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***In-vivo* observations on a topical spot-on formulation (DermSpot spot-on) for prevention of dermatological disorders in dogs**

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Abstract

Veterinary practitioners frequently encounter various types of canine dermatological disorders in their clinical practice. The incidence and prevalence of dermatological disorders vary greatly at different places; however, the treatment of such diseases is costly and usually take weeks to months for complete clinical recovery. Therefore, there is a need to explore every possible aspect and generate preventive strategies. Commercially available formulations containing a complex mixture of components suitable to maintain healthy skin can also be explored while looking for preventive solutions. With a similar view, one commercially available topical spot-on formulation (*viz.*, DermSpot Spot-on by TTK) containing a skin lipid complex, essential fatty acids, an anti-hair fall complex, emollients (including Aloe vera extract), antioxidant and biotin was evaluated in dogs *in-vivo* to observe its effectiveness in prevention of dermatological disorders. The spot-on formulation effectively prevented development of clinical dermatological disorders up to 30 days when used with a suitable protocol. The present paper highlights different aspects of *in-vivo* evaluation and a disquisition on composition of the formulation. Similar investigations are encouraged on a larger scale including a greater number of dogs in future.

Keywords: dog, dermatological disorders, prevention, spot-on, *in-vivo*

Introduction

Dogs are preferred as in-house pets or companions throughout the world. The number of small animal veterinary clinics specifically practicing canine veterinary medicine are also increasing with an increasing number and breeds of dogs in India. Canine skin disorders are very common clinical ailments recorded at organized veterinary hospitals and private veterinary clinics in urban as well as rural areas of India. Exhaustive literature is available with regards to diagnostics and therapeutics of skin diseases in dogs; however, prevention of such diseases under topical climatic conditions is a challenge where managemental factors also play a crucial role [1]. Veterinarians involved directly in treatment of skin diseases should always look for existing incidence and/or prevalence of such diseases and should plan to formulate effective preventive strategies to reduce the occurrence. Moreover, many commercial products are available in market which are being used as supportive or adjunct formulations along with specific therapeutic drugs. Various products have shown effective results as adjunct therapy improving the clinical status of dogs suffering from skin diseases. Some of the products may contain components which have established effects on general skin or coat health. Such products, if evaluated for preventive effects may show positive results in healthy dogs. With a similar point-of-view, a commercially available spot-on DermSpot (TTK) was included in a study on dermatological disorders in dogs. The owners were advised to use the formulation with a suitable protocol and results were evaluated after 30 days. The results of this pilot study described in the paper highlight the *in-vivo* observations and effectiveness of the components of the spot-on formulation.

Materials and Methods

The present study was undertaken to observe *in-vivo* effectiveness of a spot-on formulation (*viz.*, DermSpot Spot-on by TTK; 4 ml preparation for a dog >10 kg body weight; Fig.-1) for prevention of dermatological disorders in dogs brought to the Leo Animal & Bird Clinic, Vastral, Ahmedabad (Gujarat) during February to August-2020 as a part of an extensive investigation on canine dermatological disorders. Clinically healthy dogs (N=06; weighing >10 kg; irrespective of age, breed and sex; random selection) brought to the clinic were

subjected to a detailed clinical examination and found to have no ongoing dermatological disorder. All the dogs were included in the study after obtaining consent from the dog-owners.

Owners were advised to follow a scheduled strategy/protocol for topical application of DermSpot spot-on. The protocol included bathing of dog with a normal shampoo on Day-0 followed by application of the DermSpot spot-on formulation after 24 hours at two spots on dorsal midline (*i.e.*, neck and back) of dogs. Second bathing was advised after 48 hours of first application of the DermSpot spot-on and owners were advised to repeat the same protocol after 15 days.



Fig 1: The DermSpot spot-on and its application on back

The pre-application (Day-0) and post-application (Day-30) evaluation was based on clinical status, estimation of hematological parameters [*viz.*, Haemoglobin (Hb; g/dl), Total erythrocyte count (TEC; $10^6/\mu\text{l}$), Total leucocyte count (TLC; $10^3/\mu\text{l}$), Differential leucocyte count (%)] and estimation of serum biochemical parameters [*viz.*, Alanine aminotransferase (ALT; IU/L), Aspartate aminotransferase (AST; IU/L), Blood urea nitrogen (BUN; mg/dl) and Serum creatinine (SCR; mg/dl)]. The tele-follow-up was continued for additional 150 days specially to monitor clinical status of all dogs.

Results and Discussion

The application of DermSpot spot-on formulation effectively prevented development of clinical dermatological disorders in all dogs (N=06) up to 180 days (30 days of initial monitoring and status after 150 additional days). The results were visible in terms of improved appearance of the coat of the dogs without any side effects or development of skin disease (Fig.-2).



