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Histological Investigation Of The Effects Of Cinnamon Extract On Skin Of Male Sheep Affected By Mange

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Abstract

The present study has been conducted to know the histological changes and the effects on the skin of male sheep caused by the parasite Psoroptes ovis and treatment with cinnamon alcoholic extract as an alternative to the traditional treatment. The animals experimental were used(n=28) divided two groups control sheep that represent one group (n=7) and infected sheep (n=21) were treated with three different concentrations (10, 30, 50 mg / l) of cinnamon alcoholic extract were second group. The results indicated the highest rate of recovery from injury (100% of the animals) achieved at a daily treatment with the concentration of 50 mg/l for a period of four weeks. As for the two concentrations of 10 and 30 mg / l, the recovery rates for the same period reached 30% and 40%, respectively. The results of the longitudinal analysis of histological changes in the infected sheep confirmed that the parasite caused severe changes of altered skin layers, major epidermal distortion characterized by hyper keratinization and sloughing of the stratum corneum and stratum granulosum, along with many burrowing mites surrounded by inflammatory infiltrates of histiocytes. We also recorded moderate dermatitis of the sub-epidermis with abnormal architecture when compared with the control animals. However, these histological abnormalities were shifted back to the normal state as a result of the treatment with 50 mg/l of cinnamon extract. We conclude that the alcoholic extract of cinnamon with a concentration of 50 mg/l is a highly efficient alternative for the treatment of mange in animals, as clearly demonstrated by the 100% recovery rate and the restoration of the normal histological architecture.

Keywords: *Psoroptes ovis*, cinnamon, sheep

INTRODUCTION

Scabies is a common disease worldwide. Its importance lies in the fact that it is a common disease between animals and humans. It affects dogs, pigs, cats, sheep, and camels [1,2]. Scabies is a skin disease characterized by causing skin inflammation as well as hair loss, severe itching, general weakness and loss of appetite [3, 4]. The pathological problems experienced by animals are a major obstacle to the economic return of livestock [5] since they demonstrate poor quality of meat and leather along with low milk production [6].

The main cause of this disease is a very small parasite, *Psoroptes ovis*, which is an infectious parasite belongs to the Sarcoptidae family [7, 8].

Given that the disease is epidemiological in nature, it has been acquiring great importance. According to statistics, there are 300 million cases of the disease that are annually occurring around the world, whether in developed or non- developed countries [9]. Chronic infections of scabies are in the form of pimples and papillae, occurring as a result of not diagnosing the disease in its early stages, leading to the spread of the parasite to the rest of the body [10].

Clinical signs begin with red tunnels and a rash in the vicinity of the blisters. The active movement of the parasite and its toxic excretory secretions lead to severe infections causing itching and scratching, especially during the night. This results in the opening and bleeding of the blisters along with infiltration of some fluids, giving an opportunity to infectious by opportunistic microbes such as *Staphylococcus aureus* and *Streptococcus pyogenic*, which ultimately leads to hair or wool loss [11].

Cinnamon is a dense tropical tree that belongs to the invading cinnamon family. Cinnamon contains 55 - 80% cinnamaldehyde, polyphenols such as eugenol, as well as esters and small quantities of ketones and alcohols [12].

Other compounds involved in the composition of cinnamon plants are mannitol [13], gum, sugars, coumarins and tannins [14].

Cinnamon has many properties. In addition to its anti-bacterial and anti-fungal properties, it possesses anti-parasitic, anti-inflammatory and anti-viral properties. Studies have shown that cinnamaldehyde causes synthetic changes in the outer membrane of bacterial cells (Gram + & Gram -) as it can penetrate the membrane and permeate inside Gram- bacteria cells. This leads to damages to the components of the cell and thus its death due to inhibition of some cellular processes and enzymes [15].

The present investigation was designed to explore the potential of cinnamon extract to restore the normal morphological and histological characteristics in the skin of sheep infected with mange. The plant extract demonstrated a promising potential for the application as an alternative to traditional treatment approaches of mange.

