FORMULATION AND EVALUATION OF THERMORESPONSIVE HYDROGEL LOADED WITH IVERMECTIN FOR ANTIPARASITIC APPLICATION FOLLOWING MANGE INFESTATION IN CATS

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A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

2023
UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled '**Formulation And Evaluation Of Thermoresponsive Hydrogel Loaded With Ivermectin For Antiparasitic Application Following Mange Infestation In Cats**' by, Syarifah Nur'Izzati Iman Binti Syed Ramli and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course DVT 55204 – Research Project

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FORMULATION AND EVALUATION OF THERMORESPONSIVE HYDROGEL LOADED WITH IVERMECTIN FOR ANTIPARASITIC APPLICATION FOLLOWING MANGE INFESTATION IN CATS

ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

Mange infestation in cats, caused by mites, is a health concern that can lead to symptoms such as crusty lesions and hair loss. One of the current practices is administering ivermectin by injection subcutaneously. Therefore in this study, the effectiveness of a new thermoresponsive hydrogel formulation that includes 1% ivermectin (v/v) as topical ivermectin for treating mange infestations in cats was investigated. The investigation examines six formulations with different polyethene glycol (PEG) concentrations, and assesses their physical properties, gelation temperature (Tgel) analysis, and rheological behaviour. Gelation temperatures reflect sensitivity to PEG concentration, with ivermectin influencing Tgel. Rheological assessments indicate non-Newtonian, pseudoplastic behaviour which is essential for optimising hydrogel dressings. Microscopic examination confirms the presence of *Notoedres cati* in all cats prior to treatment. Initial visual lesion assessments indicate crusty lesions and alopecia, with consistent scratching behavior. Post-treatment evaluations demonstrate lesion improvement in the treatment group, highlighting the potential of ivermectin-loaded hydrogels as future topical medication.

Keywords: Mange infestation, ivermectin, cats, topical treatment, crusty lesion, thermoresponsive hydrogel.

FORMULASI DAN PENILAIAN THERMORESPONSIF HIDROGEL YANG DITERAPKAN DENGAN IVERMECTIN UNTUK APLIKASI ANTIPARASITIK DALAM PENYAKIT KURAP DALAM KUCING.

ABSTRACT

Abstrak kertas penyelidikan ini telah dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai sebahagian daripada keperluan separa penuh bagi kursus DVT 55204 - Projek Penyelidikan.

Kudis adalah penyakit kulit disebabkan tungau yang menjejaskan kesihatan kerana ia boleh menyebabkan pembentukan kerengsaan kulit dan alopesia. Salah satu rawatan semasa yang dipraktikan ialah memberi ivermectin melalui suntikan subkutaneous. Oleh itu, keberkesanan formulasi hydrogel thermoresponsif. Yang mengandungi 1% ivermectin sebagai ivermectin topical telah disiasat untuk merawat penyakit kudis pada kucing. Kajian ini mengkaji enam formulasi dengan kepekatan PEG yang berbeza and menilai sifat fizikal and analisi suhu gel (Tgel) dan karakteristic reologi formulasi tersebut. Suhu gelasi sebagai refleksi terhadap sensitiviti pada kepekatan PEG Bersama ivermectin yang mempengaruhi Tgel. Penilaian reologi menunjukan non-Newtonian, pseudoplastic yang penting untuk mengoptimumkan hydrogel sebagai *dressing*. Pemeriksaan mikroskopik memberi verifikasi kehadiran *Notoedres cati* dalam semua kucing sebelum rawatan dimulakan . Penilaian kudis secara visual sebelum rawatan memnunjukkan pembentukan kerengsaan kulit dan alopesia beserta tingkah laku menggauru yang konsisten. Penilaian kudis secara visual selepas rawatan menunjukkan pengurangan kudis di dalam group rawatan , ia menunjukkan potensi thermoresponsif hydrogel yang menagndungi 1 % ivermectin sebagai rawatan topical.

Keywords: Penyakit kudis, ivermectin, kucing , rawatan topikal, , thermoresponsif hidrogel

ACKNOWLEDGEMENT

I am deeply thankful to Dr. Luqman for his outstanding guidance and support throughout this research journey. His mentorship has played a crucial role in navigating the complexities of this study, and I feel fortunate to have benefited from his expertise.

I extend my gratitude to Dr. Mursyidah and Dr. Mimi for their valuable contributions to this research. Their diverse viewpoints and knowledgeable advice have significantly expanded the study's scope and improved its overall quality.

A special shoutout to Dr. Mariam and Dr. Abdin from UniKL for their insightful critiques, valuable contributions, and collaborative spirit. Their constructive feedback and academic insights have added a layer of excellence and depth to this thesis that I'm genuinely excited about.

To my friends Farah, Mira, Nurin, and Jazmina , thank you for being wonderful companions throughout this academic journey. Their understanding, shared enthusiasm, and support have made this experience not only academically fulfilling but also joyous.

Deepest thanks to my family, Umi, Walid and Kak Izzah for their unwavering encouragement, sacrifices, and boundless love. Their belief in my capabilities has been a constant inspiration to aim high and achieve excellence in this project. Lastly, I want to extend my heartfelt thanks to all who have been part of this journey. Your contributions have made this experience truly delightful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS	DEFINITION
IVM	Ivermectin
PEG	Polyethene glycol
PPM	Part per million
RPM	Rotation per minute
SID	Once a day
Tgel	Gelation temperature
w/w	Weight/weight

LIST OF SYMBOLS

SYMBOLS	DEFINITION
%	Percentage
°C	Celsius

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

Folej J.E (2016) indicates that cats suffering from mange may self-mutilate and change their normal behaviours such as hunting and foraging because of intense pruritus. It is caused by mites which may include *Sarcoptes scabies, Cheyletiella spp., Demodex gatoi* and *Notoedres cati* by burrowing in the epidermis to create intraepidermal tunnels to lay their eggs. The hatch mites will crawl out from the tunnel to the outermost layer of skin. Burrowing and crawling by the mites causes skin irritation which will scratch the affected area. The affected area eventually becomes more severe as the multiplication of mites and self-trauma. Skin becomes crusty, hyperkeratosis and alopecic.To reduce mange infestation, antiparasitic drugs are used to eliminate the mites on the skin. One of the drugs commonly used is extra-label formulation of ivermectin (IVM). According to Pagé et al. (2000), Administering ivermectin topically comes with advantages over oral (PO) or subcutaneous (SC) methods. Injectable ivermectin given orally might have an unpleasant taste, potentially causing animals to reject swallowing the drug. Subcutaneous administration can be time-consuming, especially when dealing with a group of animals, and may induce pain at the injection site. In this paper, IVM will be formulated into a thermoresponsive gel and evaluated for efficiency through skin lesion progression.

1.1. RESEARCH PROBLEM STATEMENT

With existing treatment for mange infestation, IVM also shows high efficiency in resolving mange lesions. However, in most cases, IVM were prescribed through parenteral injection that must be done by a certified veterinarian which is challenging for the pet owners as they are required to bring their pets to the clinic for each injection. Therefore, the limitation should be overcome by focusing on drug delivery technology. Hydrogel is known for its biocompatible properties which significantly prolong drug release and increase drug bioavailability. However, the current situation in veterinary medicine lacks of innovative formulations or products that offer comprehensive topical antiparasitic effects in a single dressing. The pluronic polymer which is a type of polymer that can transform into a hydrogel at body temperature will be used for this study. Moreover, due to its high permeability and low toxicity characteristics, pluronic polymers exhibit their potential as antiparasitic agents for veterinary purposes. Therefore, a systematic study on these effects is highly required to be carried out to understand their relationship and to produce thermoresponsive topical IVM to the animal.

