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PP-357 [Apitherapy]

Stability and permeability studies on canine skin of two liquid formulations based on apitoxin

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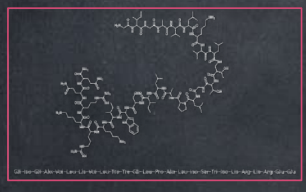
Introduction



Bee venom, a complex peptide mixture secreted by female bees of the *Apis mellifera* genus, is highly valued and recognized. This product is a solid composed primarily of peptides responsible for its biological activities, such as its major component, melittin.

It has demonstrated anti-inflammatory, antinociceptive, immunomodulatory, and antimicrobial properties, among others. *In vitro* and *in vivo* studies have demonstrated melittin's ability to inhibit the production of inflammatory mediators such as prostaglandins and cytokines, as well as to modulate the activity of immune cells such as macrophages and neutrophils. Despite these benefits, there are no commercial veterinary products on the market containing this active ingredient. In this context and considering the significant shortcomings of current anti-inflammatory treatments for chronic diseases, we set out to develop a liquid formulation for topical use in canines.

In this study, we present the results obtained from the stability analysis of apitoxin in aqueous solution and in two developed topical formulations. In addition, we present permeability studies performed on canine skin using Franz diffusion cells.

Results and discussion

Ingredient	Form 1	Form 2
Water	68.6 g	68.6 g
Glycerin	15 g	15 g
Tween 20	1.5 g	1.5 g
Propylenglycol	15 g	- g
Methylparaben	0.1 g	0.1 g
DMSO	-	15 g
Citric acid anhydride	0.5 g	0.5 g
Apitoxin	0.5 g	0.5 g

Table 1. Composition of 2 formulations developed for possible topical use in canines.

This work involved the development of a formulation for topical use in canines, using different excipients as previously presented, resulting in the formulations presented in Table 1. An HPLC method developed by the group was used to analyze the concentration of melittin in the different samples.Based on these formulations, the corresponding stability and permeability tests were performed. Some of these results are showed in this poster.

In this study, the stability of different aqueous solutions of purified bee venom was determined under different storage conditions. Melittin was used as a control because it is the majority compound and the one that has been shown to have greater lability to storage conditions. Distilled water and the mixture developed for a topical formulation 1 (Table 1) were used as solvents. Storage conditions were refrigerated (2-8°C), ambient conditions (room temperature approximately 20°C), and accelerated stability conditions according to Zone II (42°C and 75% humidity). In all cases, the product was stored in amber containers and protected from light (Figure 1 and 2).

In both solutions, melittin degrades rapidly in accelerated stability tests and ambient storage conditions. However, under refrigeration, it can maintain its potency relatively stable. For the topical solution, which contains an antioxidant agent in its formula, it remained highly potent for several months under refrigerated conditions.

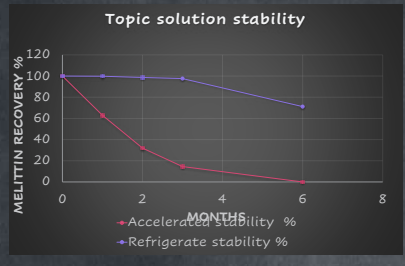


Figure 1. Graphic of melittin recovery vs storage time in different conditions.
Accelerated stability conditions according to Zone II: 42°C and 75% humidity. Refrigerated stability conditions: 2-8°C.

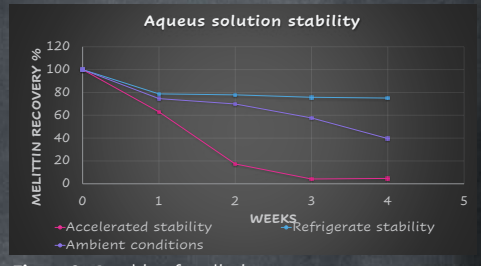


Figure 2. Graphic of melittin recovery vs storage time in different conditions.
Accelerated stability conditions according to Zone II: 42°C and 75% humidity. Refrigerated stability conditions: 2-8°C. Ambient stability conditions: room temperature approximately 20°C.

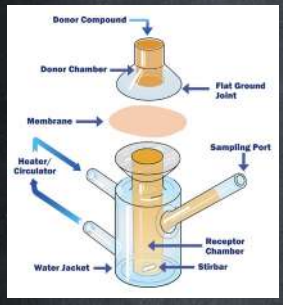


Figure 3. Franz cell diagram.
Image obtained from permgear.com, supplier of the cells used in this test.

Transepithelial permeation of apitoxin was determined using the Franz cell methodology, which consists of determining the amount of analyte that crosses a membrane (in this case, canine skin) from the donor chamber to the recipient chamber. The test was performed with two sections of canine skin: skin with abundant hair and skin with little hair. Melittin was used as a marker as the majority component. The test conditions were chosen to simulate canine physiology; this implies a temperature between 38.5-38.9 ° C ± 0.05 ° C and a pH of 5.5. Once the test began, samples were removed over time to determine if melittin passed through after a few hours. In this experiment, permeation through the canine skin was not observed for either of the 2 formulations (Form 1 and Form 2) in either of the barriers used, but absorption of the marker in the skin was observed.

Conclusions

By studying the stability of bee venom in aqueous solution and in a topical formulation developed for use in canines, it can be concluded that the latter maintains its potency in melittin after 3 months of refrigerated storage. Furthermore, no passage of melittin was observed through two canine skin sections for two topical formulations, but this compound was absorbed into the skin. For these reasons, we can conclude that the developed formulation would be adequate for preserving bee venom, but there is no evidence that it is effective for treatments that require the action of bee venom on deeper layers of the skin.

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Bee venom is a product secreted by worker and queen bees,from “Apis mellifera” highly sought after and valued. This product is a solid mainly composed of peptides responsibles for its biological activities. It has demonstrated anti-inflammatory, antinociceptive, immunomodulatory, antimicrobial properties, among others. “In vitro” and “in vivo” studies have shown the ability of melittin, the main component of apitoxin, to inhibit the production of inflammatory mediators such as prostaglandins and cytokines, as well as to modulate the activity of immune cells like macrophages and neutrophils. Despite these benefits, there are no veterinary products on the market containing this active ingredient, making its application as an anti-inflammatory of interest. In this context and considering the significant deficiencies of current anti-inflammatory treatments for chronic diseases, we aimed to develop a liquid formulation for topical use in canines. In this study, we present the results obtained from the stability analysis of apitoxin in aqueous solution and in two developed topical formulations. Additionally, we present the permeability studies conducted on canine skin using Franz diffusion cells. Stability studies in aqueous solution indicate that melittin has limited stability under these conditions, improving when stored under refrigeration. However, in the developed formulation, through the addition of stabilizing and antioxidants agents, stability was extended beyond six months at temperatures between 2–8°C. To determine the permeation capacity in canine skin, the Franz cell model was used, where a skin sample is placed in contact with the formulation being tested, and the amount of melittin capable of permeating this barrier is quantified by HPLC method. In this assay, no melittin passage through the skin was observed for one of the formulations; however, it was absorbed onto the skin used in the test. For the second formulation, evaluation is still in progress. In conclusion, we successfully established the composition and storage conditions of an apitoxin solution for topical use in canines to extend its shelf life. Additionally, we determined that one of the developed solutions allows penetration into canine skin but does not permeate through it.