Hematological, biochemical and histopathological studies on selected canine skin diseases

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1. Abstract

The skin is the largest organ of the body that has many functions. Canine dermatology remain a challenging field that requires a variety of knowledge as dermatological problems are reported to be the most common health problem in small animal practice. Blood, skin and biopsy samples were collected from 50 dogs of different breeds and sexes. The present study is designed for identification of the different etiological agents causing skin diseases in dogs, evaluation of some hematological and biochemical parameters in diseased dogs and histopathological examination of selected cases using skin biopsy. These dogs were grouped into two groups, the first one was the control group (n=10) apparently healthy dogs and the second group (n=40) diseased dogs presented with different skin ailments where 12 out of 40 dogs were suffered from demodicosis, dermatophytosis (6 cases), pyoderma (8 cases) , mixed infection (10 cases) and canine atopic dermatitis (4 cases). Evaluation of the hematological parameters revealed presence of anemia and leukocytosis with apparent neutrophilia in dogs with pyoderma and eosinophilia in the other groups. Biochemical parameters lied within the reference range in all diseased groups. Staphylococcus spp. was the most common bacterial isolate in canine pyoderma. With respect to histopathological picture, results indicated presence of large focal area of epidermal liquefactive necrosis associated with dense neutrophils infiltration. Severe dermatitis, folliculitis, perifollicular and perivascular inflammatory cells infiltration, all these changes associated with cases of pyoderma. Demodicosis cases demonstrated as heavy mite infestation in different developmental stages in the stratum conium of the epidermis and in the follicles. Laminar orthokeratotic hyperkeratosis, vacuolated epidermal prickle cells, acanthosis and vaculation of keratinocytes of the infundibula. Dermatitis characterized by moderate to marked inflammatory infiltration.

Necrosis of adnexa, folliculitis and perifollicultis were also recorded in demodecosis infected dogs. Fungal dermatosis showed pronounced laminar orthokeratotic hyperkeratosis and acanthosis. Vacuolar degeneration of keratinocytes and fungal Hyphae were seen in dermatophytosis infected dog. Ulcerative dermatitis in form of focal extensive area of epidermal necrosis and ulceration associated with inflammatory cells infiltration, acanthosis and spongiosis of prickle cell layer were noticed in mixed infection infected dogs. The most common infectious skin problems during our study were pyoderma, red mange and dermatophytosis. The predominant pathogen in pyoderma affected dogs was Staphylococcus spp. Histopathological examination was very useful in our study as they provide accurate diagnosis in a short period of time and help in clear visualization of the clinical picture.

Key words: Dermatology, Canine, Hematology, Biochemistry, histopathology.

2. Introduction

Dermatological disorders are of numerous etiologies for that, canine dermatology will remain a challenging field. However complex this field is, a large number of cases may be diagnosed and treated successfully. In order to accomplish this goal, it is necessary to follow a methodological approach. [**25].**

Dermatological problems are reported to be the most common health problem in small animal practice. It constitute about 20-75% as the chief or concurrent owner complaint [35]

Skin diseases may be infectious or non-infectious. Infections occur when there is disruption in the defense mechanism of the skin [**24]**. Infectious skin diseases of dogs classified into contagious and non-contagious infections or infestations. Contagious infections include some parasitic, bacterial, fungal and viral skin diseases. Non-contagious skin infections can develop when normal cutaneous microflora is allowed to proliferate resulting in clinical signs **[36].**

The most common manifestations of skin consisted of Pruritus, wounds, alopecia and abscesses. Pruritus is a commonly encountered problem usually associated with allergy, bacterial or parasitic skin disease **[20].**

Diagnosis of skin disorders is best made by a careful history (most important), a complete general physical examination followed by the use of laboratory and other diagnostic techniques which finally can lead to specific treatment and accurate prognosis **[17] and [39]**. Histopathological

examination conducted by cutaneous biopsy can be an advantage for the practitioner with the aid of experienced pathologist. Cutaneous biopsy has the potential to provide the greatest amount of information in the shortest period of time **[21]**.

A skin biopsy is one of the procedures that appear so simple at first glance, not appear to be dermatology "secret." It can be further increase the ability of the pathologist to visualize the clinical picture through the microscope **[16].** Literatures available on histopathological changes regarding different dermatologic canine problems are insufficient; therefore, the present study was conducted to evaluate the histopathological changes beside analysis of selected hemato-biochemical parameters in dogs suffered from number of dermatologic problems.

3. Material and methods

3.1. Ethics Statement

The use of dogs in this experiment was permitted by the owners of the dogs.. All animal procedures were performed in accordance with the Guidelines of department of Internal Medicine Faculty of Veterinary Medicine Cairo University.

3.2. Animals

The study was performed on 50 dogs of different breeds and sexes (30 males and 20 females) with great consideration to animal welfare and under owner's permission during the period from January 2020 to May 2021 with ages ranged from 6 months to 5 years. Dogs were admitted to Small Animal Clinic, Faculty of Veterinary Medicine, Cairo University, Egypt. They were divided into two groups, the first one was the control group (n=10) apparently healthy dogs came to the clinic for routine vaccination or checkup examination and the second group (n=40) diseased dogs presented with different skin ailments.