Fig 2: Improved coat condition of dogs applied with DermSpot spot-on

The pre-application (Day-0) values of hematological parameters such as Hb (g/dl), TEC ($\times 10^6/\mu\text{l}$), TLC ($\times 10^3/\mu\text{l}$), Neutrophils (%), Lymphocytes (%), Monocytes (%), Eosinophils (%) and Basophils (%) were 12.65 ± 0.92 g/dl, $05.25 \pm 0.36 \times 10^6/\mu\text{l}$, $9754.67 \pm 416.84 \times 10^3/\mu\text{l}$, $72.00 \pm 0.20\%$, $22.33 \pm 01.97\%$, $03.00 \pm 02.53\%$, $02.33 \pm 00.82\%$ and $00.33 \pm 00.52\%$, respectively. The post-application (Day-30) values of Hb (g/dl), TEC ($\times 10^6/\mu\text{l}$), TLC ($\times 10^3/\mu\text{l}$), Neutrophils (%), Lymphocytes (%), Monocytes (%), Eosinophils (%) and Basophils (%) were 12.98 ± 0.61 g/dl, $05.33 \pm 0.42 \times 10^6/\mu\text{l}$, $10092.33 \pm 499.60 \times 10^3/\mu\text{l}$, $72.17 \pm 02.14\%$, $22.33 \pm 02.94\%$, $02.67 \pm 02.50\%$, $01.50 \pm 00.55\%$ and $00.17 \pm 00.41\%$, respectively. The difference between pre-application and post-application values of hematological parameters in dogs was statistically non-significant ($P > 0.05$). The post-application levels of hematological parameters were found within the normal range established for dogs.

The pre-application (Day-0) values of ALT (IU/L), AST (IU/L), BUN (mg/dl) and SCr (mg/dl) in dogs were 31.83 ± 14.53 IU/L, 26.91 ± 13.59 IU/L, 07.49 ± 03.81 mg/dl and 00.59 ± 00.24 mg/dl, respectively. The post-application (Day-30) values of ALT (IU/L), AST (IU/L), BUN (mg/dl) and SCr (mg/dl) were 31.17 ± 04.59 IU/L, 25.79 ± 04.38 IU/L, 07.93 ± 02.73 mg/dl and 00.58 ± 00.14 mg/dl, respectively. The difference between pre-application and post-application values of serum biochemical parameters in dogs was statistically non-significant ($P > 0.05$). The post-application levels of serum biochemical parameters were found within the normal range established for dogs. Thus, it was evident that the topical application of DermSpot spot-on formulation did not have any impact on hematological and serum biochemical parameters of dogs included in the study.

The DermSpot spot-on formulation used in the present study is a topical barrier repair agent containing [i] skin lipid complex which are believed to replace lost lipids in the skin layers and to support cell-to-cell attachment for stronger barrier, [ii] essential fatty acids which keep skin cells moist and increase collagen production; [iii] anti-hair fall complex which controls hair fall; [iv] emollients which hydrate and soothe the skin; [v] vitamin-E as antioxidant and [vi] biotin to promote hair growth.

The skin lipid complex present in the DermSpot spot-on contains ceramides, cholesterol, fatty acids and phytosphingosine (as per the product brochure). Ceramide, a long-chain fatty acid links with other important molecules to promote cell functions, create a skin barrier, locks skin moisture, prevents drying, prevents irritation, develops protection on epidermal layer to protect them against damages, has anti-aging effects and soothes skin. Such aspects are generally mentioned in literature published based on studies conducted in humans. Effectiveness of ceramides are yet to be explored in depth for canine dermatological issues. On the other hand, phytosphingosine incorporated in DermSpot helps to keep skin moisture intact and prevent irritation when added in any skin formulation. It combines with some lipid components to formulate ceramide and helps to fix cracks in-between cells of the skin. Phytosphingosine has also been described to have anti-inflammatory and antimicrobial effects and it has been used in some commercial skin preparations for humans [2, 3].

The DermSpot spot-on formulation used in the present study also contains oils of hemp seed, flax seed and primrose as essential fatty acids. Such components are commonly

available in skin preparations produced and marketed commercially for humans. Oils of hemp seed are believed to have antimicrobial property, anti-inflammatory property, Omega-3 and linoleic acid which are helpful to reduce oily feeling on skin, neutralize sebum, moisturize skin without clogging skin pores, balance skin oils, provide hydration, regulate oil secretion/production and help to cure minor skin issues (e.g. acne). On the other hand, flax seed contains antioxidants and lignans which are believed to prevent wrinkles, keep skin in tight texture, prevent dandruff, rejuvenate skin and repair damaged hairs. The primrose, a common component of many human skin commercial preparations is believed to soothe and moisturize the skin, enhance the skin texture and skin elasticity to give a clear and healthy complexion. Primrose is documented to have stearic acid, Omega-6 and Omega-9 which are helpful for a stronger skin barrier.