MATERIALS AND METHODS

This study was conducted on 28 adult male sheep in one of the farms in Al-Husayniyah district, Karbala Governorate, Iraq, during the month of February 2019. The animal's infection with scabies (n=21) were clinically identified after examining the skin and confirmation of the symptoms by professional veterinarians.

Skin samples were taken from a deep section (epidermis and dermis) of the affected treated and control sheep using a sharp blade [16].

Alcohol extract for cinnamon plant *Cinnamomum zylanicum*

Collection and classification

The plant was scientifically classified by a plant taxonomist at the College of Science for Women, University of Babylon. Stems of cinnamon were collected from an herbarium in the city of Karbala and kept until the extraction process was started.

Preparation of alcoholic extracts

A weigh of 20 g of the dry matter powder was prepared and placed inside a Whatman No.1 filter paper, which was wrapped conically and sealed tightly by both ends and transferred to a Soxhlet extractor. Next, 400 ml of 96% ethyl alcohol was added and the device was run for 24 hours. The process was repeated several times to obtain a sufficient amount of the extract which was dried out at 45 ºC using a thermal oven. The dry extract was stored in the refrigerator until use [17]. For the purpose of preparing the alcoholic extract, 5 Cloud of the extract was dissolved in 5 ml of ethyl alcohol and the volume was completed to 100 ml by distilled water. Thus, the stock solution concentration became 5%, equivalent to 50 mg / ml, from which the three experimental concentrations of 50,30, and 10 mg / ml were prepared. As for the control treatment, a mixture of distilled water and solvent was used.

To investigate the effects of cinnamon extract, treatments with the three concentrations of the prepared solution were performed through the daily wiping of the entire skin (once a day).

The response rates for the treatment with the three concentrations of the extract were determined based on the healing signs diagnosed by the veterinarians, which include the disappearance of the rash in the injured area, the intensity of the itching, the extent of its persistence, and the gradual return of wool germination.

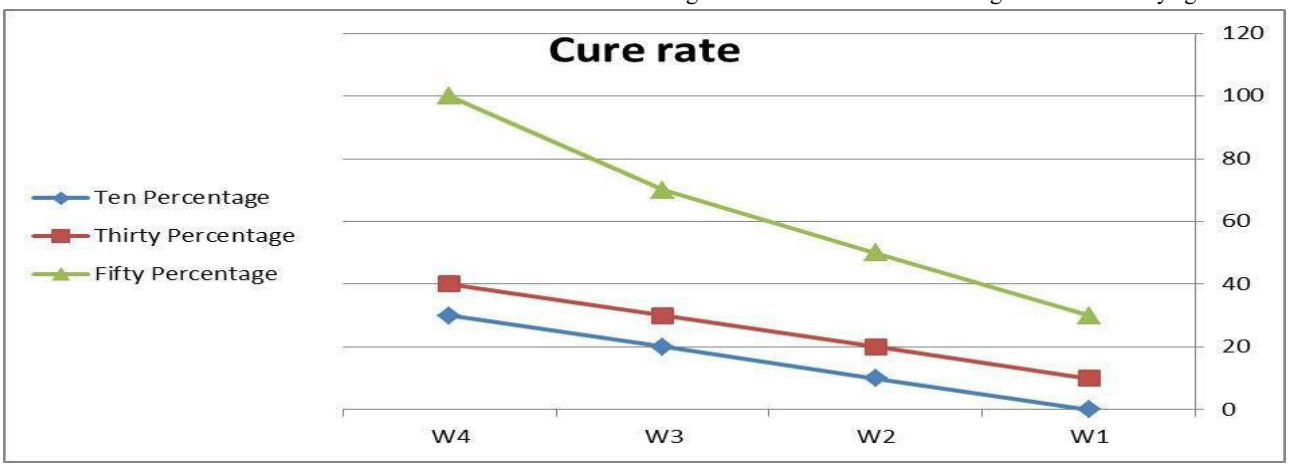
Histological changes in the treated animals

Samples were taken from infected and uninfected animals (1cm^3 deep), covering all layers of the skin (dermis and epidermis). The samples were preserved in formalin 10% and tissue sections were prepared following the classical methods, stained with hematociylin and eosin, mounted on glass slides and examined under the microscope [16].

