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Determination of the anti-inflammatory activity of nano spray-dried nanoparticles with in vivo HET-CAM assay that can be used in post-operative wound healing

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Abstract:

Ketorolac tromethamine is a non-steroidal anti-inflammatory drug (NSAID) from the heteroaryl acetic acid derivatives class that acts as a non-selective COX inhibitor. KT is often used successfully in post-surgical (postoperative) pain and surgical wounds. If the physiologically inflammatory response in wound healing is prolonged or exacerbated, it leads to a delay in the later stages of proper wound healing. In this case, anti-inflammatory agents are needed. Spray drying is one of the most extensively studied processes in the pharmaceutical field. It is frequently used for drug formulations to be used topically. Within the scope of this study, ketorolac tromethamine loaded nanoparticle based drug delivery systems, which can be effective in pain treatment and wound healing processes in postoperative conditions, were prepared. The in vivo 'Hen's Egg Test on the Chorioallantoic Membrane' assay was performed on the selected optimum formulation for anti-inflammatory activity. The in vivo anti-inflammatory activity results indicated that the nanoparticle formulation including ketorolac tromethamine as 100 µg / pellet showed significantly strong anti-inflammatory potential with 85.625 ± 11.825 % inhibition value and no embryotoxicity whereas ketorolac tromethamine showed weak activity with 60 ± 13.675 % inhibition on the chorioallantoic Membrane. With the obtained Hen's Egg Test on the chorioallantoic Membrane assay result, it has the hope of a successful nano-topical formulation especially in wound healing.

Keywords: HET-CAM, Ketorolac tromethamine, Nanoparticle, Wound healing.

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1. Introduction

Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory medication from the heteroaryl acetic acid derivatives family. It is a racemate type of cyclooxygenase (COX) inhibitor that is non-selective. The S-isomer retains the majority of its analgesic and COX inhibitory action. Orally, intramuscularly, intravenously, and as a topical ophthalmic solution, ketorolac is given as its tromethamine salt. Due to the common occurrence of gastrointestinal disturbances such as gastrointestinal bleeding, perforation, and peptic ulcers, as well as the short mean plasma half-life ($t_{1/2} \cong 5.5$ h), numerous formulation strategies for the appropriate delivery of KT have been developed (Sinha et al. 2009). Wound healing is a process of tissue regeneration that includes inflammation as an early step, whereas anti-inflammation is a positive response to a treatment that reduces inflammation, which influences the central nervous system. Acute, proliferative, and remodeling wounds are the three stages of wound healing in general. Drug therapy can sometimes hinder these wound healing processes, causing the wound to heal more slowly. As a result, novel anti-inflammatory drugs that do not impede these mechanisms may be developed (Hadagali and Chua 2014).

Spray drying is an important technology that is fast, simple, reproducible, and scalable. It has a wide application range, that is, in food, chemicals, and encapsulation of pharmaceuticals. The technology can be divided into conventional spray drying and nano spray drying. The key advantage of nano spray drying is the production of drug-loaded nanosized particles for various drug delivery applications. The recent developments in nano spray dryer technology and the market launch of the Nano Spray Dryer B-90 by Büchi Labortechnik AG in 2009 enabled the production of submicron spray-dried particles (Öztürk and Arpagaus 2021).

The chronic nature of nonhealing wounds, as well as the difficulties that come with them, has prompted the development of nanotechnology-based therapies aimed at speeding up the healing process and, ultimately, mending the damaged tissue. A number of designed nanotechnologies with unique features and numerous roles have been proposed to solve specific issues with wound repair mechanisms (Hamdan et al. 2017).

The in vivo Hen's Egg Test on the Chorioallantoic Membrane (HET- CAM) assay is very useful, inexpensive, and simple to use, sensitive to both steroidal and non-steroidal agents, and well established for screening anti-inflammatory, teratogenic, possible toxic and side effects,

and irritation potency of natural products such as plant extracts and essential oils, as well as synthetic therapeutics, drug formulations, and cosmetics (Öztürk and Kıyan 2020).

2. Materials and Methods

2.1. Materials

KT is a kind gift from Sanovel (Istanbul, Turkey). Also, the fertilized eggs used in the HET-CAM test were obtained from Has Tavuk Company (Bursa and Sivrihisar, Turkey). All other chemicals used were of analytical grade.

