic (P) Ltd.,Kerala in male and female Wistar rats. The test substance was applied as such to the shaven area of skin of group of rats (5/sex) at the dose of 2000 mg/kg body weight. Control group of animals (5/sex) were similarly treated but only with vehicle. Following dosing, the rats were observed for 21 days for mortality and clinical signs of toxicity. Body weight gain was noted weekly. At the end of the 21 days observation period rats of both the groups were subjected to necropsy.

No visible signs of treatment, such as changes in respiration, circulatory, autonomic and central nervous system, behavioral pattern were observed in the study. The body weight gain of test substance treated animals (both sexes) was not statistically different from that of the control animals. Gross pathology examination conducted on the animals at the end of 21–day observation period did not reveal any lesion that could be attributable to the toxicity of the test substance.

Since no mortality was observed in the study, under the condition of this test, it is concluded that the dermal LD50 of Agnijith (Herbal Wound Healing Ointment) for Wistar rats was >2000 mg/kg b.w.

INTRODUCTION

Like oral and inhalation route, dermal is also considered as a major route of exposure. Several xenobiotics are known to cause toxicity via dermal route. Hence it is a common practice to assess the toxicity of xenobiotic exposed via dermal route.

OBJECTIVE

To determine the acute dermal toxicity of the test substance in Wistar rats administered as single dermal dose as per OECD Guideline for Testing of Chemicals.

TEST SUBSTANCE

Common Name	:	Agnijith (Herbal Wound Healing Ointment)
Description	:	Oily ointment
Identification	:	The test substance was supplied by M/s. Padanjali Ayurvedic (P) Ltd.,Kerala.

TEST ANIMALS

The experiment was conducted in *Rattus norvegicus* (Wistar strain). Rat is a commonly used animal model in carrying out dermal toxicity studies, hence chosen for the present study.

The animals were procured from the in-house animal colony. The animals were of 8-12 weeks old at the start of the experiment.

Prior to start of experiment the animals were acclimated for 7 days.

Animals were housed in polypropylene rat cages with stainless steel top grill. Cleaned paddy husk was used as the bedding. Bedding material, cages, grills and water bottles were changed on daily basis.

Animals were housed individually.

Animals were given standard commercially available pellet feed and filtered water *ad libitum*. Animal house facility was an air-conditioned room and provided with 12h artificial fluorescent light and 12h dark.

Range Finding Experiment

No. of animals	:	Four (2 male + 2 female)

Main Experiment

Number of animals	:	Twenty (10 males + 10 females)		
No. of animals/group	:	Five males and five females per group		
Body weight at the Start of experiment	:	Male Female	: :	223 - 242 g 238 - 265 g
Identification of Animal	:	Each animals cage was properly numbered. Each animal was identified by marking.		
Randomization	:	Animals were randomly assigned to two groups.		

METHODS

Test Substance

The test substance was applied as such to the shaven area of skin of group of rats (5/sex) at the dose of 2000 mg/kg body weight. Control group of animals (5/sex) were similarly treated but only with coconut oil. Coconut oil was used as the vehicle as the test substance was prepared in coconut oil..

Test Procedure

Approximately 10% of dorsal skin area of each rat was shaved without any abrasion 24 h before the test.

The test substance was held in contact with the exposed skin under a gauze pad upto 24 h. Following this, the skin was washed with lukewarm water and wiped away with gauze.

Treatment

Range finding Study

In the range finding study, to 2 male and 2 female Agnijith (Herbal Wound Healing Ointment) was dermally administered at the dose of 2000 mg/kg b.w. Following the dosing the animals were observed for 6 days. No mortality occurred in these animals.

Main study

As no mortality was observed in the range finding study, the study was repeated as a limit test. In the limit test 5 males and 5 females were treated with Agnijith (Herbal Wound Healing Ointment) at 2000 mg/kg b.w.

Animals in control group was similarly treated but with vegetable oil alone.

OBSERVATIONS

All animals were observed during the entire observation period for any reaction at the application site, change in the fur, eyes, mucous membrane and any other overt sign of toxicity including behavioral signs.

Body weight determination of each animal was done just prior to administration of test substance (0 day) and on days 7, 14 and 21 following the administration.