3.3. Clinical examination

Dermatological examination including Case history, general clinical examination and special clinical examination of skin were performed.

3.4. Skin samples

Multiple deep skin scrapings using dull scalpel blades were performed from the periphery of the lesions or alopecic areas in the direction of hair growth until capillary bleeding. Also crusts and broken hairs were collected. The scraped sample was spread on a clean glass slide with addition of several drops of tap water or 10% KOH

solution. The sample was examined microscopically under the low (10X) and high power (40X) objectives looking for presence of mites or the spherical arthrospores of dermatophytes **[55]**.

3.5. Blood samples:

Whole blood sample was collected from each dog from the cephalic vein and divided into two aliquots**;** first one was on EDTA-containing tube for hematology. Other portion was collected on plain tube for serum separation. Serum was used for estimation of ALT, BUN and creatinine manufactured by **Stanbio®** Company (Texas, USA) using spectrophotometer (APEL, PD-303 S, Japan).

3.6. Bacterial culture:

Individual samples were collected for bacterial culture from regions of lesional skin using sterile saline-moistened swab which rubbed vigorously on sampling area, and then placed in a sterile tube containing 2 mL brain heart infusion broth and stored at 4°C. Each swab was inoculated onto mannitol salt agar and incubated aerobically at 37°C for 24 hrs. Isolates were identified based on colony morphology and Gram stain characteristics. Films from the pure suspected colonies were stained by Gram's stain and examined microscopically **[12]**.

3.7. Histopathological examination:

Skin biopsy specimens were collected (using 4 mm circular punch at a depth of 2 mm) and fixed in 10% neutral buffer formalin, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 4-5 micron thickness and stained with Haematoxylin and Eosin (H and E) for light microscopic examination (Olympus BX50, Tokyo, Japan [5]

3.8. Statistical Analysis:

Statistical analysis was performed using Student's *t* test (STATISTICA for windows, version 5.1., Stat Soft, Inc. 1984-1996). P- Values <0.05 were considered of statistical significance.

4. Results

Clinical examination and skin scraping:

Among the 40 dogs with dermatological affections, the principle clinical signs observed were in form of pruritus, alopecia, erythema, erosive or ulcerative lesions, scaling, crusting, dryness and wrinkling of the skin. During the microscopic examination of skin scraping, demodex spp. appeared as elongated cigar shaped in 12 out of 40 dogs with a percentage of 30% (fig. 1). Six out of 40 dogs (15%) were

found infected with dermatophytosis (fig. 2) as demonstrated by endothrix and ectothrix present in the infected hairs. Swabs were collected from 8 dogs (20%) showing signs of pyoderma (fig.3) and diagnosed to have bacterial infection as demonstrated by the results of bacterial culture. 10 out of 40 dogs (25%) found to suffered from mixed infection in which 7 of them were infected with pyoderma secondary to demodecosis (70%) and the other 3 (30%) were suffered from pyoderma secondary to dermatophytosis as demonstrated by bacterial culture and skin scrapings. 4 dogs (10%) found to have canine atopic dermatitis (CAD) (fig. 4) based on exclusion criteria, interpretation of detailed history and characteristic clinical findings.

Hematological and biochemical evaluation:

The mean values of hematological and biochemical parameters were illustrated in tables 1, 2 and 3. Regarding the hematological parameters of pyoderma infected dogs, the findings revealed statistically significant decrease in MCH and MCHC. Extremely statistically significance increase in WBCs, neutrophils and eosinophils. Respecting demodecosis infected dogs, results revealed presence of statistically significant decrease in Hb concentration and MCH. WBCs showed very statistically significance increase. Referring to dermatophytosis infected dogs, statistically significant decrease in Hb concentration and statistically significant increase in WBCs.. In CAD, statistically significant decrease in Hb concentration, RBCs count, PCV, MCH and monocytes were demonstrated. Dogs with mixed infection showed statistically significant decrease in MCH. The selected serum biochemical parameters did not differ significantly in all diseased groups when compared to control group.

Bacteriological examination:

Culture on mannitol salt agar with aerobic incubation at 37°C for 24 hrs, revealed appearance of creamy to golden yellow colonies with or without mannitol fermentation. Staphylococci stained with Gram stain appeared microscopically as spherical, grape-like clusters with some single and paired cocci, Gram positive, non-spore forming and non-motile.

Histopathological examination:

Regarding histopathological examination, Skin biopsy section from cases of Pyoderma (Bacterial dermatitis) revealed severe histopathological alterations exhibited by large focal area of epidermal