2.2. Preparation of nanoparticle formulation

NPs were prepared using Nano Spray-Dryer (B-90, Büchi Labortechnik AG, Switzerland).

2.3. *In vivo* anti-inflammatory activity by hen's egg test on the chorioallantoic membrane (HET-CAM)

2.3.1. Preparation of test samples

KT-loaded NP (equivalent 100 µg KT/ pellet) and pure KT (100 µg /pellet), SDS (50 mg SDS/pellet) were suspended & dissolved in a 2.5% (w/v) agarose solution. For ease of application of the pellets of these solutions (10mL) were prepared and applied dropwise on circular stainless steel supports of 5mm diameter and cooled to room temperature for solidification and applied on to the chick chorioallantoic membrane (CAM).

2.3.2. *In vivo* HET-CAM assay

The method was performed as described in the previous work. The previously incubated fertilized hen's eggs for 72 h at 36.5°C and a relative humidity of 80% were positioned in a horizontal position and rotated several times. The eggs were opened on the snub side and furthermore 10–15 mL of albumin were aspirated from them. The eggs were traced with a scalpel at the two-third of the height (from the pointed side) and after that the shells were removed with forceps. The cavity was covered with film and they were incubated at 36.5°C at a relative humidity of 80% for further 72h. Pellets (1 pellet/egg) were placed on the CAM which had approximately a diameter of 2cm. One further day later the eggs were evaluated under the stereo-microscope. A total of 10–15 eggs were utilized for each test compound and also the experiment was repeated three times for each test compound. The method is also described in **Figure 1.** Anti- inflammatory effects were evaluated by using a scoring system (**Table 1**) and followed the conversion of the score index in the proportional inhibition of inflammation (**Table 2**).

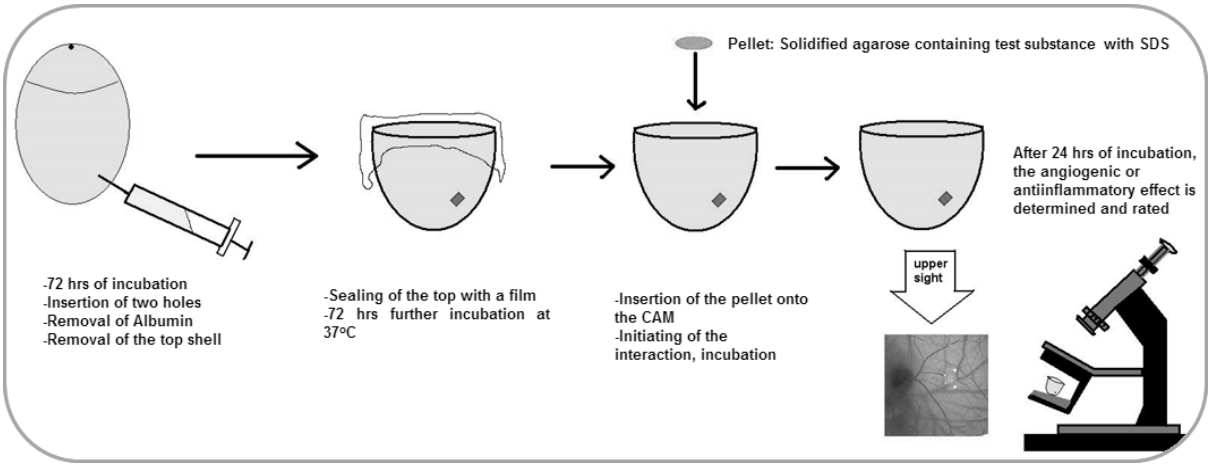


Figure 1. Evaluation of HET-CAM Assay

Table 1. The Semi quantative score system of anti-inflammatory effect on CAM after treatment

Category	Type	Effects observed on CAM after treatment
1	**Irritated**	The granuloma is strongly vascularized.
		A network of capillaries is formed star like around the granuloma.
2	**Weakly irritated**	The granuloma is poorly vascularized.
		A thin network of capillaries is formed star like around the granuloma.
3	**Weakly normalized**	The granuloma is somewhat smaller than in category 1 and 2 and only poorly vascularized.
		The star like network of vessels is hardly recognizable.
4	**Normalized**	No granuloma or only a kind of ‘‘scar’’ can be observed (if the granuloma regresses a non-vascularized scar is left).
		The network of vessels is normal (as the control).