All animals were necropsied at the end of 21- day observation period. Detailed gross examination was conducted in individual animal of both the groups.

STATISTICS

Student's t-test was employed to compare the body weight of the rats belonging to the experimental group with the control animals.

RESULTS

No mortality was observed in the treated group and control group of animals (Table 1). No visible signs of treatment, such as changes in respiration, circulatory, autonomic and central nervous system, behavioral pattern were observed in the study. No reaction was observed on the test substance-applied area of the skin. Similarly, no signs and mortality were observed in control animals (Table 2).

Compared with control, animals belonging to the test substance treated group (both sexes) did not show a significant change in body weight gain on days 7,14 or 21 (Table 3).

Necropsy examination did not reveal any abnormal lesion in any group (Table - 4).

CONCLUSION

Since no mortality was observed in the study, under the condition of this test, it is concluded that the dermal LD50 of Agnijith (Herbal Wound Healing Ointment) for Wistar rats was >2000 mg/kg b.w.

ARCHIVES

A copy of the study report, study protocol, raw data and a sample of test substance are stored.

Table 1. Mortality Data

Group No.	Total No. of rats treated	Dose (mg/kg b.w.)	Percent mortality (upto 21 days)	
			Male	Female
Group 1 (Control)	10 (5M + 5F)	(Vegetable oil)	0	0
Group 2 (Treated)	10 (5M + 5F)	2000	0	0

M - Male ; F - Female ; b.w. - Body Weight.

Table 2. Toxicity Signs

Sex : Male

Group (Treatment)	Days																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Group 1 (Control)	0/5*	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Group 2 (2000 mg/kg b.w.)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

*- No. of animals exhibited signs / No. of animals dosed

Table 2. (Contd.,) Toxicity Signs

Sex : Female

Group (Treatment)	Days																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Group 1 (Control)	0/5*	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Group 2 (2000 mg/kg b.w.)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

*- No. of animals exhibited signs / No. of animals dosed

Table 3. Weekly Body Weight Data

Sex: Male

Group	Dose (mg/kg b.w.)	Body weight (g)			
		Day 0	Day 7	Day 14	Day 21
1	(Distilled water)	253.60 ± 17.98 (n=5)	281.60 ± 15.76 (n=5)	325.00 ± 22.27 (n=5)	354.00 ± 14.54 (n=5)
2	2000	272.60 ± 10.69 (n=5)	280.80 ± 11.09 (n=5)	310.00 ± 10.91 (n=5)	330.20 ± 9.75 (n=5)

Values are Mean ± S.D.
Figures in parentheses indicate number of animals.
Values are not statistically different row-wise (P>0.05)

Table 3 (Contd.,) Weekly Body Weight Data

Sex: Female

Group	Dose (mg/kg b.w.)	Body weight (g)			
		Day 0	Day 7	Day 14	Day 21
G1	(Distilled water)	221.40 ± 4.77 (n=5)	239.00 ± 6.20 (n=5)	252.60 ± 8.68 (n=5)	266.80 ± 11.71 (n=5)
G2	2000	216.80 ± 8.32 (n=5)	232.20 ± 10.94 (n=5)	247.40 ± 10.53 (n=5)	261.00 ± 12.47 (n=5)

Values are Mean ± S.D.
Figures in parentheses indicate number of animals.
Values are not statistically different row-wise (P>0.05)

Table 4. Gross Pathology Data

Sex : Male

Dose	Animal No.	Lesions
Control	1	NAD
"	2	NAD
"	3	NAD
"	4	NAD
"	5	NAD
2000 mg/kg b.w.	11	Liver – Mottled
"	12	NAD
"	13	NAD
"	14	NAD
"	15	Lung – Emphysema

NAD - Nothing Abnormal Detected

Table 4. (contd.,) Gross Pathology Data

Sex : Female

Dose	Animal No.	Lesions
Control	6	NAD
"	7	NAD
"	8	NAD
"	9	Lung – Partial congestion Content
"	10	NAD
2000 mg/kg b.w.	16	NAD
"	17	Lung –Emphysema
"	18	NAD
"	19	NAD
"	20	NAD

NAD - Nothing Abnormal Detected