1.2. RESEARCH QUESTIONS

1.2.1. Does hydrogel IVM have the same effectiveness as other topical drugs in managing mange infestation?

1.2.2. What is the concentration needed for hydrogel IVM to reduce mange lesions?

1.2.3. Is the IVM interact well with the polymer in a thermoresponsive hydrogel formulation?

1.3. RESEARCH HYPOTHESIS

1.3.1. Thermoresponsive hydrogel loaded with IVM exhibits antiparasitic effect against ectoparasites and will minimize the skin lesions manifested by feline mange infestations

1.4. RESEARCH OBJECTIVES

1.4.1. To formulate and characterise thermoresponsive hydrogel containing IVM using polymer.

1.4.2. To investigate the effectiveness of hydrogel loaded with IVM in treating mange infestations.

1.4.3. To determine the in vivo safety effect of hydrogel during the course of treatments in cats

CHAPTER 2

LITERATURE REVIEW

2.0 AETIOLOGY AND CLINICAL SIGNS OF MITE INFESTATIONS

Feline demodicosis is a parasitic skin condition which caused by *Demodex gatoi*, *Demodex cati,* and an unidentified species have been discovered which relatively rare. The various clinical manifestation of feline demodicosis differ based on the specific mite species as there is variability in the cases when it comes to pruritus. The reports also reveal that multiple mite species coexisting in the same infestation. Among three feline demodex species, D. *gatoi* is the most common. It can be characterised by its tiny size which are 91 µm for males and 108 µm for females, and it reside in the stratum corneum, the outermost layer of skin. The primary clinical sign of infestation is pruritus, commonly manifested as excessive grooming. Even though the condition can affect any part of the body, it usually affects the ventral abdomen, inner thighs, flanks, and forelimbs because these areas are easy for the cat to groom. On some occasions, cats may exhibit miliary dermatitis or latent lip ulcers, which may be related to excessive grooming. (Beale, 2012)

Cheyletiellosis commonly referred to as Cheyletiella dermatitis is an intermittently pruritic and scaly skin condition which impacts cats, dogs, rabbits and even humans. This dermatitis is caused by *Cheyletiella spp* as shown in Figure 1. In cats, *Cheyletiella blakei* is commonly found. Mites usually transmitted through direct contact with an infected animal. Severe infestation may arise due to an environment in which considerable number of infested animals are housed together such as kennel and catteries. Grooming tools also can transfer the mites between animals. The life cycle of *Cheyletiella spp.* is believed to be

completed on the host in 35 days and consists of the following stages: egg, larva, nymph 1, nymph 2, and adult. Remarkably, these 350 µm mites have unique accessory mouthparts called palpi, that resemble hooks and allow them to adhere to their host in order to feed on tissue fluids. Adult females show resilience, surviving up to 10 days under refrigerated laboratory conditions, even though most parasite stages expire within 48 hours of leaving the host. Lesions are mainly found on the dorsum of the body, and cheyletiellosis can range in severity from clinically asymptomatic to extremely itchy. Dogs typically show more cilinical sign than cats, which may be related to cats' meticulous grooming routines that reduce the severity of infestation. Clinically, cats may exhibit severe pruritus or no symptoms at all. Cats with cheyletiellosis have small papules, crusts, mild dorsal scaling, and normal coats. (Schmeitzel, 1988)

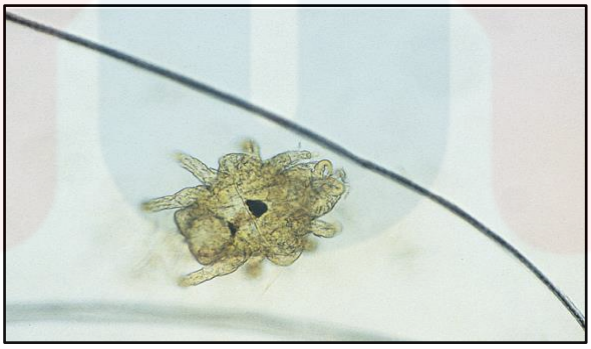


Figure 1:*Cheyletiella spp*. under microscope

Notoedric mange is an irritating and contaigous skin condition which caused by burrowing mites belonging to the Notoedres genus. The *Notoedres cati* (Figure 2) primarily affect cats but also found in other mammals which include human. It is considered uncommon in cat and predominantly diagnosed in stray cat since there are limited information on this topic. In Europe, the prevalence of the disease ranges from 0.2% in owned cats to 2.4% in stray ones (Beugnet et al., 2014).Transmission usually occurs through direct contact with rare instances of exposure to a contaminated environment. *Notoedres cati* has an impact on

cats' overall health and well-being.The itchy lesions can be escalate quickly due to scratching, potentially leading to self-injury and subsequent bacterial infection. Based on Miller et al., (2012) the lesions initiate at the ear pinna's edge and swiftly extend to the head, neck, and sometimes the legs and perineum due to the cat's grooming and sleeping patterns. According to Deplazes et al. (2016) if treatment is not received in immunocompromised humans and kittens, it can be fatal within 4-5 months. Cases are uncommon in practice and are primarily identified in cats that are malnourished, kept in unsanitary settings, or have coexisting illnesses. (Györke et al., 2022)

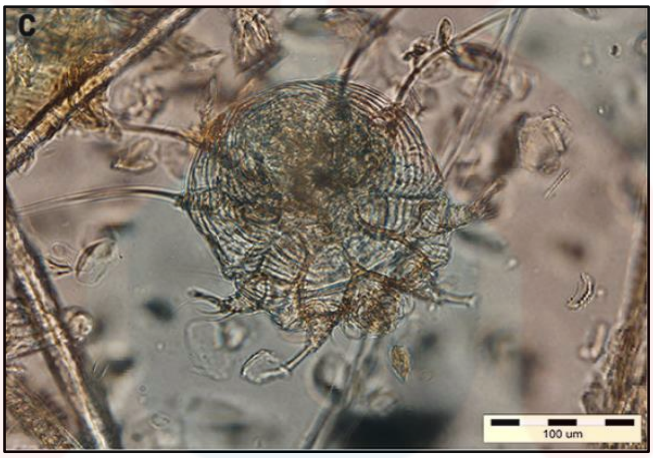


Figure 2:*Notoedres cati* under microscope

Sarcoptic mange (sarcoptic acariasis) due to *S.scabiei* has been reported rare in the cat by *S.scabiei* and *N cati* can be differentiated by their size and other features such as *S.scabiei* have longer limbs, stalks and terminal anus and they are bigger in size compared to *N* cati (Sindhuja et al., 2022). Despite that, both mites are highly infectious and it is transmitted through direct contact. Due to their nature of creating burrows, it may lead to intense pruritus, hyperkeratosis, peeling skin and lesions, especially on the face and the ears. In severe cases, the lesions can extend to the neck, limbs and other body area**.** The clinical signs are often aggravated by secondary bacterial infections, initiated by the excoriations from self trauma and the disease can even be lethal. The mite also possesses a zoonotic

potential and has been diagnosed in humans after close contact with infested animals The condition can potentially become by secondary bacterial infections caused by the excoriations from self-trauma in human and animal. As a result of close interaction with infected animals, the mite has also been identified in humans as having zoonotic potential. (Malik et al., 2006)

2.1. CURRENT SPOT-ON TREATMENT FOR MANGE INFESTATIONS.

Firstly, lime sulfur dip is recommended as a safe topical treatment for feline demodex and cheyletiellosis . Its exact mechanism of action against mites is unknown, but it is thought to involve both keratolytic effects and the formation of miticidal compounds such as polythionic acid and hydrogen sulfide (Wolverton & Wu, 2020). Despite its safety, the dip may cause gastrointestinal signs in cats, requiring precautions like the use of an Elizabethan collar during and after application. Some disadvantages are a bad smell, the possibility of staining fur, and the possibility of tarnishing jewellery. Based on Ghubash (2006), a specific dipping regimen is suggested which is applied weekly for six weeks using a 3.1% (v/v) concentration (4 ounces of dip per gallon of water). In circumstances in which Demodex or Cheyletiella is suspected, owners are strongly encouraged to commit to doing three lime sulfur dips at a minimum. It is advised to finish the entire six-treatment course if there is a noticeable improvement after the first three applications. Other differentials should be taken into consideration if the cat doesn't respond. An amitraz dip or other miticidal therapies should be investigated after a lime sulfur dip has been used as the initial course of treatment.

