پژوهشکـــده علـــوم دارویـــی
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The Institute of Pharmaceutical Sciences (TIPS)
Tehran University of Medical Sciences

۹۹/۴/۱۴۲۱
۹۹/۴/۲۰

بسمه تعالی

مدیر عامل محترم شرکت داروسازی کیمیا فام

جناب آقای محمد سعید هفت جواهریان

احتراماً پیرو درخواست جنابعالی در خصوص بررسی تست حساسیت پوستی بر روی محلول ضدعفونی کننده دست با نام تجاری نانوسیل دی۲ پلاس، نتایج بررسی نمونه به پیوست خدمتتان ارسال میگردد. یادآور می شود که نتایج این گزارش بنا به درخواست آن شرکت با نمونه ارسال شده و برای موارد اعلام شده انجام شده است و نتایج این گزارش به معنی تضمین سلامت نمونه نیست بلکه سازمان غذا و دارو، وزارت متبوع یا مراجع ذیصلاح مرتبط، صلاحیت اظهار نظر نهایی و حقوقی در تایید سلامت نمونه را دارند.

دکتر محسن امین

معاون پژوهش و فن آوری پژوهشکده علوم دارویی

Tests Results

Date: *8/June/2020*

Issued to: *Kimia Faam Co*

Product: *Hand disinfectant solution (*Nanosil D2plus®*)*

General overview of this study

Our study performed to establish the effects of the product hand disinfectant solution (Nonoil D2plus®) in skin sensitization in animal models. This investigation was performed in the TIPS, Tehran University of Medical Science. Skin sensitization was performed in a Guinea pig model for 28 days.

Animals were kept in individual cages under controlled light/dark cycle (12 h light/dark) and temperature (22-26° C). The animals received ad libitum access to standard chow pellets and water throughout the duration of the study. This experiment was carried out according to the "Regulations for using animals in scientific procedures" and all ethical concerns were taken into account. For topical administration, initially animals were intraperitoneally (i.p.) anesthetized by a combination of 10% Ketamine (90 mg/kg) and 2% Xylazine (10 mg/kg), then, their backs were shaved and 1% polyvinylpyrrolidone iodine was applied as antiseptic. After that, the sample was applied to the skin. All aspects of the studies performed in the present report, including test system facilities, equipment, reagents and materials, performance of study (study plan and conduct) and reporting of results have been set in accordance with the current GLP regulations.

Skin sensitization test

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study.

Intradermal induction phase I: A pair of 0.1 mL intradermal injections was made for each of the following, into each animal, at the injection sites (A, B and C) in the clipped extra scapular region whose fur was previously shaved. *Site A:* A 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant mixed with the chosen solvent.

Site B: The test sample (undiluted solution); the control animals were injected with the solvent alone. *Site C:* The teat sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%); the control animals were injected with an emulsion of the blank liquid with adjuvant.

Topical induction phase II: The maximum concentration that can be achieved in intradermal induction phase *I* did not produce irritation, animals are pretreated with 10% sodium dodecyl sulfate 24 (±2) hours before the topical induction application.

At 7 d after completion of the intradermal induction phase (on the 8th day), 0.5 mL of the test article was administrated via topical application to the intrascapular region of each animal, using a patch of area approximately 8 cm^2 (absorbent gauze), so as to cover the intradermal injection sites. The patches were secured using an occlusive dressing. The dressings and patches were removed after 48 h.

The control animals administrated distilled water patches alone were treated similarly.

Challenge phase: At 14 d after completion of the topical induction (on the 24th day) all the control and test animals were challenged with the test sample. The test article (0.5 mL) was applied topically to sites which were not treated during the induction (on the right flank). An absorbent gauze measuring 2.5cm × 2.5 cm was soaked in the test article solution. The patches were secured via an occlusive dressing similar to the previous stage. The dressings and patches were removed after 24 h.

Evaluation of results: GMPT grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals.

If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.

If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge.

The outcome of the test is presented as the frequency of positive challenge results in test and control animals.

Table 1. GMPT

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and continent erythema	2
Intense erythema and/or swelling	3

Table 2: Rating of sensitization response

Percentage sensitized	Grades	Classification
0-8	I	No difference from control
9-28	II	Mild
28-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

Results

Individual results of dermal scoring for the challenge appear in Table 3. The result showed that hand disinfectant solution (Nanosil D2plus®) had no sensitivity to guinea pig skin compared to controls. Guinea pig scoring is shown in Table 3 and 4.

Table 3. Guinea pig sensitization dermal reactions

Group	Animal Number	24±2h before phase II patch application		24±2h following challenge phase		48±2h following challenge phase	
		Left	Right	Test sites	Control sites	Test sites	Control sites
Test Group	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	7	0	0	0	0	0	0
	8	0	0	0	0	0	0
	9	0	0	0	0	0	0
	10	0	0	0	0	0	0
Negative Control	11	0	0	0	0	0	0
	12	0	0	0	0	0	0
	13	0	0	0	0	0	0
	14	0	0	0	0	0	0
	15	0	0	0	0	0	0

Table 4: Scores of skin sensitization test

Group	Test	Control
Challenge phase 24 ± 2 (Mean±SEM)	0 ±0.000	0±0.000
Challenge phase 48 ± 2 (Mean±SEM)	0 ±0.000	0±0.000

Results are expressed for ten animals in test group.

Reference

Reference: OECD Guidelines for the Testing of Chemicals (Skin sensitization-406). Accessed on September 28, 2019.

Conclusion

This study was performed to test skin sensitization of the hand disinfectant solution (Nanosil D2plus®) in guinea pig. Under the conditions of this study, the test article showed no significant evidence of causing skin sensitization in the guinea pig.

It must be emphasized that this report is not a certificate of safety or efficacy of the tested sample and any certification must be made by responsible authorities of the Iranian Food and Drug Administration or related authorities.

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