liquefactive necrosis associated with dense neutrophils infiltration. The dermis showed severe dermatitis, folliculitis, perifollicular and perivascular inflammatory cells infiltration mainly neutrophils, lymphocytes and macrophages (Figure 5). Parasitic dermatosis (demodicosis) was demonstrated in skin biopsy specimens where lesions summarized as heavy mites infestation in different developmental stages in the stratum conium of the epidermis and in the follicles. Laminar orthokeratotic hyperkeratosis, vacuolated epidermal prickle cells, acanthosis and vacuolation of keratinocytes of the infundibula. Furthermore, the dermis showed dermatitis characterized by moderate to marked inflammatory infiltration of the dermis by macrophages, lymphocytes and eosinophils (fig. 6, 7, 8, 9&10). Necrosis of adnexa, folliculitis and perifollicultis were also recorded in examined cases (fig. 11). Some cases showed crust formation with parakeratotic hyperkeratosis (fig. 12). Fungal dermatosis sections of skin biopsy showed pronounced laminar orthokeratotic hyperkeratosis and acanthosis. Vacuolar degeneration of keratinocytes and fungal Hyphae were seen in some examined cases. Slight dermal edema associated with few inflammatory cells infiltration were also recorded (Figures 13, 14, 15 & 16). Light microscopic examination of skin biopsy section from some cases revealed lesions of Ulceration (ulcerative dermatitis) in form of focal extensive area of epidermal necrosis and ulceration associated with inflammatory cells infiltration (fig. 17 & 18). Severe dermatitis exhibited by extensive inflammatory infiltrate in the dermis which mainly lymphocytes, macrophages and plasma cells (Figure 18). Moreover, acanthosis and spongiosis of prickle cell layer were noticed (Figure 17). Microscopically, skin biopsy specimen from one case exhibited acanthosis and spongiosis of epidermal prickle cell layer with formation of papillary projections in the dermis and connective tissue core (Figure 19).

5. **Discussion**

Skin is the most sensitive tissue of dog's body and has tremendous aesthetic value. In small animal practice, a majority of ailments are constituted by dermatological abnormalities as the concurrent or chief owner's complaint [48]In addition the skin is synergistic with internal organ system and thus reflect pathologic processes that are either primary or shared with other organs of the body [31] .

Regarding the hematological parameters of Pyoderma infected dogs, the findings revealed statistically significant

decrease in MCH and MCHC. Extremely statistically significance increase in WBCs and eosinophils. Leukocytosis observed was due significant increase in the neutrophils and eosinophils Increased eosinophils were observed only in cases of pyoderma secondary to FAD. Our findings were compatible with [58] who explained that inflammation is the most frequent cause of neutrophilia, left shifts of great magnitude may occur in dogs that have pyoderma .Dogs with bacterial pyoderma often reveal neutrophilia and lymphopenia [30], [4], [23] and [28] but dissimilar to results of [53]who found that pyoderma infected dogs had significantly lower mean value of total leukocyte count (TLC) and [32] who found leukopenia with neutropenia in dogs with pyoderma. Leucocytosis was attributed to a higher cutaneous inflammatory reaction and release of substances such as leukotaxins from cell injury and leucocytosis promoting factors from blood into the injured area resulting in release of more neutrophils in the blood stream. Also attributed this elevation to the development of cellular and humoral responses [44]. The present study revealed statistically significant decrease in values of Hb and MCH in cases of dogs with demodicosis which is in accordance with other reports of [41], [54], [7], [43] and [49]. The decrease in the values of Hb and MCH might be due to anemia caused by the loss of skin protein or due to stress arising from the disease as reported by [13].

Generalized inflammation and response of leukocytes to prolonged antigenic stimulus and hypersensitivity reaction subsequent to Demodex persistence in tissues could lead to elevated leukocytes and eosinophils [47] and [15]. Slight elevation of eosinophils may be also connected with the presence of *Toxocara canis* in diseased puppies as demonstrated by direct fecal smear examination [19].

Concerning dermatophytosis infected dogs our results revealed presence of statistically significant decrease in the level of Hb concentration. These results agreed with [59], [22] and [14]. However, our results contraded those reported by [27], [45], [56] who didn't find any significant changes in erythrogram of dermatophytosis infected dogs. The reason of haemoglobin decrease may be attributed to low food intake caused by clinical illness and discomfort in mycotic infections [14]. Leukocytosis might be due to inflammation and secondary bacterial infections [3] and [37].

Regarding canine atopic dermatitis, there was statistically significant decrease in Hb concentration, RBCs count, PCV, and MCH.. Results disagreed with [52] and [10] who found that Hb concentration did

not changed significantly in affected dogs. Eosinophilia was observed in atopic dogs by [10]who attributed this to hypersensitivity reaction because of raised histamine concentration which causes release of eosinophils in the blood circulation. [60] also found that atopic dogs expressed high level of eosinophils because of the mast cells stimulation via IgE receptors whereas some researchers like [38] and [11] reported that eosinophilia may not always be related to allergic diseases. Regarding histopathological examination of skin biopsy section from cases of pyoderma (Bacterial dermatitis), our results come in accordance with [46], [6], [42] and [2] who demonstrated epidermal necrosis and edema, dermal collagen necrosis, and severe inflammatory cells infiltration, mainly neutrophils in the epidermis, dermis, and around the hair follicles either aggregate or in a diffuse manner.

Respecting parasitic dermatosis (demodicosis), our results agreed with[46], [51], [29] and [9] who found large numbers of intrafollicular mites, acanthosis and follicular keratosis, Purulo-granulomatous or lymphoplasmacytic perifolliculitis, Subepidermal edema and marked infiltration of mast cells, eosinophils, lymphocytes and dermal fibrosis.

