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Evaluation of Dermal Irritation and Skin Sensitization of Icaritin in Rabbits

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Abstract
The cutaneous irritation and skin sensitization toxicity potential of a novel molecule, Icaritin (ICT) loaded scaffold, was investigated in rabbits by the guidelines established by the Organization for Economic Cooperation and Development (OECD). Rabbits were dermally connected to an ICT-loaded scaffold for 72 hours or repeated application to evaluate the level of dermal irritation. During the test, there were no instances of adverse responses such as erythema or oedema that were seen, as shown by the data. The skin sensitization test was performed on rabbits, and they were exposed to ICT laden scaffold for twenty-four hours. It was discovered that the ICT-loaded scaffold did not exhibit any sensitization response, however, the positive group exhibited a significant amount of sensitization symptoms. The results of this study indicate that the scaffold loaded with ICT does not result in cutaneous irritation or skin sensitization toxicity, and it seems to be safe for usage on animals.

Keywords
Icaritin, Dermal irritation, Skin sensitization

INTRODUCTION

Icaritin (ICT), is a prenylflavonoid chemical that is isolated from the Epimedium plant. It is used in traditional Chinese medicine because to its aphrodisiac, anti-ageing, and immunomodulatory properties [1]. In recent years, it has garnered a large amount of interest attributable to the potential therapeutic uses it has in the treatment of cancer, osteoporosis, and inflammatory diseases [2]. The chemical structure of ICT is comprised of a flavonoid ring system that is connected to a prenyl group. This boosts the bioavailability and stability of the compound, which makes it a good option for the creation of new drugs [3]. Using a variety of different modes of action, ICT demonstrates benefits that include anticancer, anti-inflammatory, and antioxidant capabilities [4]. ICT can prevent the generation of inflammatory cytokines, as well as cancer cell proliferation and angiogenesis, as well as the development of osteoclasts [5]. Additionally, it

can stimulate apoptosis, and oxidative stress targeting cancer cells [6]. It is a potential option for the treatment of a wide range of disorders due to the pharmacological qualities that it has. Furthermore, it has been shown that sub-micromolar doses of ICT demonstrated estrogen-like action in estrogen receptors[7].

The efficacy and safety of ICT for clinical application, however, need more investigation. It is necessary to do a suitable toxicological review on newly discovered chemicals before they are consumed by humans and animals, particularly those that are used on a regular basis. REACH, which stands for Registration, Evaluation, Authorization, and Restrictions on Chemicals, is an effort that focuses on chemicals that are used in the preclinical testing of pharmaceuticals [8]. One of the most important issues is the prediction of adverse effects. Due to the fact that animal skin is very sensitive to the majority of chemicals, it is imperative that any novel formulations be tested on the skin for a certain amount of time in order to determine whether or not any irritation or erythema will develop. For the purpose of conducting a minimal set of toxicity screening, which offers a basic assessment of the possible dangers posed by ICT, studies on skin irritation and skin sensitization are crucial components. On the other hand, there is still a paucity of information on research on dermal irritation and skin sensitization, as well as acute, sub-chronic, reproductive, and development studies that are conducted in accordance with the related toxicological standards. It is essential to do a risk assessment of ICT. As a result, the current research reveals a safety profile for the topical application of ICT on rabbits in accordance with the parameters established by OECD 404.

MATERIALS AND METHODS

PNP Biotech Co., Ltd. in Chengdu, China, manufactured Icaritin with the Cas No. number 118525409 and a purity level of 98.5%. Animals collected from the JSSCPO animal house were healthy adult Swiss Albino rabbits weighing between 2 and 3 kilograms and twenty weeks old. In addition to being fed with regular laboratory feed and water on an ad libitum basis, they were furnished with polypropylene cages. A temperature range of 22 °C to 24°C, with a relative humidity of 55% ± 10%, and a light/dark cycle of 12 hours were maintained in the animal facility throughout the experiment. The animals were allowed to acclimatize for a period of 10 days before the first application. JSSCPO/OT/IAEC/01/2023-24 is the approval number that was given to this research by the IAEC, JSS College of Pharmacy in Ooty.