Second, Amitraz is not approved for use in cats, and neither the manufacturer nor the author recommend using it because of possible negative side effects. As studied by Mueller (2004)

cats may experience drowsiness, diarrhea, hypersalivation, anorexia, and lethargy as side effects. Although the drug has not received official approval, there are cases reported in the literature where it has been successful in treating feline demodex infections. Based on Foil et al. (2003) for a duration of 4 to 6 weeks, the suggested treatment entails applying a 0.0125 to 0.025% solution every 5 to 7 days. However, weekly amitraz solution dips for six to eight treatments have been found to be an effective strategy for managing this specific parasite when it comes to cheyletiellosis. According to Muller et al. (2001) , a 0.025% solution (1 vial/2 gallons of water, 250 ppm) is the recommended dose, but it should be noted that this is an off-label use of the medication.

Thirdly, fipronil is an insecticide that belongs to the phenyl pyrazole class and inhibits GABA receptors in parasites. It shows promise as a safe and efficient treatment for Cheyletiella mites as research by Chadwick (1997) the 10% spot-on product and the 0.25% spray both work well. Based on Broek (2007) the recommended dosages for the 0.25% fipronil spray are one to two pumps per pound every two weeks for three to four treatments, and the Frontline 10% spot-on product should be applied every thirty days for three treatments. A few dermatologists choose to apply spot-on products more frequently, once every two weeks, and report faster clinical responses without any negative side effects. In sarcoptic mange in canine, a spray form at 3 mL/kg every 3 weeks for three treatments or a sponge form at 6 mL/kg once weekly for two weeks are the two treatment approaches. According to Curtis, (2012) fipronil is a useful option for treating sarcoptic mange in dogs, but it's important to remember that this treatment is advised for early-stage situations or when other options are inappropriate.

Lastly, the widely used parasite medication selamectin (Revolution, Pfizer) shows promise as a treatment for feline notoedric mange. According to Itoh et al. (2004), it can be applied every 2 weeks for three treatments, offering a favorable balance of safety and ease of use and standing out as the author's preferred option. Selamectin was shown to be effective against cheyletiellosis in a household containing fifteen affected cats studied by Chailleux & Paradis (2002) . When three treatments were given every 30 days without concurrent environmental decontamination, the study discovered that after 60 days, all cats had shown clinical resolution and were free of mites. Selamectin has proven to be a safe and efficient treatment for cheyletiella in cats; notably, no relapses were reported even a year later.

Based on Hardy et al. (2012), Selamectin (Stronghold; Pfizer Ltd) should be administered 14 days after Imidocloprid (Advantage; Bayer) on two separate occasions, separated by four weeks, after feline sarcoptic mange was diagnosed. One week following the first selamectin treatment, pruritus and lesions improved, and after the second application, crusting completely resolved. Furthermore, two to four weeks after the cat's first selamectin application, the owner's pruritic rash disappeared on its own.

2.2. IVERMECTIN FOR MANGE INFESTATION

IVM operates by selectively attaching to specific receptors for neurotransmitters present in the peripheral motor synapses of parasites. This action leads to an insecticidal impact, effectively eradicating both internal and external parasites. It accomplishes this by inducing paralysis in nematodes, arthropods, and insects through the inhibition of nerve impulse conduction in nematode interneuron synapses and arthropod and insect nerve-muscle synapses. To treat sarcoptic mange, IVM can be administered orally or subcutaneously. The recommended treatment regimen involves an oral dosage of 0.2 to 0.4 mg/kg every 7

days for a total of three to four treatments, or a subcutaneous dosage every 14 days for two to three treatments (Dourmishev et al., 2005).

A study was conducted by Page et al, (2000) to evaluate the effectiveness of different administration routes for treating cheyletiellosis by using an extra-label formulation of IVM through oral administration, topical (pour-on) and subcutaneous injection as treatment methods. All methods showed a positive result in reducing *Cheyletiella* spp. infection in sixteen Persian cats. However, subcutaneous injection required a longer time during treatment and induced pain at the injection site. In contrast, the oral route posed difficulties as cats were reluctant to swallow the drugs due to the unpalatable taste of IVM. Additionally, some cats were reported in experiencing topical (pour-on) side effects which localized alopecia and mild scaling on the site of application of the drugs. Despite that, these side effects were ameliorated in 1- 2 months.

Early research indicated that the most effective protocol for treating demodicosis was to administer IVM orally once a day for 0.4 mg/kg. However, oral administration on a daily basis at 350 µg/kg and 400 µg/kg proved only 30% and 48% cure rates, respectively, suggesting poor efficacy as the results could have been adversely affected by concurrent drug administration and sample size restrictions. (Mueller et al., 2020)

For three weeks, all of the affected cats received weekly subcutaneous injections of injectable IVM (Inj. Neomec, 10%) at a dose of 200µg/kg body weight. Supportive therapy consisted of applying a topical skin ointment and taking oral multivitamins (syrup zipvit) and minerals (syrup zincovit) every day for at least ten days. Following a three-week course of subcutaneous IVM treatment at a dosage of 200µg/kg body weight, the affected cats

recovered completely and showed signs of healthy coat and hair growth. All signs of severe pruritus, alopecia, scales, or crusts entirely disappeared from the affected body parts, proving that IVM is an effective treatment for notoedric mange in domestic cats.(Ozukum et al.,2019)

2.3. PLURONIC GEL AS THERMORESPONSIVE GEL

Based on Akash & Rehman (2015). Pluronic, particularly the widely studied Pluronic P127, is renowned for its remarkable thermoreversible qualities as it made of polyethelene oxide and polypropylene oxide as shown in Figure 3. It exhibits a unique behaviour, transforming from a free-flowing liquid state at lower temperatures to a semi-solid gel state at higher temperatures. This exceptional property positions it as an excellent thermoresponsive polymer for topical drug delivery systems. (Ban et al., 2017). These thermoresponsive gels, which undergo a phase transition from liquid to gel upon contact with the skin or body temperature, have garnered significant attention in wound care applications. The ability to rapidly form a protective gel barrier and adapt to wounds of varying sizes and locations makes them highly desirable for enhancing wound self-medication. According to Haliza Katas et al. (2017), to achieve the desired gelation temperature (Tgel) suitable for topical applications, the chemical composition is modified by incorporating specific excipients such as polyethene glycol 400 (PEG 400) and/or starch. PEG 400, a water-soluble polymer, forms stable micelle clusters with Pluronic P127, effectively elevating the Tgel. Additionally, starch, with its capability to form inclusion complexes with hydrophobic substances, is widely utilized to further adjust the gelation temperature. The advantages of thermoresponsive topical drug delivery systems extend beyond their temperature-dependent gelation. These systems offer excellent flow properties upon administration,

enabling even distribution across various wound sizes and locations. Moreover, they facilitate the controlled release of active substances, allowing for targeted and sustained therapeutic effects. (Moreno et al., 2014)