Fungal dermatosis sections of skin biopsy showed hyperkeratosis, spongiosis and marked dermal edema, skin bullae in the epidermal layer and accumulation of collagen fibers. Spores and hyphae of *Microsporum* were found in the stratum basale layer of epidermis and infiltration of eosinophils in the dermis layer directly beneath the basal layer of epidermis. These findings were reported by [1] and [34] which were similar to our finding. Light microscopic examination of skin biopsy section from CAD cases revealed lesions of Ulceration (ulcerative dermatitis) in form of focal extensive area of epidermal necrosis and ulceration associated with inflammatory cells infiltration. Severe dermatitis exhibited by extensive inflammatory infiltrate in the dermis which mainly lymphocytes, macrophages and plasma cells (Figure 14). Moreover, acanthosis and spongiosis of prickle cell layer were noticed. Microscopically, skin biopsy specimen from one case exhibited acanthosis and spongiosis of epidermal prickle cell layer with formation of papillary projections in the dermis and connective tissue core. These results were diagnosed as CAD and the findings come in accordance with [46], [50], [38], [40], [18] and [57] and [8] who found mild to severe infiltration of inflammatory cells in the epidermal, sub epidermal, dermal region as well as around the sebaceous glands and

cellular infiltrate on mainly consisted of plasma cells, lymphocytes, mast cells, eosinophils and polymorphs followed by spongiosis. Perivascular dermatitis was observed in some cases, epidermal hyperplasia followed by hyperkeratosis. Allergic skin diseases produce multifocal spongiosis and it is characterized by edema of the intercellular spaces of the epidermis and superficial follicular wall. Inflammatory cell influx accompanies spongiotic change. Epithelial hyperplasia, or acanthosis, may be due to internal factors (metabolic, hereditary) or external injury (self-trauma).

6. Conclusion

The most common infectious skin problems during our study were pyoderma, red mange and dermatophytosis. The predominant pathogen in pyoderma affected dogs was Staphylococcus spp. . Histopathological examination was a very useful in our study as it provides an accurate diagnosis in a short period of time and help in clear visualization of the clinical picture.

7. References

1- Alkaragoly H. (2014). The histopathological changes at skin of German shepherd dogs associated with ringworm infection in directorate of K9 in Al-Diwanyia province. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 13(1):48. DOI: 10.29079/ vol13 iss1art277.

2- Arbaga A, El-Bahrawy A, Elsify A, Khaled H, Hassan HY, Kamr A (2021). Biochemical and histopathological changes related to the topical application of Aloe vera ointment for canine pyoderma, Veterinary World, 14(5): 1354-1362.

3- Arora, N.; Vohra, S.; Singh, S.; Potliya, S.; Lather, A.; Gupta, A.; Arora, D. and Singh, D. (2013). Therapeutic management of chronic generalized demodicosis in a pug. Adv. Anim. Vet. Sci., 1(2S):26 –28.

4- Aujla, R.S.; Singh, N.; Sood, N.; Gupta, P.P. and Sodhi, S. (1997). Bacterial dermatitis in dogs in Punjab: Prevalence and clinico-pathological studies. Ind. Vet. J., 74:837-840.

5- Bancroft, J. D. and Gamble, M. (2008). Theory and practice of histological techniques, 6th edition. Philadelphia, PA: Churchill Livingstone/Elsevier, Elsevier Health Sciences. 6-Bäumer, W.; Bizikova, P.; Jacob, M. and Linder, K.E. (2016). Establishing a canine superficial pyoderma model. Journal of Applied Microbiology, 122(2):331-337.

7- Beigh, S.A.; Soodan, J.S.; Singh, R.; Khan, A.M. and Dar, M.A. (2014a). Evaluation of trace elements, oxidant/antioxidant status, vitamin C and

β- carotene in dogs with dermatophytosis. Mycosis, 57:358-365.

8- Bhagya, BK., Ansar Kamran, C., Suguna Rao and Veeregowda BM. (2021). Histopathology of skin lesions of canine atopic dermatitis in pugs. JEZS; 9(1): 603-606.

9- Bond, R., Morris, D.O., Guillot, J., Bensignor, E.J., Robson, D., Mason, K.V., Kano, R. and Hill, P.B. (2020). Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats. Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Veterinarydermatology, 31 (1), pp.27-e4.

10- Brar, R. K., Dhaliwal, P. S., Kumar, A., Chhabra, S., and Uppal, S. K. (2017). Clinico pathological Studies on Atopic Dermatitis in Dogs. Journal of Animal Research, 7(3), 507.

11- Collie, D.S.; DeBoer, D.J.; Muggenberg, B.A. and Bice, D.E. (1997). Evaluation of association of blood and bronchoalveolar eosinophil numbers and serum total Ige concentration with the expression of non specific airway reactivity in dogs. Am. J. Vet. Res., 58:34-39.

12- Cruickshank, R.; Duguid, J.P.; Marmion, B.R. and Swain, R.H.A. (1975). Medical microbiology. 12th living stone, London New York.

13- Deb, A.R.; Jha, M.K. and Prasad, K.D. (2000). Clinical and hematological changes in dogs infected with Demodex canis. J. Res. Birsa Agric. Univ., 12: 281-283.

14- Devi, T. and Vijayakumar, K. (2013b). Prevalence of canine dermatophytosis: Heamato-Biochemical changes in infected dogs. J. Vet. SCi., 1(2):41-45.

