

**A STUDY TO EVALUATE PRIMARY SKIN IRRITATION WITH
AGNIJITH (HERBAL WOUND HEALING OINTMENT) IN NEW
ZEALAND WHITE RABBITS**

Project No : TRC 108/04

Sponsor

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

Experiment Guidelines

OECD Guidelines for Testing of Chemicals
(Revised Guideline 404, Adopted 24th April, 2002).

TEST FACILITY

Department of Pharmacology,
Vel's College of Pharmacy,
Pallavaram,
Tamil Nadu,
India.

Date: 11.10.2004

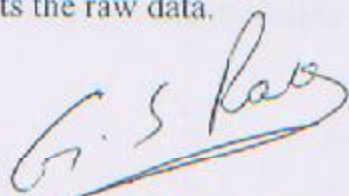
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CERTIFICATE

It is certified that this study report entitled, "A Study to Evaluate Primary Skin Irritation with Agnijith (Herbal Wound Healing Ointment) in New Zealand White Rabbits" 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.



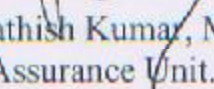
Dr.G. Srinivasa Rao, M.Pharm., Ph.D.
STUDY DIRECTOR.

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QUALITY ASSURANCE STATEMENT

This is to certify that the final report of the study entitled "A Study to Evaluate Primary Skin Irritation with Agnijith (Herbal Wound Healing Ointment) in New Zealand White Rabbits" 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. Sathish Kumar, M.Tech. (Biotechnology),
Quality Assurance Unit.

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S U M M A R Y

Primary Skin Irritation potential of Agnijith (Herbal Wound Healing Ointment) supplied by M/s. **Padanjali Ayurvedic (P) Ltd., Kerala** was tested in New Zealand white rabbits. A volume of 500 μ l of the test substance was evenly applied as such to the clipped area of skin of the rabbits under a gauze patch. At the end of 4 h, the gauze patch was removed and application site was wiped with water to remove the residual test substance, without altering the integrity of the epidermis.

Initially, the test substance was applied to the shaven area of skin of one animal under a gauze patch. At the end of four hours, it was observed that the test substance did not cause any skin reaction. The experiment was repeated using two additional animals, for an exposure period of four hours.

Animals were observed for erythema and edema at 1, 24, 48 and 72 h following the removal of gauze patch.

None of the animals exhibited any skin reaction at any of the above time points of observation. Primary skin irritation index was calculated as 0.

In the light of above observations, it is concluded that the test substance, Agnijith (Herbal Wound Healing Ointment) was **Non-Irritant** to the skin of rabbit.

INTRODUCTION

Primary skin irritation is a pharmacological effect involving the interaction of chemicals with sensory receptors in skin or mucosae at the site of contamination.

The current study was carried out to assess the primary skin irritation potential of the test substance applied to the shaven skin sites of rabbits in a single dose. Rabbit was used in the study as it is a commonly recommended animal model for carrying out primary skin irritation test, which is also acceptable as per OECD guideline for testing of chemicals.

OBJECTIVE

To determine the primary skin irritation potential of the test substance applied to the shaven skin sites.

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 ANIMAL

Species	:	<i>Oryctolagus cuniculus</i> (New Zealand White Strain)
No. of animals allotted for the study	:	3 Males
Body weight at the start of experiment	:	2.3 – 3.1 kg
Identification of animal	:	Each animal cage was properly numbered.

Each animal was identified by marking.

Animal house facility was an air-conditioned room and provided with 12h artificial fluorescent light and 12h dark.

Animals were housed individually in standard rabbit cages.

Standard pellet feed and filtered water were provided to the animals *ad libitum*.

METHODS

Preparation of Animals for the Experiment

All rabbits were shaved at dorsal and lateral surfaces in the abdominal region approximately an area of 6 cm² 24 h prior to initiation of the experiment.

Preparation of Test Substance

A volume of 500 µl of the test substance was evenly applied to the test site.

Application of the Test Substance

The test substance (500 µl) was evenly applied to an area of about 6 cm² of skin of left lateral surface of the rabbits under a gauze patch. Right untreated shaven surface was considered as control area which was also covered with a gauze patch.

The gauze patches of the both left and right surfaces of the animal was loosely held in contact with the skin by cotton gauze and held in place with a non irritating tape.

At the end of the 4 h exposure period, the residual test substance was removed, using water without altering the integrity of the epidermis.

Initial test

Initially, the test substance was applied to the shaven area of skin of one animal

under a gauze patch. At the end of four hours, it was observed that the test substance did not cause any skin reaction in this animal.

Confirmatory test

The experiment was repeated using two additional animals, for an exposure period of four hours.

OBSERVATIONS

Dermal responses were observed and scored in initially tested animal immediately after 4 h exposure and thereafter at 24, 48 and 72 h after the removal of gauze patch. Confirmatory test animals were observed at 1, 24, 48 and 72h after the removal of gauze patch.

Clinical observations and grading of skin reaction

The dermal responses were scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal. For the initial test in one animal, the test site was examined immediately after the patch had been removed. Dermal reactions were graded and recorded according to the grades in the Appendix 1.

In addition to dermal responses, clinical signs of toxicity and body weight were recorded.

Euthanasia

On termination the animals were euthanised.

SKIN REACTION IN TREATED AREA**Table 1a. Initial Test : Skin Reaction ***

Rabbit No.	Erythema				Edema			
	0 h*	24 h	48 h	72 h	0 h*	24 h	48 h	72 h
1	0	0	0	0	0	0	0	0

* Immediately after the removal of patch

Table 1b .Confirmatory Test: Skin Reaction*

Rabbit No.	Erythema				Edema			
	1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0

Total Mean Value

$$\text{Primary Skin Irritation Index} = \frac{\text{Total Mean Value}}{4}$$

$$= \frac{0}{4} = 0$$

*Scores given (*vide* Appendix 1).

SKIN REACTION IN CONTROL AREA**Table 2a. Initial Test : Skin Reaction ***

Rabbit No.	Erythema				Edema			
	0 h*	24 h	48 h	72 h	0 h*	24 h	48 h	72 h
1	0	0	0	0	0	0	0	0

* Immediately after the removal of patch

Table 2b. Confirmatory Test: Skin Reaction*

Rabbit No.	Erythema				Edema			
	1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0

Minimum score is 0

$$\begin{aligned} \text{Primary Skin Irritation Index} &= \frac{\text{Total Mean Value}}{4} \\ &= \frac{0}{4} = 0 \end{aligned}$$

*Scores given (vide Appendix 1).

APPENDIX 1. SCORE CARD

SKIN REACTION	VALUE
ERYTHEMA AND ESCHAR FORMATION	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Maximum possible: 4	
EDEMA FORMATION	
No edema	0
Very slight edema	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm extending beyond the area of exposure)	4
Maximum possible : 4	