Acute dermal irritation

The research on acute skin irritation was carried out in compliance with the guidelines contained in OECD Guidelines 404, which are titled "Acute dermal irritation/corrosion." An irritant consisting of a 0.8% weight-to-volume aqueous solution of formaldehyde was administered to a positive control group. A placebo patch was administered to a control group, and a polymeric scaffold loaded with ICT (0.5 g/kg) was administered to a treatment group. To generate a paste preparation for cutaneous application, ICT was combined with a minimal quantity of CMC, which was in the amount of 0.5g/kg. A portion of the rabbit's trunk of about 5 cm x 5 cm was removed to experiment. Following that, the test article was placed in one intact location on each rabbit, which was subsequently covered with an occlusive dressing and covered with a gauze patch of 2.5 cm × 2.5 cm. During the application process, Elizabethan collars were placed on the animals under consideration. Following a period of four hours during which the test object was subjected to skin attachment, the wrappings and patches were removed. At the conclusion of the exposure time, the remaining test items were removed from the test site during the scoring process for cutaneous responses. This was accomplished by gently rinsing them with water that had been steeped in lukewarm water. Three minutes, one hour, and four hours after the patch was removed, there were no cutaneous responses seen. Considering that the rabbits who participated in the original test did not display any cutaneous response, the experiment was carried out once again with two more rabbits in order to validate the results of the first test. During this time, three separate studies about repeated dermal application were carried out. Applications were submitted for a period of seven days in a row. ICT was administered to rabbits in a single dermal trial, and then again in a repeated dermal research. The test sites were evaluated for erythema and edema after one hour, twenty-four hours, forty-eight hours, and seventy-two hours after the exposure. The dermal reactions were evaluated in accordance with the guidelines provided by the OECD. On a scale ranging from 0 to 4, erythema and edema were rated, with 0 indicating that there was no impact and 4 indicating that the symptoms were significant. After removing the patches from each animal, the cutaneous reaction scores were tallied at one hour, twenty-four hours, forty-eight hours, and seventy-two hours. These numbers were then divided by three to get the mean irritation score for each time point. These findings were compared to those obtained from the animals that served as the control and were given distilled water. For the purpose of calculating the main irritation index, the mean scores were added together and then averaged [9].

Skin sensitization experiment

The Rabbits were also used for skin sensatization experiment as per OECD guidelines for 28 days. A positive control group rabbit was administered 0.1% w/v 1-chloro-2,4-dinitrobenzene (CDNB) dissolved in 10% propylene glycol as a conventional skin sensitizing agent. For treatment group, ICT loaded Scaffold was applied to the shaved area, covered with an impermeable adhesive plaster, and secured with an elastic bandage for 6 hours. Hair removal and induction were done weekly on days 6-7 and 13-14. The test was conducted on day 28. Reactions at treated sites were observed and scored 24, 48, and 72 hours after patch removal. All reactions were evaluated using a standard scoring code. They were monitored for signs of toxicity, systemic effects, and abnormal behaviour [9]

RESULTS

Dermal irritation

Table 1 provides a summary of the findings from the studies that were conducted to investigate acute cutaneous irritation (single and repeated exposure). In none of the groups who were treated with ICT loaded scaffold, there were any clinical indicators or changes in body weight that were noticed. No cutaneous reactions, erythema or edema, were seen in rabbits (Fig. 1).

Table 1 Dermal irritation test of ICT-loaded Scaffold at different time intervals in rabbit

ICT-loaded Scaffold	Dermal irritation	1hr	24h	48h	72h
Dermal irritation on rabbit with single dose at different time interval	Erythema Score	0	0	0	0
	Edema Score	0	0	0	0
Dermal irritation on rabbit with repeated dose at different time interval	Erythema Score	0	0	0	0
	Edema Score	0	0	0	0