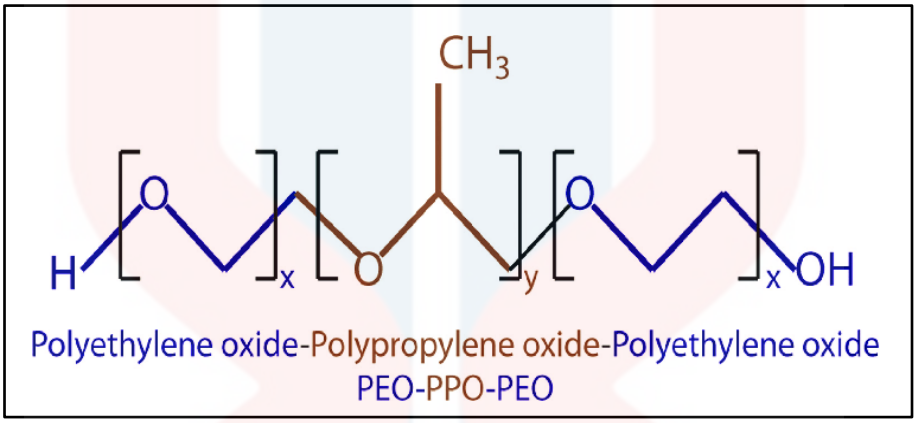


Figure 3: The molecular structure of Pluronic p127

2.4. PLURONIC GEL AS WOUND HEALING

Pluronic polyols are a class of non-ionic surfactants that are widely used in the drug and pharmaceutical industries. They are produced when polymeric oxypropylene and oxyethylene condense. Due to their unique physical properties, these polyols show potential as carriers of materials for osseous grafting (Hokett et al., 2000). Unpublished data supports their potential benefits, which include promoting collagen formation, aiding in wound healing, and facilitating early fibroblast attachment. Furthermore, pluronic polyols have the potential to decrease PGE2 production by monocytes in culture, improve microcirculation in elevated skin flaps, and lessen edema in injured tissues. Furthermore, their 20% concentration serves as an ideal vehicle for assisting in graft placement and maintenance at the area of interest due to their known ability to form a gel at body temperature.(Fowler et al., 2002)

Based on research by Nalbandian et al. (1987), when combined with humectants and antibiotics, Pluronic P127 meets the requirements for an effective short-term skin replacement. In addition to its positive wettability characteristics, this non-ionic polymers can be applied as a liquid that conforms to the wound's shape before going through in situ gelation. It can remain liquid at room temperature and change into a transparent, colorless gel at body temperature due to its special negative temperature coefficient gelation property. The final outcome is a gel that promotes faster reepithelialization of the wound, lessens pain and inflammation, and maintains a thin layer of wound fluid to stop the loss of heat, plasma, water, and electrolytes. According to Dr. J. James Rowsey, it is suggested that pluronic P127 will stimulate the body's natural production of epithelial growth factor (EGF), which will promote faster healing of wounds. In certain cases, the experimental use of Pluronic P127 preparations showed accelerated wound healing. (Park,1978)

CHAPTER 3

RESEARCH METHODOLOGY

3.0 MATERIALS AND METHODS

3.1.1. FORMULATION OF THERMORESPONSIVE GEL

The thermoresponsive gels (25% w/w) were prepared using cold method which established by Haliza Katas et al.(2017). 12.5g of Pluronic P127 was measured and poured into 50mL of cold distilled water. The combination undergoes continuous magnetic stirring at 200 rpm, 4 °C for 4 hours to prepare thermoresponsive gel (25% w/w). Then, it was held at 4 °C overnight.10 mL of each different concentration PEG 400 (1% , 5% ,10 % and 22%) were prepared by dilution using cold distilled water. Then, added into 50 mL of Pluronic gel. The preparation was conducted under continuous magnetic stirring at 200 rpm , 4 °C for 4 hours as shown in Figure 4.

Figure 4: Implementation of procedure

3.1.2. PREPARATION OF THERMORESPONSIVE HYDROGEL LOADED WITH IVERMECTIN

The solubility of IVM was tested in 90%, 80% and 70% of ethanol. In this paper, 80% of ethanol was chosen to prepare 1 % of IVM as according to study , this concentration is effective against scabietic treatment (González Canga et al., 2008). 10mg of IVM was measured and diluted using 1 mL of 80% ethanol. 1% of IVM were added into thermoresponsive gel under cold method. The mixture was stirred for 4 hours at 4 °C before being kept in a 4-8 °C refrigerator until further analysed.

3.1.3. DETERMINING GELATION TEMPERATURE

Each of thermoresponsive hydrogel with different concentration of PEG 400 was added into beaker. Each hydrogel was continuously stirred under magnetic stirring at different temperature (initial temperature, 20 °C and 37 °C) as shown in Figure 5.

The speed of stirrer was observed to determine time and temperature of sol- gel transition. Time and temperature of sol – gel transition was recorded.

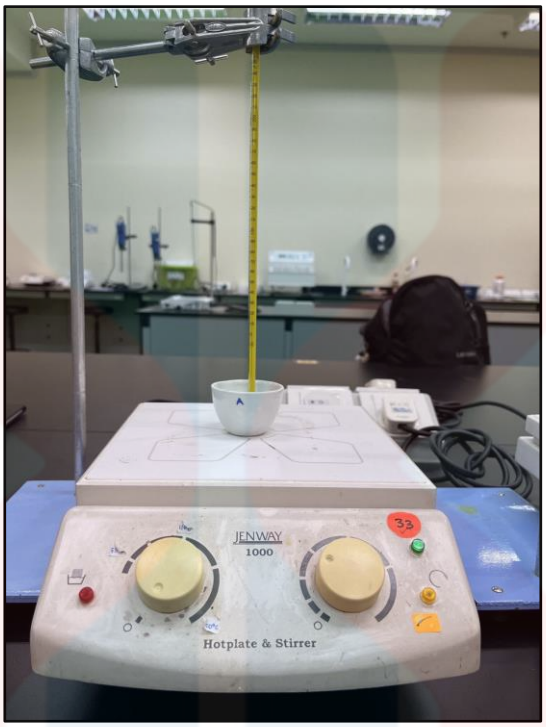


Figure 5: Determination of gelation temperature.

3.1.4. EVALUATION OF FORMULATION

All the parameters were conducted under standard procedure. All six formulation were inspected for their appearance, colour , odour, physical state and pH which was measured by using Eutech pH 700 (USA).

3.1.4.1. Determination of pH

The pH meter was calibrated using neutral pH reading. The probe was immersed into 30 ml of hydrogel formulation. The pH readings were measured for thrice and average pH was recorded.

3.1.4.2. Viscosity studies

All six formulations were tested for their rheological studies using Molecular Compact Rheometer 202 (Anton Paar, Germany) as shown in Figure 6. Shear rate versus shear stress was applied for 1 -100 shear rate per second with 10 second measuring point for each formulation.

Figure 6: Rheometer

3.2. STUDY POPULATION

The study population comprised of 12 stray cats collected from the wet market in Kota Bharu, selected for their exposure to environmental factors that may contribute to mange infestations. These cats were grouped into 3 group which each group consist of 4 cats.

3.3. STUDY ANIMAL

The research subjects in this project are stray cats that manifested the clinical signs of mange infestation were collected from different markets in Kota Bharu.

3.4. SELECTION CRITERIA OF ANIMAL

3.4.1.1. Inclusion criteria

The inclusion criteria for subjects was the detection of a mange lesion by a through superficial and deep skin scraping. This ensured that only cats with clinical signs of mange were included in the study, allowing for a targeted investigation of the efficacy of ivermectin as a treatment.