RESULTS AND DISCUSSION

The effects of cinnamon alcoholic extract

The results presented in figure 1 indicate that the rates of cure from scabies infection show different values depending on the concentration and duration of the once-per-day treatment. It was observed that the use of 10 mg/l extract caused varied cure responses, starting from 0% in the first week and reaching a maximum of 30% in the fourth week. Relatively better outcomes were recorded with the use of 30 mg/l extract, with the cure rate developing from 10% in the first week to 40% in the fourth week. Importantly, treatment with the highest concentration of 50 mg/l revealed very good outcomes as compared to the lower concentrations, with a cure rate of 30% in the first week that reached to the maximum of 100% of the treated animals in the fourth week.



Model 1. The effects of different concentrations of the alcoholic extract of cinnamon on the skin of sheep infected with scabies. The X axis represents duration of treatment in weeks while the Y axis reflects the cure rate by number of animals.

Upon following up the cases until two weeks after recovery from the injury, it became clear that there were no relapse events occurred within the 100% animals recovered at the fourth week. Previous studies revealed that cinnamaldehyde extracted from the bark tissue of Chinese cinnamon by methylene chloride has effective properties as an insecticide. These results are consistent with those previously reported by other authors [19] who attributed the ant-parasitic effects of cinnamon extract to the fact that this plant contains substances and compounds that have powerful killing abilities upon attachment to the parasite, through affecting the nervous system and thus causing paralysis and death of the parasite. In addition, some of the secondary compounds present in the plant extract were shown to have damaging effects on egg production organs in insects.

HISTOPATHOLOGICAL CHANGES

The histological examinations for the control group of our study revealed that the structure of the skin was composed of the normal architecture of epidermis and dermis layers. The epidermis consisted of stratum corneum (layers of dead, scaly, keratinized cells) and stratum granulosum (layers of flattened keratinocytes). The dermis was formed of the reticule layer, hair follicles (hair root and hair shaft) as well as sebaceous and sweat glands (Figure 2). This structure represents the commonly known normal histology of the skin in sheep and goats [20, 21, 22].

In contrast, tissue sections of biopsy specimens from scabies lesions of the infected animals showed an altered histology that is typical for this parasitic infection. This was represented by a folded skin with a wave-like epidermis, hyperkeratosis, epidermal thickening and sloughing of stratified squamous epithelial, i.e. stratum corneum and stratum granulosum, leading to loss of skin flexibility. Furthermore, the epithelial cells of the epidermis were intensely basophilic with many tunnels of mites surrounded by inflammatory cells including infiltrates of eosinophils, macrophages, lymphocytes and histiocytes. In addition, the examination revealed the occurrence of sub-epidermal moderate dermatitis and disorganized structures when compared with control group (Figures 3 and 4). Such altered histology of the infected skin was also described by a group of authors [23, 22] who reported mites burrowing into the stratum corneum of the epidermis along with thickening of the epidermal tissue and intensive cellular infiltrates.

Nevertheless, the microscopic examination of the skin sections from sheep treated with 50% cinnamon showed a histological architecture similar to that of the control groups, with restoration of the normal thickness of the epidermis and dermis layers (Figure 5). These results provide an extended support to the previous reports of the possibility of using *Cinnamoum zeylanicum* extract as an alternative to the topical treatment of psoroptic mange in rabbits as well as other skin diseases, such as that caused by *Sarcoptes scabiei*, in different animals and in humans . The extracted oil showed also its *in vitro* effectiveness in treating skin diseases caused by *Psoroptes cuniculi*, which was very high at a concentration that ranged between 0.10 and 10 % [25.26].

