A STUDY TO EVALUATE IRRITATION WITH AGNIJITH (HERBAL WOUND HEALING OINTMENT) TO MUCOUS MEMBRANE IN NEW ZEALAND WHITE RABBITS

Project No :.TRC 109/04

Sponsor

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

EXPERIMENT GUIDELINE

OECD GUIDELINES FOR TESTING OF CHEMICALS
(Revised Guideline 405, 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 Irritation with Agnijith (Herbal Wound Healing Ointment) to Mucous Membrane 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 Irritation with Agnijith (Herbal Wound Healing Ointment) to Mucous Membrane 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. Satish Kumar, M. Tech. (Biotechnology).
Quality Assurance Unit.

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SUMMARY

Mucous membrane irritation potential with Agnijith (Herbal Wound Healing Ointment) supplied by **M/s. Padanjali Ayurvedic (P) Ltd., Kerala** was tested in male New Zealand white rabbits. In the initial test 0.1 ml of the test substance was instilled in to the conjunctival sac of the left eye of 1 rabbit. The right eye of the rabbit was served as the control. This animal presented no ocular lesion at 1 and 24 h. To confirm the findings of the initial test, the study was repeated in two additional animals. In this confirmatory test, 0.1 ml of test substance was instilled in to the conjunctival sac of left eye of these animals. Right eye served as the control.

The eyes were examined and lesions were graded after application of the test substance at 1, 24, 48 and 72 h. The ocular irritation potential was evaluated by Draize's method.

None of the animals presented any lesion throughout the observation period. The maximum mean score for ocular lesions observed in the experiment was 0. Hence, the test article, Agnijith (Herbal Wound Healing Ointment) was considered as **Non-Irritant** to the mucous membrane of rabbits.

INTRODUCTION

The eyes may be a target or route for toxicity under various circumstances. Injury by direct contact with a chemical may present clinically from occupational and domestic incidents. Hence, in the assessment and evaluation of toxic characteristics of a substance, determination of the irritant and/or corrosive effects on eyes of mammals is an important initial step.

The current study was carried out and to assess the irritation/corrosive potential of test substance applied in to the conjunctival sac of the eyes of rabbits in a single dose. Rabbit was used in the study as it is a commonly recommended animal model for carrying out acute eye irritation/corrosive test, as per OECD guideline.

OBJECTIVE

To determine the ocular irritant/corrosive potential of the test article, in rabbit as an experimental model, when applied in to the conjunctival sac of eyes as per OECD guideline.

TEST ARTICLE

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	:	*Oryctolagus cuniculus* (New Zealand White Strain)
No. of animals allotted for the study	:	3 Males
Body weight at the start of experiment	:	2.2 – 2.8 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

Initial test

A volume of 0.1 ml of the test substance was instilled in to the conjunctival sac of left eye of one animal after gently pulling the lower lid away from the eyeball. The lids were gently held together for about one second in order to prevent loss of the test substance. The right eye, which remained untreated, served as control.

In the initial test carried out in the above animal, no ocular lesion was observed at 1 and 24 h following instillation of the test substance.

Confirmatory test

Since the animal in the initial test did not present any ocular reaction, the test was repeated in two additional animals as described above.

OBSERVATIONS

Grading of eye reactions

The eyes of the animals in the initial and confirmatory tests were examined at 1, 24, 48, and 72 hours after test substance application.

Since the animals did not develop any ocular lesion, the observation period was not extended beyond 72 h.

The grades of ocular reaction (conjunctivae, cornea and iris) were recorded at each time point of examination. Scale for scoring ocular lesions is given in Appendix 1.

Euthanasia

On termination, the animals were euthanised.

RESULTS

The summary of mean ocular lesions is given in Table – 1. None of the animals showed any lesion throughout the observation period (Table 2 – 5).

CONCLUSION

The maximum mean score for ocular lesions observed in the experiment was 0. Hence, the test article, Agnijith (Herbal Wound Healing Ointment)was considered as **Non - Irritant** to the mucous membrane of rabbits as per the classification by first approximation given in Appendix 2.

