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Research Article

In-Vivo Acute Dermal Toxicity Study of Formulations on Rabbits

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ABSTRACT

Purpose: To develop an Aceclofenac transdermal gel with a capability for topical drug delivery and
The purpose of this study is to obtain scientific information regarding health hazards that may result from a acute dermal exposure to the agricultural chemical for providing the basis of laying down the measure of safety protection in application.

Methods: Aceclofenac gel formulations, incorporating various permeation enhancers, were prepared using carbomer as a gelling agent. The formulations were examined for their in vitro characteristics including viscosity, pH and drug release as well as in-vivo pharmacological activities.

Results: The formulations containing 5 % of either Propylene glycol as permeation enhancers gave drug release patterns comparable to that of the reference product. Propanol increased the apparent viscosity of the test gels to the same extent as that of the reference. Drug release from the formulations fitted best to the Higuchi model. A significant in vivo analgesic effect was produced by the test formulations and the effect was superior to that obtained with the reference product.

Conclusion: Aceclofenac gel preparations containing propylene glycol respectively, exhibited pronounced analgesic activity and could be further developed for topical delivery of Aceclofenac.

Keywords:
Transdermal gel, toxicity study, Primary dermal irritation study Aceclofenac,Penetration enhancer

INTRODUCTION

The test substance is applied to the skin of the test animals in different doses and the animals are observed for effects and mortality. Animals showing severe signs of toxicity/distress are humanely killed. At the end of the test all surviving animals are to be sacrificed. Necropsy is performed on all dead, killed and sacrificed animals. To test the irritancy potential of experimental Aceclofenac gels, rabbits were used, the selected gels were applied to the shaved skin on the rabbits back. The selection of rabbits for this study is described as follows.

MATERIALS AND METHODS

Preparation of Aceclofenac gels

Required quantities of Carbomer 940, 971, 974 were soaked in some amount of distilled water for 2 to 3 hrs. Then it was neutralized by adding sufficient quantity of triethanolamine while stirring with help of overhead stirrer (Phase I).

Aceclofenac was dissolved in the mixture of ethanol (99.9%), either of the PEG 200,400,600 and propylene glycol (Phase II), Transcutol & DMSO were added in some formulations as penetration enhancers. (Phase II) was added slowly to the (phase I) while stirring with help of overhead stirrer, the remaining quantity of distilled water was then added to make up the final 100gm weight. pH of all formulations was maintained in the range of 6.8 to 7.4.

Determination of gel viscosity

The viscosity of the formulations and reference was performed using a Brookfield digital viscometer (Model DV-II, USA) equipped with spindle S27. The apparent viscosity was measured at 17 seconds−1 shear rate (50 rpm) and room temperature, after a 3-min rest time.

Evaluation of in vitro ibuprofen release

A synthetic hydrophilic membrane was mounted on a Franz diffusion cell (PermeGear, Riegelsville, PA, USA). The receptor compartment contained 6.5 mL of phosphate buffer (pH 7.4). One gram of the test formulation or reference was applied to the membrane over an area of 1.131 cm2 area across the donor compartment. The donor cell was exposed to ambient temperature and covered with parafilm to prevent evaporation. The temperature of the receptor compartment was maintained at 32 ºC while the buffer solution was stirred continuously with a Teflon-coated magnetic bar. Samples (0.5 ml) were withdrawn from the release medium at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h and replaced with an equal volume of fresh buffer solution to maintain sink conditions. The samples were analyzed by high performance liquid chromatography (HPLC) using the operating parameters described below (see 'HPLC analysis'). Cumulative amount of the drug permeated versus time (Zero order) and also versus square root of time (Higuchi model)

Housing and feeding conditions:

Temperature - 22±3ºC for rodents and 20° (± 3°) for rabbits
Relative Humidity – 50-60% (However, humidity should not be below 30% or exceed 70% at any given point of time).
Lighting – 12 hours light and dark cycle
Diet and water – Standard laboratory diet specific to the species and filtered water free from contamination.