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Figures

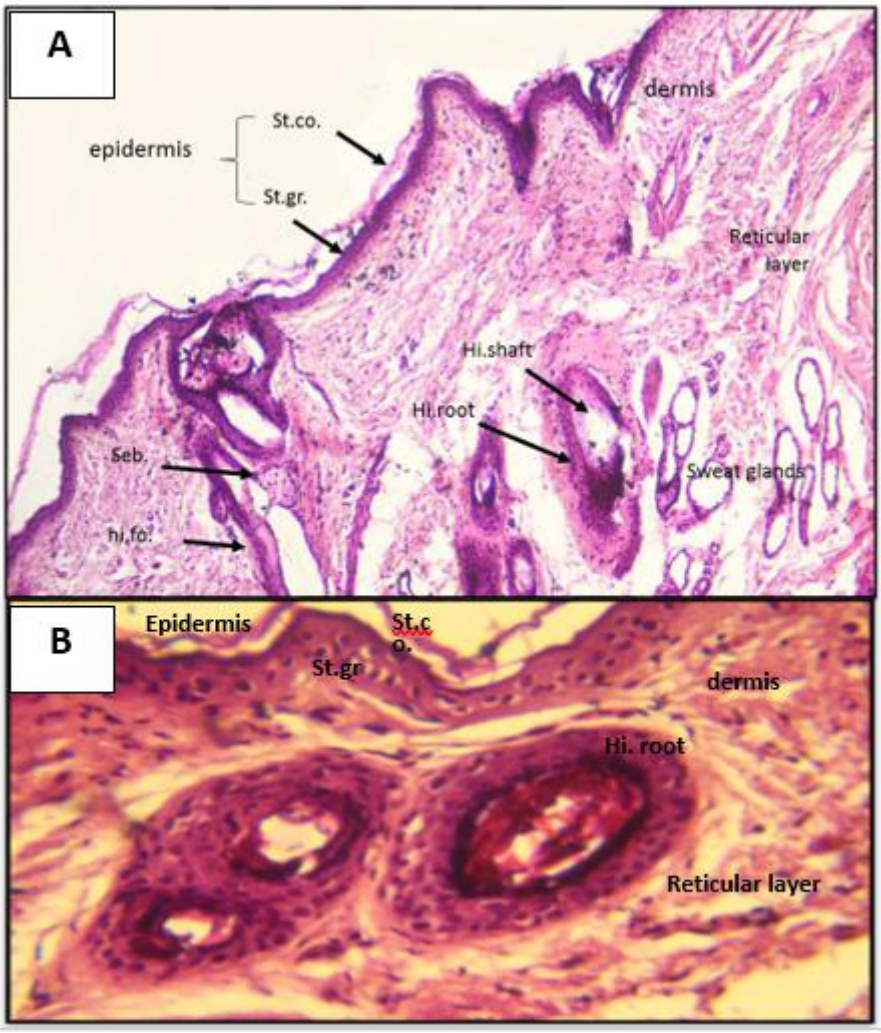

Figure 1. (A,B) Histological examination of normal sheep's skin consisting of epidermis (stratum corneum, st.co, and stratum granulosum, st.gr.) and dermis which contains the reticular layer; hair follicles (Hi.fo.); hair root (Hir.root); hair shaft (Hi.shaft); sebaceous glands and sweat glands. H&E stain; A:10X, B:40X.