15- Dhume GV, Sharode DB, Dakshinkar NP, et al. (2002). Haematobiochemical investigation in canine demodicosis. The Blue cross book. ;19(3):16–17.

16- Dunstan, R. W., Mauldin, E. A., Davenport, G. M. Credille K. M. (2004);A Guide to Taking Skin Biopsies: A Pathologist's Perspective in: Small Animal Dermatology Secrets (34-42).

17-Grant, D.I. (1990). Skin diseases in the dog and cat. 2nd Ed., Blackwell Scientific Publications, Oxford, London, U.K., 193 p.

18-Gross T.L., Ihrke P.J., Walder E.J. and Affolter V.K. (2005). Skin diseases of the dog and cat clinical and histopathologic diagnosis. 2nd Ed, Blackwell Science Ltd. P.p.,200-205.

19-Hagiwara, M.K. and P.M.L. Germano, (1974). Electrophoresis of serum proteins of normal dogs and dogs with demodectic mange. Revista da Facultale de MedicinaVeterinaria e Zootecnia de Universidade de Sao Paulo, 11: 69-81.

20- Haithem, A.M., Wael, M.K. and Mahmoud, E. (2011). Field Survey on Most Common Medicinal and Surgical Diseases in Police Guard and Explosive Dogs from 11/ 2007- 2/ 2010. J. Am. Sci., 7(4):816-826.

21- Hnilica, K.A. and Patterson, A.P., (2016). Small Animal Dermatology: A Color Atlas and Therapeutic Guide, 4th ed., Saunders imprints. 652 p.

22-Ibrahim H.S.M.; Hassan, M.S. and Hassan, N.K. (1984). Hematological and biochemical changes of ringworm infected buffaloes. Assiut Vet. Med. J., 12:161.

23-Ihrke, P.J. (2006). Bacterial infections of the skin. In: Infectious Diseases of the Dog and Cat, 3rd Ed., (Ed. Greene, C.E.), Saunders Elsevier, Canada, pp. 807-823.

24-Janeway, C.A.; Travers, P.; Walport, M. and Shlomchik, M.J. (2001). Immunobiology: the immune system in health and disease. 5th ed., Garland publishing, New York, pp. 1-16.

25-Jasmin, P. (2011). Clinical Handbook on Canine Dermatology. 3rd Ed., Virbac. S.A., Carros, France.175 p. 26-Kanitakis, J. (2002). Anatomy, histology and immunohistochemistry of normal human skin. European journal of dermatology, 12(4), 390-401.

27-Khosla, R.; Gupta, M.P.; Dhablania, D.C. and Jand, S.K. (1989). Clinic-diagnostic features of dermatophytosis in dogs with particular reference to therapeutic measures. Ind. Vet. J., 66:1157.

28-Lodh, C. and Das, S. (2014). Diagnostic significance of haemato-biochemical changes in canine dermatitis. Ind. J. Canine Pract., 6(2):99- 102.

29-Machado L.H.A.; Fabris V. E.; Neto R. T.; Rodrigues J. C. and F. C. Oliveira (2012). Histopathology in veterinary dermatology: Historical records of thirty years of diagnosis at the Department of Pathology of Botucatu Medical School, UNESP (1977-2007) Vet. e Zootec.; 19(2): 222-235.

30-Mason, I.S. (1991). Canine pyoderma. J. Small Anim. Pract., 32:381-386.

31-Miller, W.H.; Griffin, C.G. and Campbell, K.L. (2013). Muller and Kirk's small animal dermatology. 7thEd., Elsevier Mosby, St Louis, Missouri, U.S., 952 p.

32- Min, S.H.; Kang, M.H.; Sur, J.H. and Park, H.M. (2014). Staphylococcus pseudintermedius infection associated with nodular skin lesions and systemic inflammatory response syndrome in a dog. Can. Vet. J., 55:480–483.

33-Monteiro-Riviere, N. A. (2010). Structure and function of skin. Toxicology of the Skin.

34- Moriello, K.A.; Coyner, K.; Paterson, S. and Mignon, B. (2017). Diagnosis and treatment of dermatophytosis in dogs and cats. Veterinary Dermatology, 28(3):266-e68.

35-Muller, G.H., Kirk, R.W., and Scott, D. W. (1989). Cutaneous parasitology. In Small Animal Dermatology. W.B. Sanders Company: P. 347-426. 36-Munjal, R.S. (2012). Common dermatological diseases by bacteria and fungi in pet dogs. Ind. J. Fund. Appl. Life Sci., 2(2):207-209

37-Nikee K., Anil k., Shashi K., Archana and G. D. Singh. (2018). Therapeutic Management of Generalized Demodicosis in a Female Rottweiler Dog. International Journal of Current Microbiology and Applied Sciences 7: 3463-3466.

38- Nimmo Wilkie J.S, Yager J.A, Eyre P, and Parker W.M. (1990). Morphometric analysis of the skin of dogs with atopic dermatitis and correlations with cutaneous and plasma histamine and total serum IgE. Veterinary Pathology; 27:179-186.

39- Nuttall, T.J.; Harvey, R.G. and McKeever, P.J. (2009). A Color Handbook of Skin Diseases of the Dog and Cat. 2nd Ed., Manson publishing/the Veterinary Press, London, UK., 337 p.