The anti-hair-fall complex of the DermSpot spot-on formulation contains apigenin (*i.e.*, 4',5,7-trihydroxyflavone) which is helpful to increase density and elasticity of dermal layers and inhibit cytotoxic effects of ultraviolet light. It is also documented to have antioxidant, anti-carcinogenic and anti-inflammatory properties which are helpful to protect skin from inflammatory reactions and damages caused by free radicals. In humans, apigenin is documented to restore human dermal fibroblast viability and decrease collagenase expression [4]. The oleanolic acid (a pentacyclic triterpenes) used in the anti-hair-fall complex of the spot-on formulation is documented to be non-toxic in nature, has good biological activity, has scavenging action, has antibacterial property, has antiprotozoal property and is hepatoprotective [5, 6].

Glycerin, Polyethylene glycol (PEG) - PEG200 and Aloe vera extract have been incorporated as emollients in the DermSpot spot-on formulation used in the study. Glycerin is known to be used as sunscreen, traps moisture, provides glow, fights acne, defends skin against pimple development, soften and soothes skin, tones and cleanse the skin in humans. The PEG-200 is evaluated for deleterious effects (e.g., teratogenic in high doses) nowadays; however, it has been described by scientists to have non-toxic and non-allergic properties in past [7]. Meyer and Sturmer [7] have documented that application of PEG-200 once for a day (24 hours) or repeated application for 14 days – every second days for 24 hours does not cause any type of skin irritation. On the other hand, Aloe vera extract used as an emollient in the formulation also has proven moisturizing effects, preventive effects against sunburn, antioxidant property, protective effects against radiation, anti-inflammatory property (due to C-glucosyl chromone) and wound healing properties by increasing collagen formation (due to glucomannan) [8].

Additionally, the spot-on formulation used in the study also has vitamin-E as an antioxidant which is a fat-soluble vitamin having anti-inflammatory property and supportive effects for the immune system. Vitamin-E is also believed to support different cellular functions and maintain skin health by combating with free radicles. This property also ensures prevention of skin against damage caused by ultraviolet radiation. Vitamin-E is also used in various commercial skin products because it gets absorbed easily into the skin and may get stored in sebaceous glands. The oils containing vitamin-E are hard and thick which spread on skin and prepare good moisturizing barrier. The biotin or vitamin-H added into the DermSpot spot-on has effect on fat metabolism, maintains healthy skin appearance and has some proven effects in cases

of psoriasis in humans.

Dermatological effects of all the components used in DermSpot spot-on formulation, are not fully understood or studied exclusively in canine dermatological disorders in Gujarat. In past, various scientists have used different types of barrier repair agents or moisturizers containing different components and they have evaluated their applicability in clinical cases such as canine atopic dermatitis. Frigg *et al.* [9] conducted a clinical study on effect of biotin on skin condition in dogs where they opined that use of biotin for treatment of fur related issues and skin disorders in dogs can provide favorable outcome. Piekutowska *et al.* [10] opined that treatment with skin lipid complex can stimulate the production and secretion of endogenous skin lipids which further contribute to improved epidermal barrier. Ceramide-based moisturizer has been used by Jung *et al.* [11] who opined that such a moisturizer is effective for treating skin barrier dysfunctions. Blaskovic *et al.* [12] used a spot-on formulation containing polyunsaturated fatty acids and essential oils in case of atopic dermatitis where they suggested that use of such a product can be effective in ameliorating clinical signs of atopic dermatitis and is safe for use without adverse reactions. Kapun *et al.* [13] supported practice of supplementing vitamin-E in dogs with atopic dermatitis based on their study on determination of levels of oxidative stress markers, blood glutathione peroxidase, erythrocyte superoxide dismutase, plasma and skin vitamin-E concentrations in atopic dermatitis.

Thus, presence of a combination of some proven and effective components in the DermSpot spot-on could have prevented possible damage to the skin barrier in dogs included in the study. The cooperation from dog-owners of the dogs included in the study could also have resulted in effective clinical outcome.

Based on this study, it can be undoubtedly said that the spot-on formulation contains effective composition to improve skin barrier in dogs. The product should also be considered for large-scale clinical trial in future. The findings of such studies may show noticeable effectiveness of the formulation *in-vivo*. Encouraging the use of such products would benefit veterinarians to reduce incidence and prevalence of canine dermatological disorders. Moreover, the use of such barrier repairing spot-on formulation may also reduce treatment costs by preventing development of severe skin diseases in dogs.

Conclusion

In the present study, a topical spot-on formulation (*viz.*, DermSpot Spot-on) containing skin lipid complex, essential fatty acids, anti-hair fall complex, emollients, antioxidants and biotin effectively prevented development of dermatological disorders *in-vivo* in 06 dogs for 30 days without showing any side effects. Regular use of such preparations is encouraged to prevent clinical disease development, to reduce incidence or prevalence and to reduce treatment cost associated with dermatological disorders in dogs.

Conflict of interest

Authors declare no conflict of interest with regards to funding. All authors have equally contributed during preparation of this research manuscript. Necessary permissions were received before initiating the work.

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