A STUDY TO EVALUATE ACUTE ORAL TOXICITY WITH AGNIJITH (HERBALWOUND HEALING OINTMENT) IN WISTAR RATS

Project No :.TRC 106/04

Sponsor

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

EXPERIMENT GUIDELINE

OECD GUIDELINES FOR TESTING OF CHEMICALS
(Section-4, No.420, Adopted 17th December, 2001).

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 Acute Oral 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 Oral Toxicity with Agnijith (Herbal Wound Healing Ointment) in Wistar Rats" has been examined with respect to 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

Acute Oral Toxicity study with Agnijith (Herbal Wound Healing Ointment) supplied by **M/s. Padanjali Ayurvedic (P) Ltd., Kerala** was evaluated in Wistar rats. The test substance was administered at 2000 mg/kg b.w. (limit dose as given in the guideline), sequentially to 5 female rats. Following the dosing each animal was observed at 30 min, 1, 2, 3, and 6 h, and thereafter once in a day for clinical signs of toxicity and mortality. Prior to dosing (day 0) and on days 7 and 14 following dosing the body weight of individual animal was measured.

At the end of the 14 days observation period all the animals were subjected to necropsy.

No visible signs of treatment such as changes in respiratory, circulatory, autonomic and central nervous system, behavioral pattern were observed in the study. No mortality was observed in any animal.

Body weight measured in test substance treated animals on days 7 and 14 showed an increasing trend. Gross pathology examination conducted on the animals at the end of 14 – 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 oral LD50 of Agnijith (Herbal Wound Healing Ointment) for Wistar rats was >2000 mg/kg b.w. and the test substance was classified according to the Globally Harmonized System (GHS) category as **5/unclassified**.

INTRODUCTION

Accidental exposure to xenobiotics by oral route is common. Therefore it is important to have a prehand information on the acute toxicity of a xenobiotic. Also this information is considered useful in fixing dose levels for chronic toxicity experiments.

OBJECTIVES

The objective of the present study was to evaluate acute oral toxicity of the test substance in Wistar rats as per OECD guidelines for testing of chemicals.

Considering the ethical aspects of using animals in experiments and regulatory requirements the study was designed to use less number of animals as possible to achieve the objective of the study without compromising proper scientific evaluation of the findings.

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 oral 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 individually 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 given standard commercially available pellet feed and filtered water *ad libitum*, except during fasting. During fasting drinking water alone was given. Animal house facility was an air-conditioned room and provided with 12h artificial fluorescent light and 12h dark.

Total no. of animals received for the study	:	5
Sex	:	Female
Body Weight at Start of Experiment	:	200 - 227 g

Sighting Study

No. of animals : 1

Main Study

Number of Animals : 4

Identification of Animal : Each animals cage was properly numbered. Each animal was identified by marking.

Randomization : Animals were picked up randomly for the sequential dosing.

METHOD

Test substance

Known weight of the test substance was mixed with vegetable oil, and thereafter administered to group of rats at the desired dose level.

Treatment

In the sighting study, one overnight fasted female rat was given the test substance suspended in vegetable oil at the dose of 2000 mg/kg b.w. by oral intubation. This animal was observed for 14 days. In the main study, to overnight fasted four female rats the test substance suspended in vegetable oil was given the dose of 2000 mg/kg b.w.

OBSERVATIONS

All animals were observed individually, daily for 14 days for signs and mortality.

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

All animals were subjected to necropsy at the end of 14 - day observation period. Detailed gross examination was conducted in each animal.

STATISTICS

As per the study design there was no scope for applying any specific statistical tool to the data.

RESULTS

Animal treated with the test substance at 2000 mg/kg b.w. in the sighting study as well as those treated with the test substance at 2000 mg/kg b.w. in the main study did not show any mortality throughout the observation period of 14 days (Table-1).

No visible signs of treatment such as changes in respiratory, circulatory, autonomic and central nervous system, behavioral pattern were observed in the study (Table-2).

All animals showed an increasing trend in the body weight gain on days 7 and 14 (Table-3).

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

CONCLUSION

Since no mortality was observed in the study, under the condition of this test, it is concluded that the oral LD50 of Agnijith (Herbal Wound Healing Ointment)for Wistar rats was >2000 mg/kg b.w., and the test substance was classified according to the Globally Harmonized System (GHS) category as **5/unclassified**.

ARCHIVES

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

Table 1. Mortality Data

Study	Total No. of rats treated	Dose (mg/kg b.w.)	Percent mortality (upto 14 days)
Sighting	1	2000	0
Main	4	2000	0

Table 2. Toxicity Signs

Sighting Study

Dose	Days														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2000 mg/kg b.w.	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

Main Study

Dose	Days														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2000 mg/kg b.w.	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

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

Table 3. Weekly Mean Body Weight Data

Study	Dose (mg/kg b.w.)	Body Weight (g)		
		Day 0	Day 7	Day 14
Sighting	2000	215.00 (n=1)	241.00 (n=1)	284.00 (n=1)
Main	2000	221.67 ± 6.11 (n=4)	249.33 ± 8.08 (n=4)	268.00 ± 7.93 (n=4)

Values are Mean ± S.D.
Values given in parentheses are no. of animals.

Table 4. Gross Pathology Data

Animal No.	Study	Lesions
1	Sighting	NAD
2	Main	NAD
3		NAD
4		Lung – Stray petechiae
5		NAD

NAD - Nothing Abnormal Detected