40--Olivry T, Naydan DK, and Moore PE. (1997). Characterization of the cutaneous inflammatory infiltrate in canine atopic dermatitis. Am. J Dermatopathol; 19:477-486.

41- Patel, J.; Maiti, S.K.; Sanyal, P.K. and Tiwari, S.P. (2005). Haematobiochemical and mineral profiles in generalized canine demodicosis. Intas Polivet, 6:331– 334. 42-Rafatpanah, Sh; Rad, M.; Movassaghi, A. R. and Khoshnegah, J. (2020). Clinical, bacteriological and histopathological aspects of first time pyoderma in a population of Iranian domestic dogs: a retrospective study IJVR, 21 (2), Ser. No. 71:130-135.

43- Reddy, B.S., K.N. Kumari and S. Sivajothi, (2014). Haemato-biochemical findings and thyroxin levels in canine demodicosis. Comp. Clin. Pathol., DOI: 10.1007/s00580-014-1893-y.

44-Reddy, B.S., Kumari, K.N. and Sivajothi, S., (2015). Haemato-biochemical findings and thyroxin levels in canine

demodicosis. Comparative Clinical Pathology, 24(2), pp.287-290.

45- Remi, R.A.; Thoha, T.B.; Adefolake, O.O.; Sikirat, M.O. and Oluwadun, A. (2012). Investigatin on some hematological parameters and the biochemical system in pupils with dermatophytosis. Report and Opinion, 4(9):67-69 (Cited after Devi and Vijayakumar, 2013).

46- Rojko J.L, Hoover E.A, and Martin S.L. (1978). Histologic interpretation of cutaneous biopsies from dogs with dermatologic disorders. Veterinary Pathology;15:579-589.

47- Sakina, A., R.K. Mandial and Q. Mudasir, (2012). Haematobiochemical changes in canine demodecosis. Vet. Scan, 7: 75-78.

48- Sakina, A. and Mandial, R.K. (2011). Prevalence and clinical observations of mange in dogs. Vet Pract, 12(2), pp.248-50.

49- Salem, N.Y., Abdel-Saeed, H., Farag, H.S. and Ghandour, R.A., (2020). Canine demodicosis: Hematological and biochemical alterations. Veterinary World, 13(1), p.68. 50- -Scott DW. (1981) . Observations on canine atopy. J. Am. Anim. Hosp. Assoc; 17:91

51- Scott, J.C.; Nelson, R.W.; Bruner, J.M. and Williams, D.A. (1996). Serum canine thyrotropin concentration (cTSH) in euthyroid, hypothyroid and sick euthyroid dogs (abstract). Proceedings of 14th ACVIM forum, San Antonio. p 768.

52- Sharma, R., Hussain, K., Chhibber, S., Kumar, M., & Singh, R. (2015). Clinico-haematological and biochemical studies in allergic dermatitis in dogs. Indian J. Canine Pract, 7(2), 124-129.

53- Shyma, V.H. and Vijayakumar, K. (2011). Hematobiochemical studies in dogs affected with bacterial dermatitis. J. Vet. Anim. Sci., 42:20-22.

54- Singh, S.K., U. Dimri, M.C. Sharma, D. Swarup, B. Sharma, H.O. Pandey and P. Kumari, (2011). The role of apoptosis in immunosuppression of dogs with demodicosis. Vet. Immunol. Immunopathol., 144: 487-492.

55- Solusby, E.J.L. (1986). Helminths, Arthropods and Protozoa of Domestic Animals, 7th ed. Bailliere, Tindall, London, 809p.

56- Sykes, J.E. and Outerbridge, C.A. (2014). Dermatophytosis. In: Canine and Feline Infectious Diseases (Eds. Sykes, J. E.), Elsevier Saunders Inc., St. Louis, Missouri, U.S., pp. 558-569.

57- Vaseem S.A.S. (2008). Studies on diagnosis and therapeutic management of atopic dermatitis in dogs. Master thesis,

Karnataka Veterinary, Animal, and Fisheries Sciences University, Bidar.

58- Weiss, D. and Wardrop (2010). Schalm‘s Veterinary Hematology, 6th ed., Blackwell Publishing Ltd, Singapore.

59- Wilkinson, G.T. (1979). Multiple dermatophytes infections in a dog. J. Small Anim. Pract., 20:111.

60- Wuersch, K.; Brachelente, C.; Doherr, M.; Reist, M.; Sattler, U.; Forster, U.; Bertoni, G.; Peel, J.E. and Welle, M. (2006). Immune dysregulation in flea allergy dermatitis: A model for the immunopathogenesis of allergic dermatitis. Vet. Immunol. Immunopathol., 110:311–323.

Table 1. Erythrogram of dogs with selected skin problems compared with that of apparently healthy dogs (Mean ± SD).