3.4.1.2. Exclusion criteria

Cats with pre-existing health conditions were excluded from the research by thorough physical examination and assessing blood parameters to ensure that the evaluation of ivermectin's efficacy was not influenced by underlying health issues. The cats that were excluded were released to the origin place.

3.5. STUDY DESIGN

Table 1 : Categorisation of Treatment Intervention

Group	Number of cats	Treatment given	Frequency	Duration
Positive Control	4	Spot on Frontline	SID for every 2 weeks	2 weeks
Negative Control	4	Thermoresponsive gel only	SID for every 2 weeks	2 weeks
Treatment	4	Thermoresponsive gel loaded (1%) IVM	SID for every 2 weeks	2 weeks

3.6. TREATMENT APPLICATION

All cats in the treatment group were administered a thermoresponsive gel loaded with 1% ivermectin, applied exclusively to the affected areas

3.7. ETHICAL CONSIDERATION

All cats be handled in strict accordance with guidelines and approval by Institutional Animal Care and Use Committee (IACUC), Universiti Malaysia Kelantan with approval code UMK/FPV/ACUE/FYP/007/2023

CHAPTER 4

RESULT

4.0 EVALUATION OF FORMULATION

Table 2 shows result of the physical properties of the thermoresponsive gel where all formulation exhibit colourless, odourless and semisolid characteristics. However, the formulation of Pluronic P127 shows the highest pH value followed by Pluronic P127 with 5% of PEG , Pluronic P127 with 1% PEG, Pluronic P127 with 10% PEG, Pluronic P127 with 22% of PEG.

Table 2: Physical properties of formulation

Formulation	**Physical appearance**			**pH**
	Colour	**Odour**	**Form**	
Pluronic P127	Colourless	Odourless	Semisolid	7.19
Pluronic P127 + 1%	Colourless	Odourless	Semisolid	7.03
Pluronic P127 + 5%	Colourless	Odourless	Semisolid	7.06
Pluronic P127 +10 %	Colourless	Odourless	Semisolid	6.98
Pluronic P127 + 22%	Colourless	Odourless	Semisolid	6.92
Pluronic P127 + 22% + 1 % IVM	Colourless	Odourless	Semisolid	6.86

4.1. GELATION TEMPERATURE

Based on Table 3 the gelation temperature (Tgel) at the skin temperature was achieved when Pluronic P127 was mixed with 22% w/v PEG 400 and increased to 37.3 ± 2.5 °C after adding 1 % IVM.

Table 3: Gelation Temperature (Tgel °C)

Formulation	Tgel (°C)
Pluronic P127 (F1)	37.0 ± 0.0
Pluronic P127+ PEG 400 1% (F2)	39.5 ± 0.7
Pluronic P127 + PEG 400 5% (F3)	37.0 ± 2.8
Pluronic P127 + PEG 400 10% (F4)	34.0 ± 5.7
Pluronic P127 + PEG 400 22% (F5)	35.5 ± 0.7
Pluronic P127 + PEG 400 22% + IVM 1%	37.3 ± 2.5

4.2. VISCOSITY STUDIES

In this case, the hydrogel loaded with IVM has transitional properties between solid and liquid states, with characteristics that require a comprehensive study of its rheological properties. Viscosity plays a key role in defining the substance's flow resistance, impacting its ease of application and spread. Studying shear rate and shear stress, provides invaluable insight into the flow behaviour of the hydrogel.

Therefore, it is imperative to consider these factors to develop effective hydrogel dressings. A hydrogel dressing with precisely calibrated viscosity levels enhances animal compliance and exhibits a non-Newtonian and pseudoplastic profile. This profile indicates that perceived viscosities decline with increasing shear rate as illustrated by the Figure 7.

The graph illustrates a non-linear plot and gradient (n) which represents the apparent viscosity. All materials display n <1, reflecting the non-Newtonian and pseudoplastic profile.

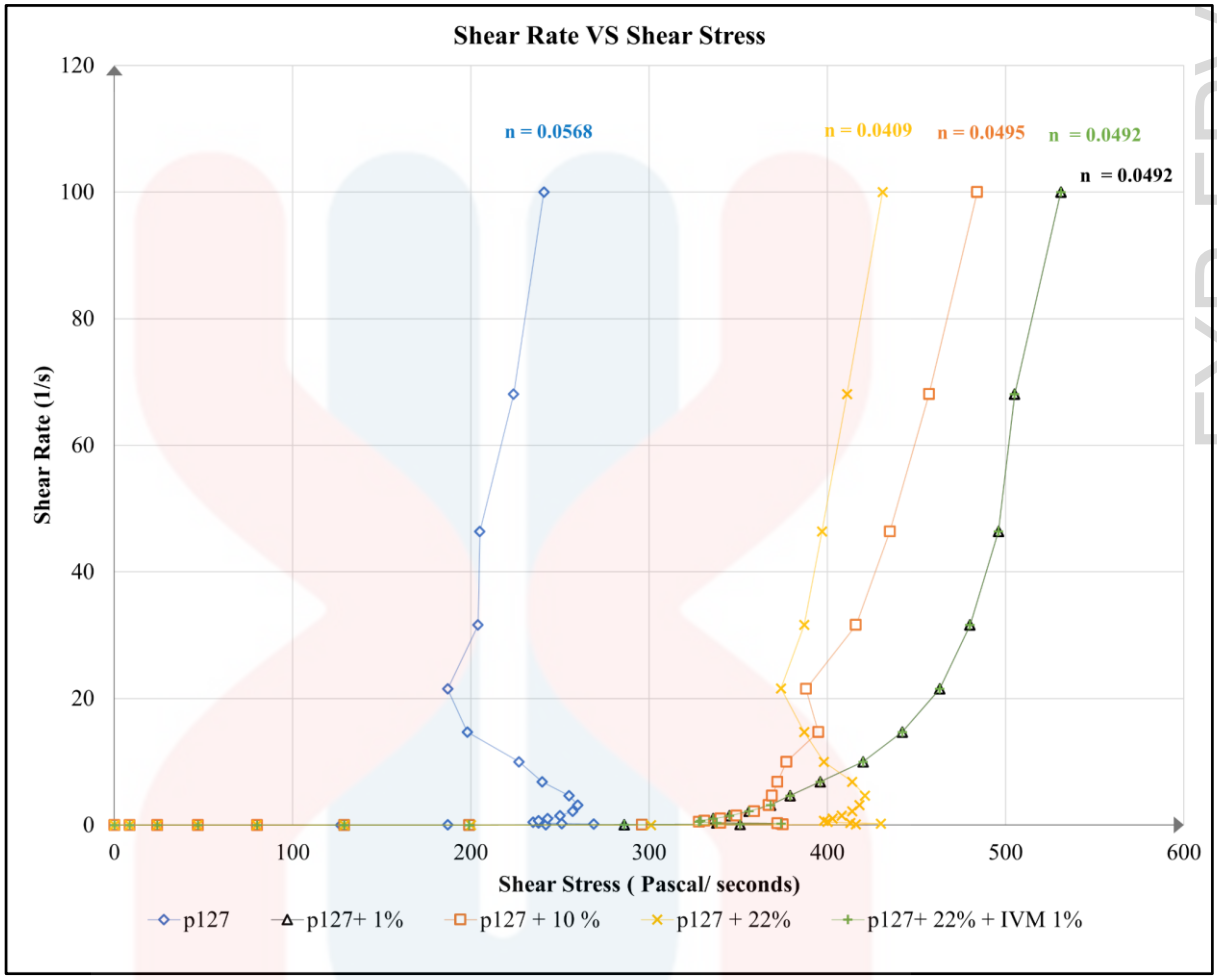


Figure 7: Viscosity of Formulations

4.3. MICROSCOPIC EXAMINATION

Notoedres cati was detected in all cats, encompassing both positive and negative control groups, including the treatment group as shown in Figure 8 and 9. The characteristics observed were the thumbprint body pattern and the dorsal anus.