Table 2. The score index in the proportional inhibition of inflammation

İnhibition (%)	Anti-inflammatory Effect
≤ 40	No anti-inflammatory effect
40-55	Uncertain anti-inflammatory effect
55-70	Weak anti-inflammatory effect
70-85	Good anti-inflammatory effect
> 85	Strong anti-inflammatory effect

3. Results

We aimed to provide a high anti-inflammatory effect with a low dose to be determined by in vivo hen's egg test on Chorioallantoic Membrane (HET-CAM) analysis that a new, popular and *in vivo* animal experiment alternative method. Figure 2 shows anti-inflammatory effects of KT and KT-loaded NP.

The in vivo anti-inflammatory activity results indicated that the nanoparticle formulation including ketorolac tromethamine as 100 µg/pellet showed significantly strong anti-inflammatory potential with 85.625 ± 11.825% inhibition value and no embryotoxicity whereas ketorolac tromethamine showed weak activity with 60 ± 13.675% inh. on the CAM.

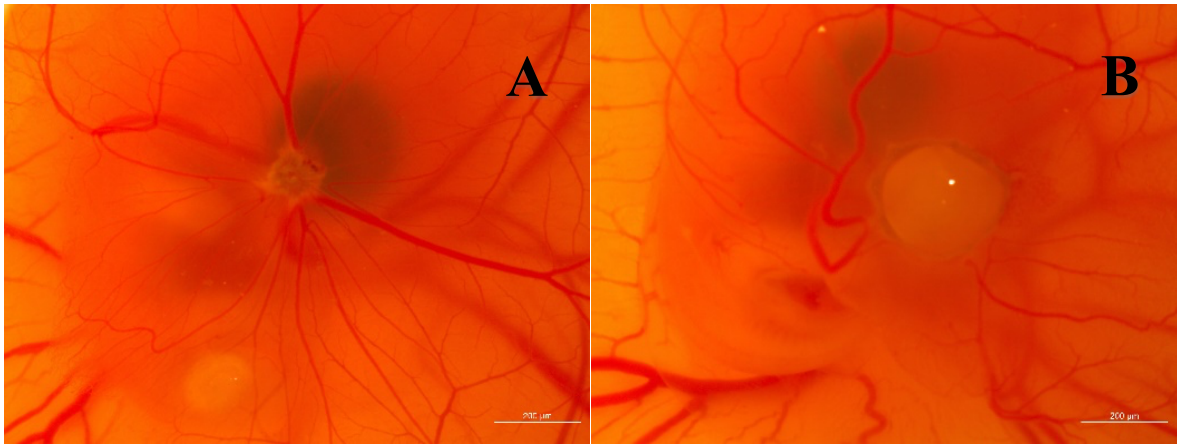


Figure 2. The in vivo anti-inflammatory effects of KT and KT-loaded NP on the CAM. (*A: Weak anti-inflammatory effect of KT at 100 µg/pellet, B: Strong anti-inflammatory activity of KT-NP including KT as 100 µg/pellet on the CAM*)

4. Discussion

The anti-inflammatory activity of KT loaded NP formulations was examined using the in vivo HET-CAM assay. For the assay, SDS was used to cause irritation and hemorrhage on the CAM. The anti-inflammatory results are in Figure 2. For the evaluation of the anti-inflammatory effect, a semi- quantitative score system is used as shown in Table 1 and a score index described in Table 2. In line with the HET-CAM results, it is thought that the anti-inflammatory activity has been increased with the nanoparticle structure and thus a successful treatment can be obtained in the wound healing process. Similar results are found in the literature (Öztürk and Kıyan 2020; Öztürk et al. 2020a; Öztürk et al. 2020b).

5. Conclusion

KT-loaded nanoparticles were produced in a nano-spray-drying device. With the obtained HET-CAM result, it has the hope of a successful nano-topical formulation especially in wound healing.

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Congress President

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