Group	PCV (%)	Hb (g/dl)	RBCs(x106)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	45.60±1.51	17.12±0.89	7.00±0.31	65.14±1.66	24.40±1.04	35.72±0.23
Pyoderma	44.00±4.24 (0.54)	15.90± 0.80 (0.05)*	7.12±0.33 (0.57)	66.12±4.21 (0.64)	22.33±0.35 (0.003)**	34.04±1.30 (0.02)*
demodicosis	36.35±10.33 (0.08)	12.70±3.72 (0.03)*	5.66±1.64 (0.10)	64.29±2.18 (0.49)	21.84±1.05 (0.004)**	34.85±2.00 (0.36)
Dermatophytosis	37.63±8.26 (0.06)	12.90±2.72 (0.01)**	5.80±1.12 (0.05)*	55.72±22.83 (0.36)	22.21±2.20 (0.09)	34.30±0.66 (0.004)**
CAD*	32.55±4.67 (0.0006)***	10.97±1.72 (0.0002)***	4.75±0.81 (0.0007)***	68.91±5.28 (0.17)	23.21±2.08 (0.29)	33.67±0.84 (0.001)***
Mixed infection	34.52±15.39 (0.14)	12.02±5.41 (0.07)*	5.37±2.29 (0.15)	63.78±1.70 (0.26)	22.24±0.84 (0.01)**	34.86±1.01 (0.10)

SD: Standard Deviation**, P-value:** Probability value. ***P**= ≤0.05, ****P**= ≤0.01, *****P**= ≤0.001.

CAD : canine atopic dermatitis

Table 2. Leukogram of dogs with selected skin problems compared with that of apparently healthy dogs (Mean ±SD).

Group	**TLC (x10^3)**	**Neutrophils (%)**	**Lymphocytes (%)**	**Monocytes (%)**	**Eosinophils (%)**	**Basophils (%)**
Control	9.84±1.47	59.20±7.60	31.60±5.17	6.60±2.51	2.60±1.34	0.00±0.00
Pyoderma	21.16±4.22 (0.0005)***	64.00±16.76 (0.05)*	28.32±14.52 (0.64)	4.12±1.60 (0.09)	7.80±2.28 (0.002)**	0.00±0.00
Demodicosis	20.51±5.61 (0.002)**	33.80±25.78 (0.06)	59.71±28.92 (0.06)	5.33±3.78 (0.53)	6.20±3.27 (0.05)*	0.00±0.00
Dermatophytosis	16.00±5.10 (0.03)*	52.56±30.85 (0.64)	39.67±30.24 (0.56)	4.33±2.31 (0.25)	3.43±1.50 (0.44)	0.00±0.00
CAD *	15.87±10.01 (0.21)	49.55±27.08 (0.46)	39.15±29.98 (0.59)	3.30±1.24 (0.04)*	8.00±5.29 (0.06)	0.00±0.00
Mixed infection	17.37±7.62 (0.06)	43.50±27.09 (0.24)	48.75±27.78 (0.21)	3.50±1.73 (0.13)	4.25±0.96 (0.07)	0.00±0.00

SD: Standard Deviation, P-value: Probability value. *P= ≤0.05, **P= ≤0.01, ***P= ≤0.001.

CAD :canine atopic dermatitis.

Table 3. Selected biochemical parameters in healthy and dermatology affected dogs : (Mean ±SD).

Group	Creatinine	BUN	ALT
Control	0.87±0.14	16.40±2.51	39.00±18.29
Pyoderma	1.13±0.27 (0.09)	20.60±3.51 (0.06)	55.22±15.05 (0.18)
Demodicosis	0.76±0.19 (0.32)	15.48±5.09 (0.72)	36.00±23.36 (0.83)
Dermatophytosis	0.79±0.17 (0.50)	13.50±2.50 (0.16)	36.40±16.65 (0.85)
CAD *	0.79±0.06 (0.37)	14.50±4.91 (0.47)	30.87±18.91 (0.55)
Mixed infection	0.77±0.13 (0.34)	13.92±4.46 (0.32)	28.85±7.58 (0.34)

SD: Standard Deviation, P-value: Probability value. *P= ≤0.05, **P= ≤0.01, ***P= ≤0.001. CAD: canine atopic dermatitis.