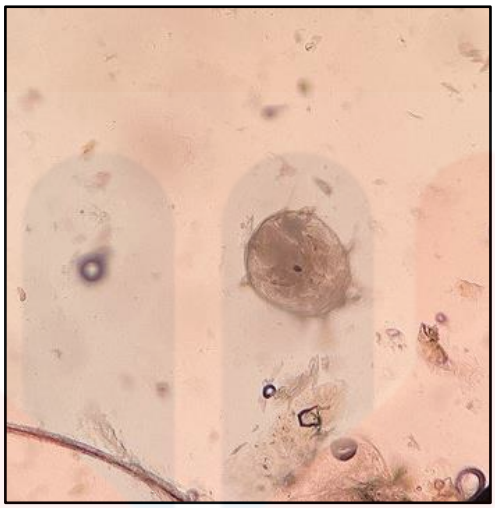


Figure 8: *N.cati* under 40x magnifaction

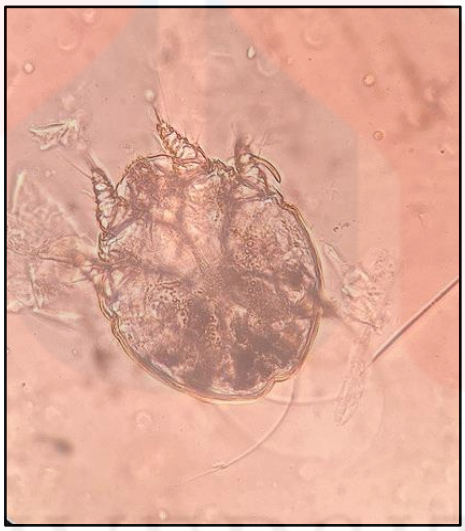


Figure 9: *N.cati* under 100x magnification

4.4. VISUAL LESION ASSESSMENT

4.4.1. Initial assessment

Four (4) cats were used as sample study for treatment group. Cat 1 presented with a substantial 0.4 cm thick crusty lesion on both ear pinnae margins, covering an area of approximately 1.5 cm x 2 cm compared to Cat 2 which had comparable affected area, yet with a thinner 0.2 cm crusty lesion at the ear margins. Cat 3 displayed multifocal, elevated crusty lesions on the external ear pinna, progressing

into alopecia while Cat 4 exhibited thick crusty lesions along the left ear margin and the rostral border of the right ear helix, approximately 0.2 cm in thickness. .Upon observation, a consistent scratching behaviour in all cats was noted, suggesting a uniform response to the discomfort and irritation associated with the presence of crusty lesions. Table 4 show the condition of the lesion at day 0 for treatment group , negative control group and positive control group.

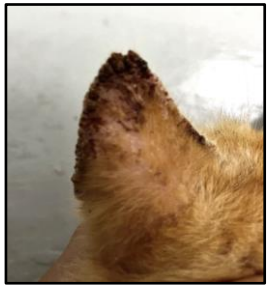
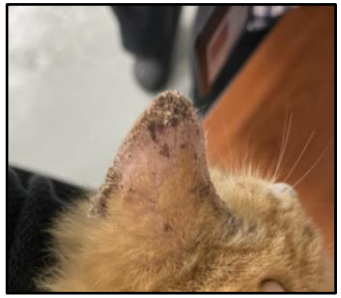
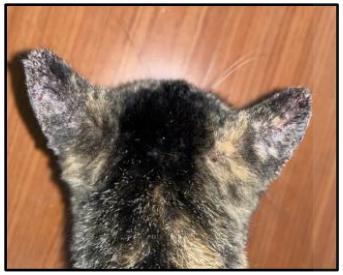
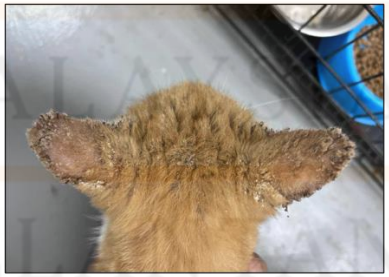
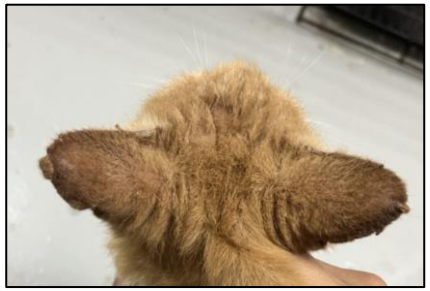
4.4.2. Post Treatment

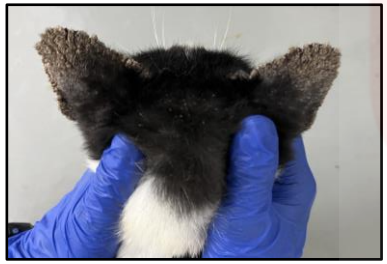
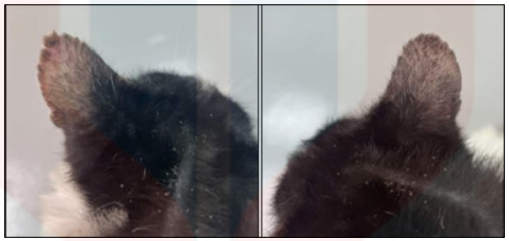
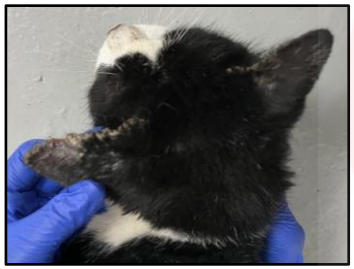
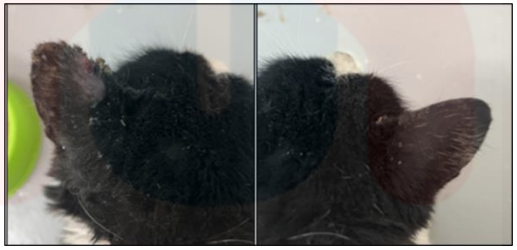
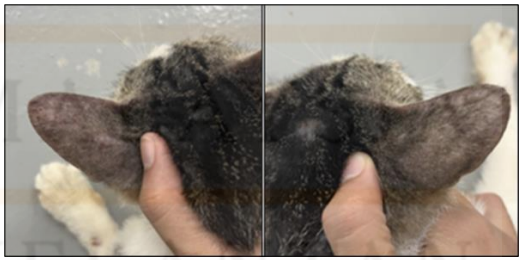
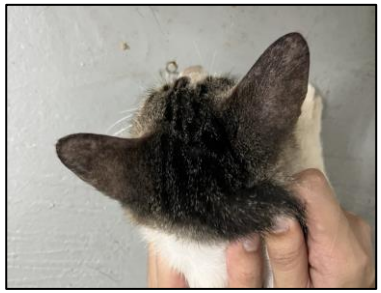
After two weeks of treatment, a re-evaluation was conducted to assess the lesion. Based on the Table 4 , the post-treatment assessment has demonstrated a reduction in the severity of lesions in the treatment group and positive control group in comparison to the day 0. Nine days post-treatment, noticeable improvements were observed,in treatment group which characterized by reduced lesion thickness and affected area, particularly in the alopecic region in Cat 1 while in Cat 2 there was a discernible decrease in both lesion thickness and affected area. The comparison between Cat 1 and Cat 2 indicates the effectiveness of the hydrogel may be influenced by the thickness of the lesion. For Cat 3 and Cat 4, a significant reduction in lesions was observed in the right ear, while the left ear lesions progressed into the alopecic region. The comparison of Cat 3 and Cat 4 implied that lesion distribution and location play a pivotal role in influencing the performance of hydrogel with the ear margin requiring an extended period for the thermoresponsive gel to manifest its effects.