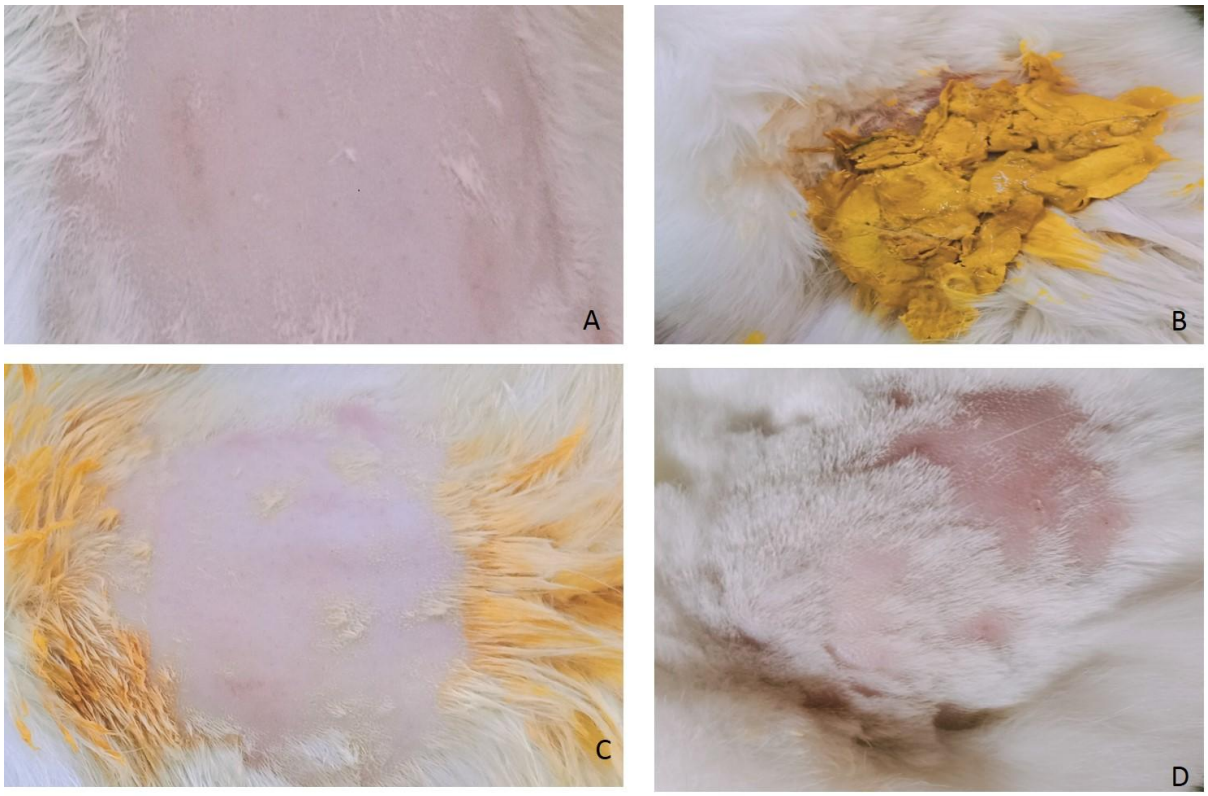

Fig. 1 Dermal irritation and sensitization test on rabbit
1A represents before treatment, **1B** treatment with ICT-loaded Scaffold,
1C – after 72 hrs of ICT-loaded treatment, **1D**- at the end of 14th day of treatment

Skin sensitization

No clinical manifestations or alterations in body weight were seen in any of the groups. There were no statistically significant mean weight differences in body weights between the control and the treated groups from the first day of treatment until the completion of the trial. The skin sensitization tests were confirmed by the positive control group (CNDB), which showed positive dermal sensitization reactions. No sensitization was seen in rabbits confronted with ICT loaded Scaffold treatment group. In this investigation, there was no evidence of erythema or edema after the challenge.

DISCUSSION

Risk assessment is a function of the data pertaining to hazards and exposures. The method of testing that is advised for creating scientifically solid data on the irritation caused by the drug is the stepwise testing strategy. This method is used for novel chemicals that are launched into the market. This research was done to investigate whether ICT might produce cutaneous irritation and skin sensitization. The hyperemia of superficial capillaries is the root cause of erythema, which is characterized by redness of the skin or mucous membranes. Edema denotes swelling induced by fluid in body's tissue. When rabbits were subjected to cutaneous irritation, the results revealed that there were no skin reactions, including erythema and edema. The findings of the skin sensitization experiment demonstrated that ICT does not result in skin sensitization in rabbits, with the exception of the group that served as the positive control. From the very first day to the very last day of the trial, not a single one of these animals displayed any clinical indications or any overt evidence of toxicity in the skin irritation investigation (singled and repeated treatment). In the subsequent fourteen days, prior to the removal of the patch administration, the ICT loaded scaffold treated group of rabbits did not exhibit any disordered

behavior that was associated with the therapy. The loss in body weight is a significant indicator of gross toxicity, which indicates that a significant amount of toxicity or interference with the absorption of nutrients will be reflected in the reduction in body weight. Between the control group and the treatment group, there were no statistically significant variations in mean body weights between the two groups from the very first day of patch administration to the very last day of the trial.

CONCLUSION

Therefore, it is possible to draw the conclusion that the scaffold that is loaded with ICT does not have a propensity to cause significant tissue damage, nor does it seem to interfere with the mechanism by which nutrients are absorbed. Based on the study of all the available parameters that were investigated, that the ICT loaded scaffold was well tolerated by the experimental rabbits, and that there was no dermal irritation or skin sensitization in the animals.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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