Selection and randomization:

Animals were randomly placed in cages upon receipt and then randomized according to the body weights, Animals

Author for Correspondence

considered unsuitable because of outlaying body weights were excluded from the study. The animal backs were carefully shaved using sterilized shaving blade to remove the fur. The selected animals were grouped into 4 groups. And were marked with identification codes using diluted picric acid solution (1.2%w/v). Circular areas of 2.54 cm (1 inch diameter) were marked on the back of each animal using marker ink one spot on right side and one spot on left side of vertebral column. The spot on right side was abraded using shaving blade and the spot on left side was kept intact 1gm quantity of the selected gels was applied on marked spots using absorbent cotton wool. The toxic manifestations if any on the skin were then assessed by observing the skin at pre-selected time intervals after treatment e.g. 1 hrs,4 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hours, The observations were recorded as numerical scores as for each animal as follows

0 = no visible reaction
1 = mild erythema
2 = intense erythema
3 = intense erythema with edema
4 = intense erythema with edema and vesicular erosion

The score for treatment group and control group were then compared

Table 1: Composition of the Aceclofenac gel formulations

Name of ingredient	Weight (gm)								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Aceclofenac	1	1	1	1	1	1	1	1	1
Ethanol (99.9 %)	23.94	23.94	23.94	23.94	23.94	23.94	23.94	23.94	23.94
PEG 400	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40
Carbopol 974	0.5	1	2	-	-	-	-	-	-
Carbopol 971	-	-	-	0.5	1	2	-	-	-
Carbopol 940	-	-	-	-	-	-	0.5	1	2
Propylene glycol	15.52	15.52	15.52	15.52	15.52	15.52	15.52	15.52	15.52
Triethanol amine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water (up to 100 gm\ )	100	100	100	100	100	100	100	100	100

Table 2: The selection of rabbits for this study with Dose for topical Application.

Test animal	Healthy Rabbits
Strain	New Zealand white rabbits
Age	22 weeks
Sex	Male
Weight	2-2.5 kg
No of animals per groups	3 animals
Doses	1 gm per square inch topically
No. of Groups	Group I = F_1 Gel base treated control Group II = 1gm F_3 Formulation (without penetration enhancer) Group III = 1gm F_6 Formulation (with penetration enhancer) DMSO 1%w/w. Group IV = 1gm F_9 Formulation (with penetration enhancer) Transcutol 1%w/w,

G - Gel base, PEG 200,400 & Transcutol - penetration enhancer, Carbopol 974,940 & 971 - as Gelling Agent

Table 3: Numerical score for irritancy potential for vehicle treated control Group–I (F_1 Formulation)

Sr. No.	Animal code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded
1	A	0	0	0	0	0	0	0	0	0	0	0	0
2	B	0	0	0	0	0	0	0	0	0	0	0	0
3	C	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Numerical score for irritancy potential for Group- II treated with [F_3 Formulation] which containing no penetration enhancer

Sr. No.	Animal code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded
1	D	0	0	0	0	0	0	0	0	0	0	0	0
2	E	0	0	0	0	0	0	0	0	0	0	0	0
3	F	0	0	0	0	0	0	0	0	0	0	0	0

Table 5: Numerical score for irritancy potential for Group- III treated with [F_6 Formulation] which containing DMSO as penetration enhancer

Sr. No.	Animal code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded
1	G	0	0	0	0	0	0	0	0	0	0	0	0
2	H	0	0	0	0	0	0	0	0	0	0	0	0
3	I	0	0	0	0	0	0	0	0	0	0	0	0

Table 6: Numerical score for irritancy potential for Group - IV treated with [F_9 Formulation] which containing Transcutol as penetration enhancer

Sr. No	Animal code	1 hrs		4 hrs		12 hrs		24 hrs		48 hrs		72 hrs	
		Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded
1	J	0	0	0	0	0	0	0	0	0	0	0	0
2	K	0	0	0	0	0	0	0	0	0	0	0	0
3	L	0	0	0	0	0	0	0	0	0	0	0	0

RESULTS

Table no 1, 2, 3, 4 & 5 gives these scores for Group I, Group II, Group III and Group IV respectively

Observations and Examination: The following for 72 hrs. For vehicle control gel base animals those treated with selected formulations didn't indicate any manifestation of skin irritation such as redness of skin or inflammation at site of application. Both the abraded areas & intact areas when applied with vehicle or selected formulation were found to be free of any sign of irritation. Thus it can be concluded that all of the selected formulae are safe for topical application. However this study was performed using a single application of gel products Hence, it is needed to confirm safety after repeated applications also. *Finding:* Only a very silent erythema reaction was noted on animals at exposure observation time. No dermal irritation was noted at the 24, 48 or 72 hour examination.

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