On the day 14 of post treatment, the significant improvement of all cats in the treatment group were observed. The crusty lesion and alopecic region on the day 9 post treatment is replaced by the extensive hair growth. However, in Cat 1 and

Cat 4 the crusty lesion is still present on the edge of ear margin. Among all cats, Cat 4 exhibits excessive scratching on the affect region which exacerbates the existing wound from day 9 post treatment. The hair growth on the previous alopecic area was observed and significant reduction of crusty lesion also noticeable in day 14 post treatment of positive control group. In contrast, the negative control group did not show significant improvement .

Table 4: The comparison of initial assessment and post treatment

Group	Initial Assessment (Day 0)	Post Treatment (Day 9)	Post Treatment (Day 14)
Positive Control		None	
Negative Control		None	
Treatment group	Cat 1 	Cat 1 	Cat 1 

	Cat 2 	Cat 2 	Cat 2 
	Cat 3 	Cat 3 	Cat 3 
	Cat 4 	Cat 4 	Cat 4 

CHAPTER 5

DISCUSSION

The optimal formulation of thermoresponsive gels was selected based on Tgel and physical properties which were supported by rheological analysis before being incorporated with IVM 1%. Based on the result in Table 3, all formulations exhibit similar physical properties which appear colourless, odourless and semisolid state however, there are slight differences in pH. Formulation F1 shows the highest value (7.19) followed by F3 (07.06), F2 (7.03), F4 (6.98), F5 (6.92). Despite that, these formulations are safe to be applied on the skin as the pH within the normal pH of the cat skin, is 6.6 -7.4 which may not irritate (Bourdeau et al., 2004).

In contrast, the Tgel result shows F2 , shows the highest Tgel (39.5 ± 0.7 °C) followed by F3 (37.0 ± 2.8°C), F1 (37.0 ± 0.0°C), F5 (35.5 ± 0.7°C) and F4 (34.0 ± 5.7 °C). The fluctuation of the formulations was due to the concentration of PEG which PEG 400 alters the micellar structure of Pluronic P127 by forming bonds between the ester group of PEG 400 and the hydrophilic chains of Pluronic P127. This interaction induces dehydration in the hydrophobic polypropylene (PPO) block, leading to heightened entanglement among neighbouring micelles as reported by Faris Taufeq et al. (2023)..However, in his studies showed different result as Tgel of Pluronic P127 falls below 25°C followed by Pluronic P127 with 5% PEG (27.3 ± 0.6 °C), Pluronic with 10% PEG (30.0 ± 1.0°C) and Pluronic P127 with 22% PEG (37.0 ± 1.0°C). Despite triplicate measurements was carried out but the variability of the result may due human factors as the Tgel was measured when magnetic stirrer stopped stirring under constant rotation per minute.

F1, F4 and F5 were selected for further analysis to incorporate 1% IVM as stated by Ibrahim et al. (2012) that acceptable Tgel is considered when it falls within the temperature range of 25 to 37 °C. Tgel that is higher than 37 °C may remain liquid during application which cause quick drainage on the application site. However, after 1% IVM was incorporated, F1, F4 and F5 showed elevation of Tgel but F5 was selected as it was the nearest range to the acceptable range,37.3 ± 2.5℃. This occurs due to 80% of ethanol content in preparing 1% IVM which affects the micellar structure of Pluronic PF127. The findings from studies, ethanol is a short alkyl group which suppresses the micelle formation as it binds with copolymer, thus increasing the gelation temperature (Kwon et al., 2001). However, this can be overcome as comparing to study by Faris Taufeq et al. (2022), the loaded component is extracted and dried freeze the to increase the solubilty of loaded component with themoresponsive gel and eliminating other content.

Despite the influence of ethanol after loading 1% IVM in F5 (P127 + 22%) , it reveals that this combination still exhibits a thermoresponsive nature as the graph illustrated in Figure 7 shows a pseudo-plastic or non-Newtonian curve which is similar to the F2 (P127 +1%) graph. However, the pattern of the F2 and F5 with 1% IVM (P127+22%+1%IVM) curves is different from other formulations. Compared to research by Katas et al. (2017), all formulations with and without containing the loaded component show similar rheological curves.To address this potential inconsistency, it is recommended to conduct repeated rheological studies, as the present measurement was undertaken only once during experimentation.

The performance of thermoresponsive gel loaded 1 % IVM was assess through visual assessment of the animal as shown in Table 4. All cat in treatment group shows positive

outcome as reduction of crusty lesion through the distribution and thickness. However, Cat 3 still exhibits scratching behaviour throughout the treatment course. In contrast, the lesion in positive control group has slow progressive healing despite applying commercial product, Frontline. In comparison to the research by Knaus et al. (2014), instead of visual assessment, mite counting and clinical lesion scoring were conducted to determine the efficacy percentage of the treatment through the number of live mite post treatment which more significant value. Plus, a single course of treatment was inadequate to cure the mange infestation as it required over 8 weeks to determine the effectiveness of the treatment after thorough clinical examination and negative test for mite infestation.

It is also imperative to compare the outcomes of the negative control group, which was treated with Pluronic P127 gel, with those of the treatment group. As Pluronic P127 has wound healing capabilities, its impact on the treatment results should be considered. Despite this, there was no amelioration in the lesions for any of the cats in this group and the crusty lesions intensified after the treatment. It is important to emphasise that the wound-healing potential of Pluronic P127 gel may not be fully achieved, as pointed out by Li et al. (2023), who noted that hydrogels derived from P127 often face challenges such as deficiencies in mechanical strength, adhesion and self-healing capabilities.

Lastly, several factors that may influence lesion progression must also be considered. Throughout the treatment period, all cats in each group still displayed scratching behaviour on the ear and tended to rub the affected area against the cage wall. Although the hydrogel was in a semisolid form upon contact with the skin, frequent scratching and rubbing of the affected area can cause the gel to disengage and eventually fade away. Furthermore, the investigation into the bioavailability of 1% ivermectin in the hydrogel to the skin was not conducted in this

study. As stated by Canga et al. (2007), ivermectin's pharmacokinetic variables differ depending on the formulation, route, and animal species. The choice of formulation vehicle plays a significant role in absorption kinetics and plasma availability, with even minor variations having the potential to affect disposition kinetics and impact the efficacy of ivermectin against both endoparasites and ectoparasites in livestock. Additionally, it should be noted that ivermectin is not ovicidal, which means that eggs can hatch on the skin surface and cause a recurrence of mange infestation. This is due to the poor bioavailability of ivermectin in hydrogel on the skin, which may be insufficient to combat a high number of infestations.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

Based on the research finding through visual lesion evaluation, it was observed that the severity of the lesion decreased, as evidenced by the reduction in the appearance of crusty, alopecic areas which shows potential of thermorepsonsive hydrogel loaded with 1% IVM in treatment mange infestation as topical medication. However, there are several limitations were identified, including the lack of investigation into the sustained release of IVM in the hydrogel and its solubility. The poor bioavailability of IVM in the hydrogel on the skin may lead to insufficient efficacy against infestations. As recommendations, repeated studies such as rheological measurement and Tgel are required to overcome inconsistent data..Plus, duration of study should be prolonged in order to evaluate first post treatment and number of treatment needed to completely sure mange infestation. For future studies, investigation of stability of the drugs and sustained released of the drugs should be conducted.