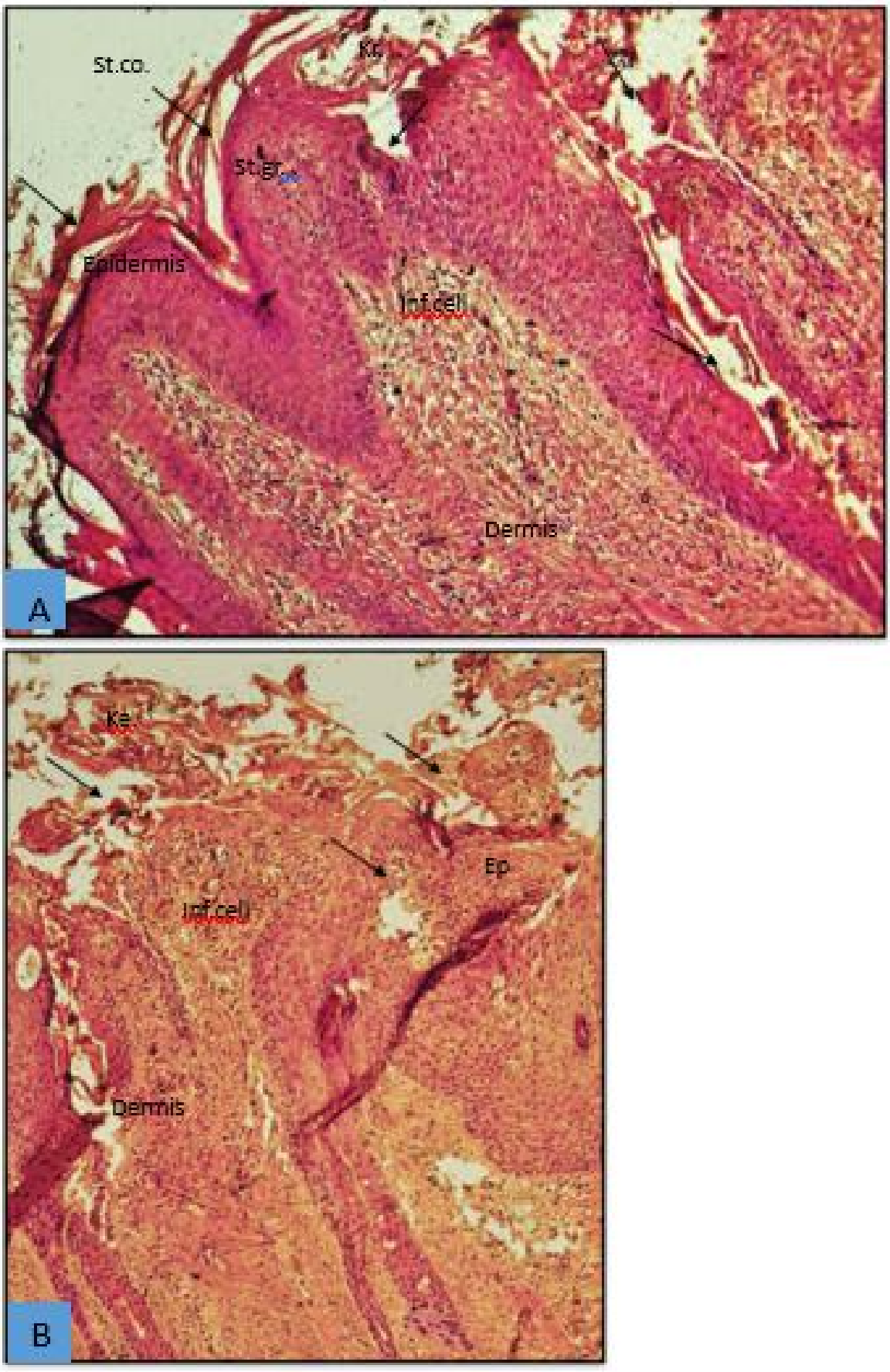

Figure 2. (A, B) Histological examination of infected sheep's skin showing hyperkeratosis, sloughing of stratified squamous epithelial, and epidermal thickening (stratum corneum, st.co.; stratum granulosum, st.gr). Also, many tunnels of mites (black arrows), dermal disorientation structures, and infiltration of inflammatory cells (inf.cell) are observed. H&E stain; 10X.