ARCHIVES

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

Table 1. Summary of Ocular Lesions

Time of Observation	Ocular Lesions			
	Total Score		Mean ± S.D.	
	Treated	Control	Treated	Control
1 hour	0	0	0	0
24 hour	0	0	0	0
48 hour	0	0	0	0
72 hour	0	0	0	0

Final Classification: Non -Irritant

Table 2. Ocular Lesions of Individual Rabbits: Positive Draize's Scores*

1 h after application

Treated – Left eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

Control – Right eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

* - *Vide* Appendix 1 for grading system of ocular lesions.

Table 3. Ocular Lesions of Individual Rabbits: Positive Draize's Scores*

24 h after application

Treated – Left eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

Control – Right eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

* - *Vide* Appendix 1 for grading system of ocular lesions.

Table 4. Ocular Lesions of Individual Rabbits: Positive Draize's Scores*

48 h after application

Treated – Left eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

Control – Right eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

* - *Vide* Appendix 1 for grading system of ocular lesions.

Table 5. Ocular Lesions of Individual Rabbits: Positive Draize's Scores*

72 h after application

Treated – Left eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

Control – Right eye		Sex	Ocular Lesions									Total Score
			Cornea			Iris		Conjunctivae				
Rabbit No.	Test		A	B	AxBx5	A	Ax5	A	B	C	(A+B+C)X2	
1	Initial	M	0	0	0	0	0	0	0	0	0	0
2	Confirmatory	M	0	0	0	0	0	0	0	0	0	0
3	Confirmatory	M	0	0	0	0	0	0	0	0	0	0

Total score =0
Mean score ± S.D. = 0 ± 0

* - *Vide* Appendix I for grading system of ocular lesions.

Appendix 1. Scale for Scoring Ocular Lesions *

Sl.No	Lesions			Score
1	Cornea	A	Opacity-degree of density (area most dense taken for reading) No opacity	0
			Scattered of diffuse area, details of iris clearly visible	1
			Easily discernable transculent areas, details of iris slightly obscured	2
			Opalescent areas, no details of iris visible, size of pupil barely discernable	3
			Opaque, iris invisible	4
		B	Area of cornea involved One quarter (or less) but not zero	1
			Greater than one quarter, but less than half	2
			Greater than half, but less than three quarters	3
			Greater than three quarters, up whole area	4
	Score equals A x B x 5		Total maximum	80
2		A	Values Normal	0
	Iris		Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
			No reaction to light, haemorrhage, gross destruction (any or all of these)	2
	Score equals Ax5		Total maximum	10
3	Conjunctivae	A	Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris) Vessels normal	0
			Vessels definitely injected above normal	1
			More diffuse, deeper crimson red, individual vessels not easily discernible Diffuse beefy red	2
			Diffuse beefy red	3
		B	Chemosis No swelling	0
			Any swelling above normal (includes nictitating membrane)	1
			Obvious swelling with partial eversion of lids	2
			Swelling with lids about half closed	3
			Swelling with lids about half closed to completely closed	4
		C	Discharge No discharge	0
			Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
			Discharge with moistening of lids and hairs just adjacent to lids	2
			Discharge with moistening of the lids and hairs and considerable area around the eye	3
	Score equals (A+B+C)x 2		Total maximum	20

The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva.
Total maximum score possible = 110.

*Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Assoc. Food and Drug Officials of the U.S., Austin, Texas, 1959.

Appendix 2. Classification by First Approximation

Maximum mean score	Classification by first approximation
0	N (non-irritant)
2.5	PN (possibly non-irritant)
2.5 to 15	M1 (mild-irritant -1)
16 to 25	M2 (mild-irritant-2)
26 to 50	M3 (mild-irritant-3)
51 to 80	S (slightly irritant)
81 to 100	E (extremely irritant)
101 to 110	Mx (maximum irritant)