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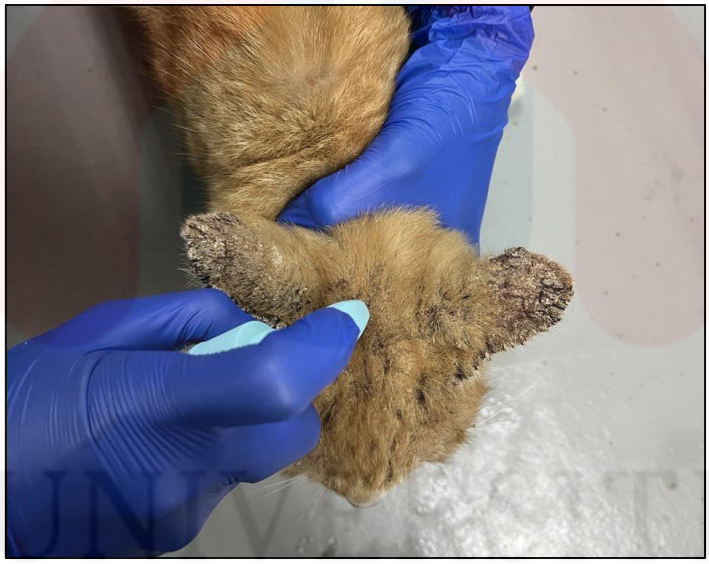
APPENDICES

Appendix 1: Formulation three concentrations of Pluronic P127

Appendix 2: Evaluation of gelation temperature

Appendix 3 : Observation of magnetic stirrer as indication of gelation temperature



Appendix 4: Application of the formula.

Measuring Points	Sheart Stress (Pascal)	Shear Rate (1/s)	Viscosity (Pascal/ second)
0	0.00	0.000000	0.00
1	8.66	0.000152	56800.00
2	24.20	0.000284	85000.00
3	46.90	0.000474	98800.00
4	80.10	0.000745	108000.00
5	129.00	0.001380	93100.00
6	199.00	0.004920	40500.00
7	286.00	0.039000	7310.00
8	351.00	0.112000	3120.00
9	374.00	0.245000	1530.00
10	338.00	0.346000	974.00
11	328.00	0.479000	684.00
12	329.00	0.691000	477.00
13	336.00	1.010000	334.00
14	345.00	1.470000	234.00
15	356.00	2.160000	165.00
16	368.00	3.170000	116.00
17	379.00	4.650000	81.60
18	396.00	6.810000	58.10
19	420.00	10.000000	42.00
20	442.00	14.700000	30.10
21	463.00	21.500000	21.50
22	480.00	31.600000	15.20
23	496.00	46.400000	10.70
24	505.00	68.100000	7.41
25	531.00	100.000000	5.31

Appendix 5 : Rheological Measurement of Pluronic P127 Gel

Measuring Points	Sheart Stress (Pascal)	Shear Rate (1/s)	Viscosity (Pascal/ second)
0	0	0	0
1	8.66	0.000152	56,800
2	24.2	0.000284	85,000
3	46.9	0.000474	98,800
4	80.1	0.000745	108,000
5	129	0.00138	93100
6	199	0.00492	40,500
7	286	0.039	7,310
8	351	0.112	3,120
9	374	0.245	1,530
10	338	0.346	974
11	328	0.479	684
12	329	0.691	477
13	336	1.01	334
14	345	1.47	234
15	356	2.16	165
16	368	3.17	116
17	379	4.65	81.6
18	396	6.81	58.1
19	420	10	42
20	442	14.7	30.1
21	463	21.5	21.5
22	480	31.6	15.2
23	496	46.4	10.7
24	505	68.1	7.41
25	531	100	5.31

Appendix 6: Rheological Measurement of Pluronic P127 and PEG 1%

Measuring Points	Sheart Stress (Pascal)	Shear Rate (1/s)	Viscosity (Pascal/ second)
0	0	0	0
1	8.66	0.000152	56800
2	24.2	0.000284	85000
3	46.9	0.000474	98800
4	80.1	0.000745	108000
5	129	0.00138	93100
6	199	0.00492	40500
7	286	0.039	7310
8	351	0.112	3120
9	374	0.245	1530
10	338	0.346	974
11	328	0.479	684
12	329	0.691	477
13	336	1.01	334
14	345	1.47	234
15	356	2.16	165
16	368	3.17	116
17	379	4.65	81.6
18	396	6.81	58.1
19	420	10	42
20	442	14.7	30.1
21	463	21.5	21.5
22	480	31.6	15.2
23	496	46.4	10.7
24	505	68.1	7.41
25	531	100	5.31

Appendix 7: Rheological Measurement Pluronic P127 and PEG 5%

Measuring Points	Sheart Stress (Pascal)	Shear Rate (1/s)	Viscosity (Pascal/ second)
0	0	0	0
1	8.68	0.00015	57700.00
2	24.2	0.00028	86500.00
3	46.9	0.000486	96500.00
4	80.1	0.000828	96700.00
5	129	0.0015	85700.00
6	199	0.0036	55200.00
7	296	0.0201	14700.00
8	375	0.122	3070.00
9	372	0.251	1490.00
10	340	0.348	977.00
11	328	0.478	687.00
12	331	0.688	481.00
13	340	1.01	338.00
14	349	1.47	237.00
15	359	2.16	166.00
16	367	3.17	116.00
17	369	4.65	79.30
18	372	6.82	54.50
19	377	10	37.70
20	395	14.7	26.90
21	388	21.5	18.00
22	416	31.6	13.10
23	435	46.4	9.37
24	457	68.1	6.71
25	484	100	4.84

Appendix 8: Rheological Measurement of Pluronic P127 and PEG 10%

Measuring Points	Sheart Stress (Pascal)	Shear Rate (1/s)	Viscosity (Pascal/ second)
0	0	0	0
1	8.64	0.000155	55600.00
2	24.1	0.000313	77000.00
3	46.8	0.000492	95100.00
4	80	0.000813	98400.00
5	129	0.00135	95400.00
6	200	0.00269	74100.00
7	301	0.00891	33800.00
8	416	0.0879	4740.00
9	430	0.241	1790.00
10	413	0.343	1200.00
11	400	0.484	826.00
12	398	0.693	575.00
13	403	1.01	400.00
14	408	1.48	276.00
15	414	2.17	191.00
16	418	3.17	132.00
17	421	4.65	90.50
18	414	6.83	60.60
19	398	10	39.70
20	387	14.7	26.40
21	374	21.6	17.30
22	387	31.6	12.30
23	397	46.4	8.56
24	411	68.1	6.03
25	431	100	4.31

Appendix 9: Rheological Measurement of Pluronic P127 and PEG 22%

Measuring Points	Sheart Stress (Pascal)	Shear Rate (1/s)	Viscosity (Pascal/ second)
0	0	0	0
1	8.66	0.000152	56,800
2	24.2	0.000284	85,000
3	46.9	0.000474	98,800
4	80.1	0.000745	108,000.00
5	129	0.00138	93,100
6	199	0.00492	40,500
7	286	0.039	7.31
8	351	0.112	3,120
9	374	0.245	1,530
10	338	0.346	974
11	328	0.479	684
12	329	0.691	477
13	336	1.01	334
14	345	1.47	234
15	356	2.16	165
16	368	3.17	116
17	379	4.65	81.6
18	396	6.81	58.1
19	420	10	42
20	442	14.7	30.1
21	463	21.5	21.5
22	480	31.6	15.2
23	496	46.4	10.7
24	505	68.1	7.41
25	531	100	5.31

Appendix 10: Rheological Measurement of Pluronic P127 , PEG 22% and IVM 1%