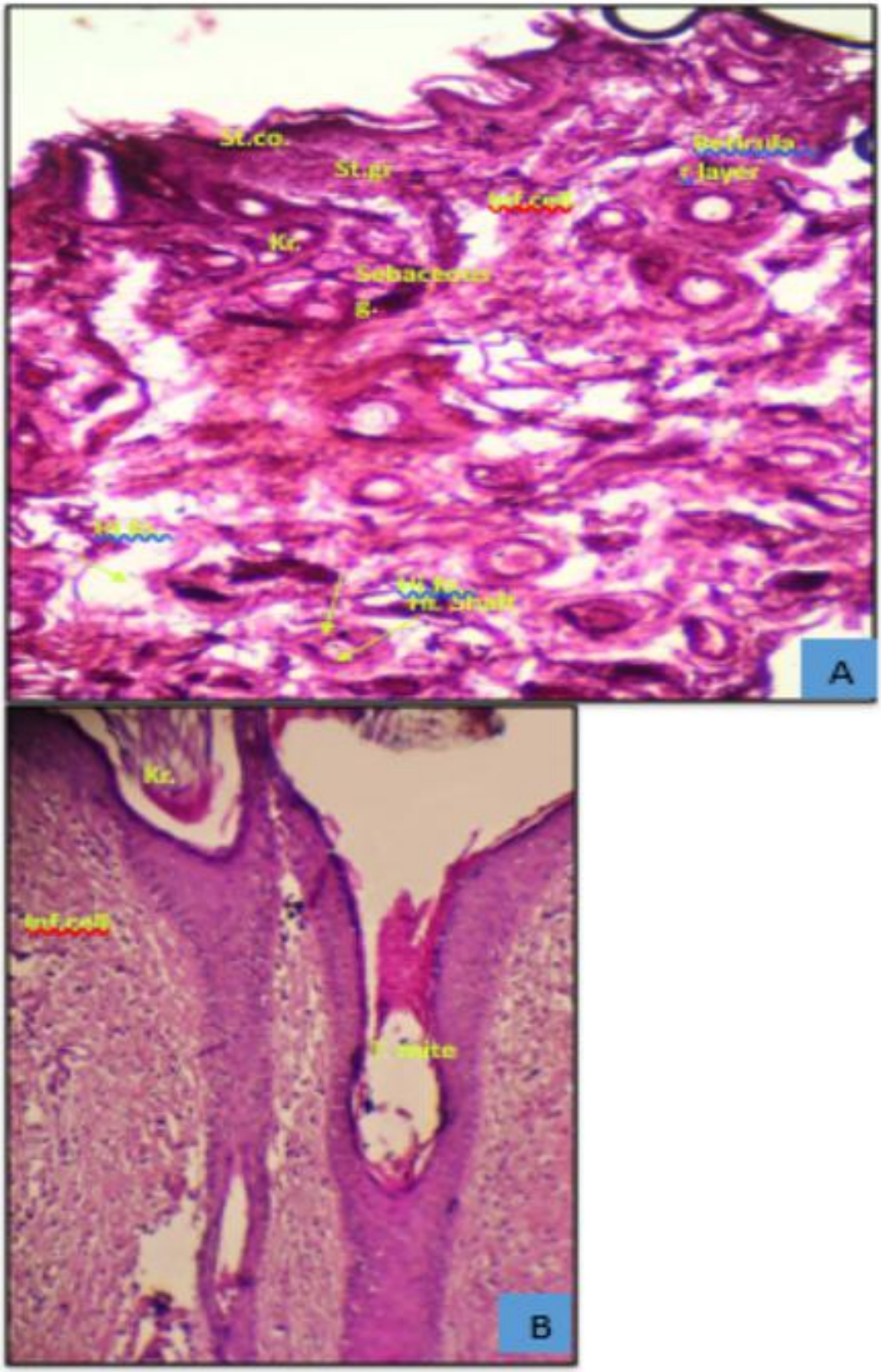

Figure (3). (A, B) Histological examination of infected sheep's skin revealing hyper-keratinization (Kr) with tunnels of mites in the stratified squamous epithelium (stratum corneum, st.co; stratum granulosum, st.gr.). Also, moderate dermatitis and disorientation structures appear in the reticular layer, hair follicles (Hi.fo.), hair root (Hir.root) and hair shaft (Hi.shaft). H&E stain;10X.

Figure 4. Histological examination of treated sheep's skin showing restored normal structure which is similar that of the control. Epidermis (stratum corneum, st.co.: stratum granulosum, st.gr.), dermis (reticular layer; hair follicles, black arrows). H&E stain; 10X.