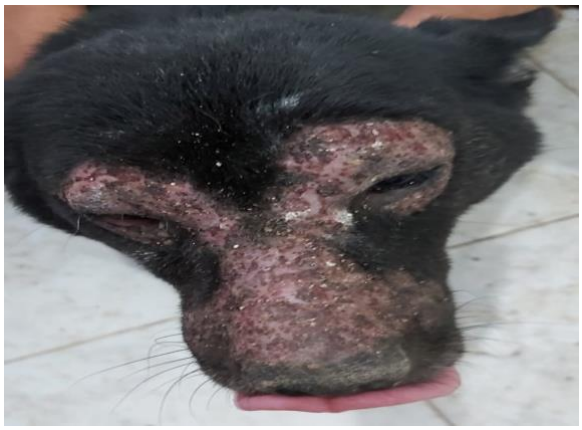

Fig. (1): Localized demodicosis in a dog

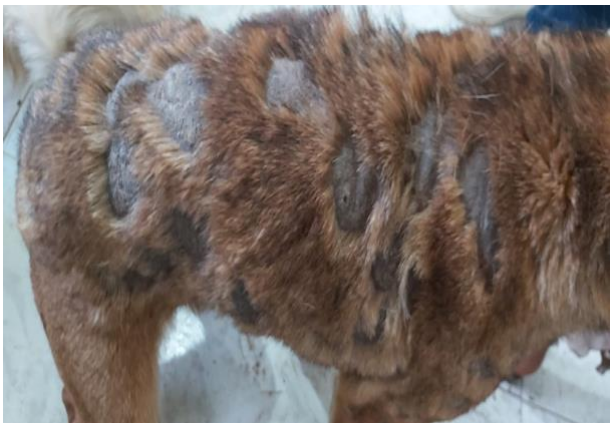

Fig. (2): Dog with generalized dermatophytosis

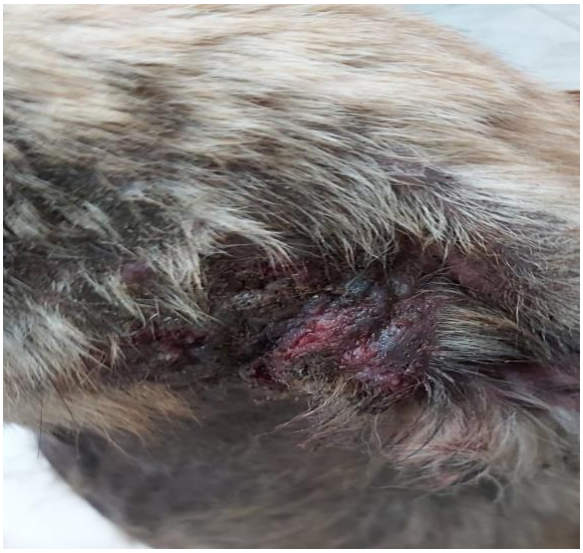

Fig. (3): Pyoderma in a dog

Fig. (4): Atopic dermatitis in a dog

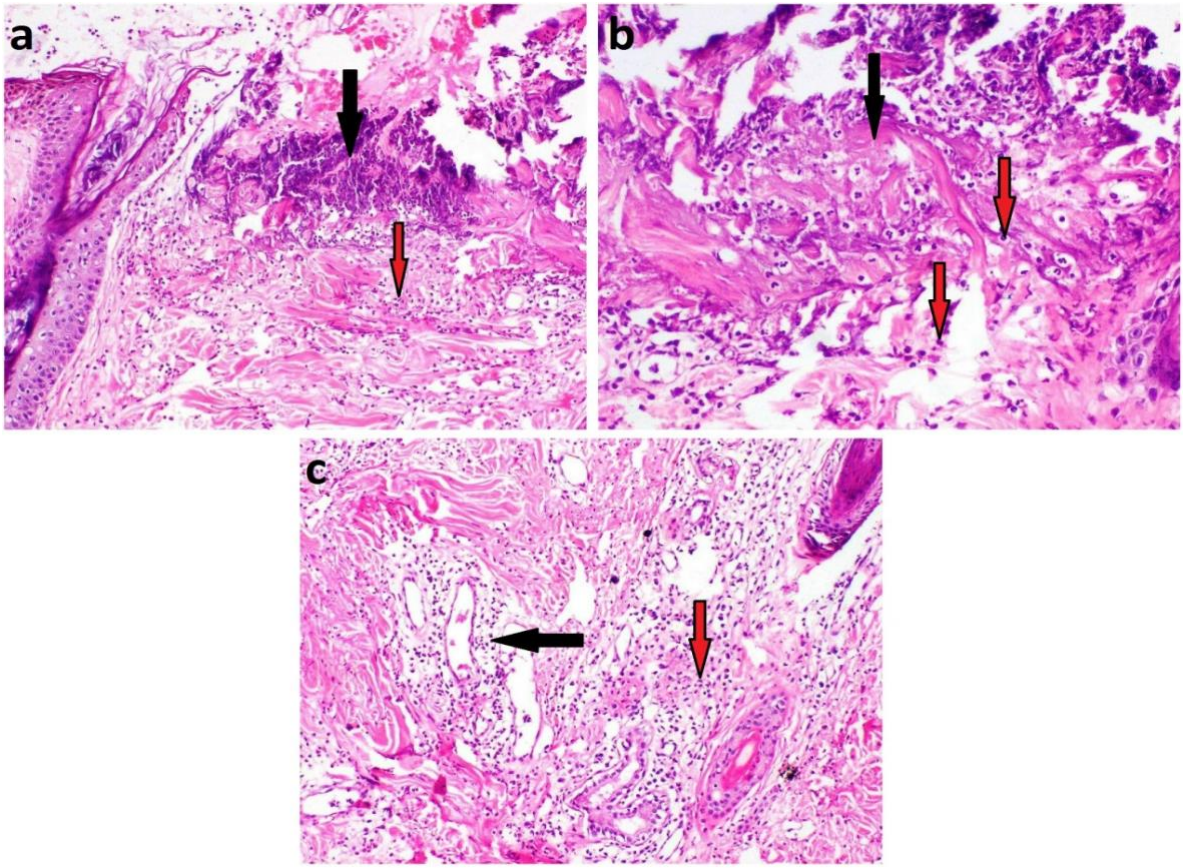

Fig. (5): Histopathology of skin biopsies of pyoderma: Large focal area of epidermal liquifactive necrosis (black arrow) associated with dense neutrophils infiltration (red arrow). (c) Severe dermatitis exhibited by perifollicular and perivascular massive neutrophils, lymphocytes and macrophages infiltration. Smears stained with (H & E stain, X 100 (a & c) & X 200 (b).

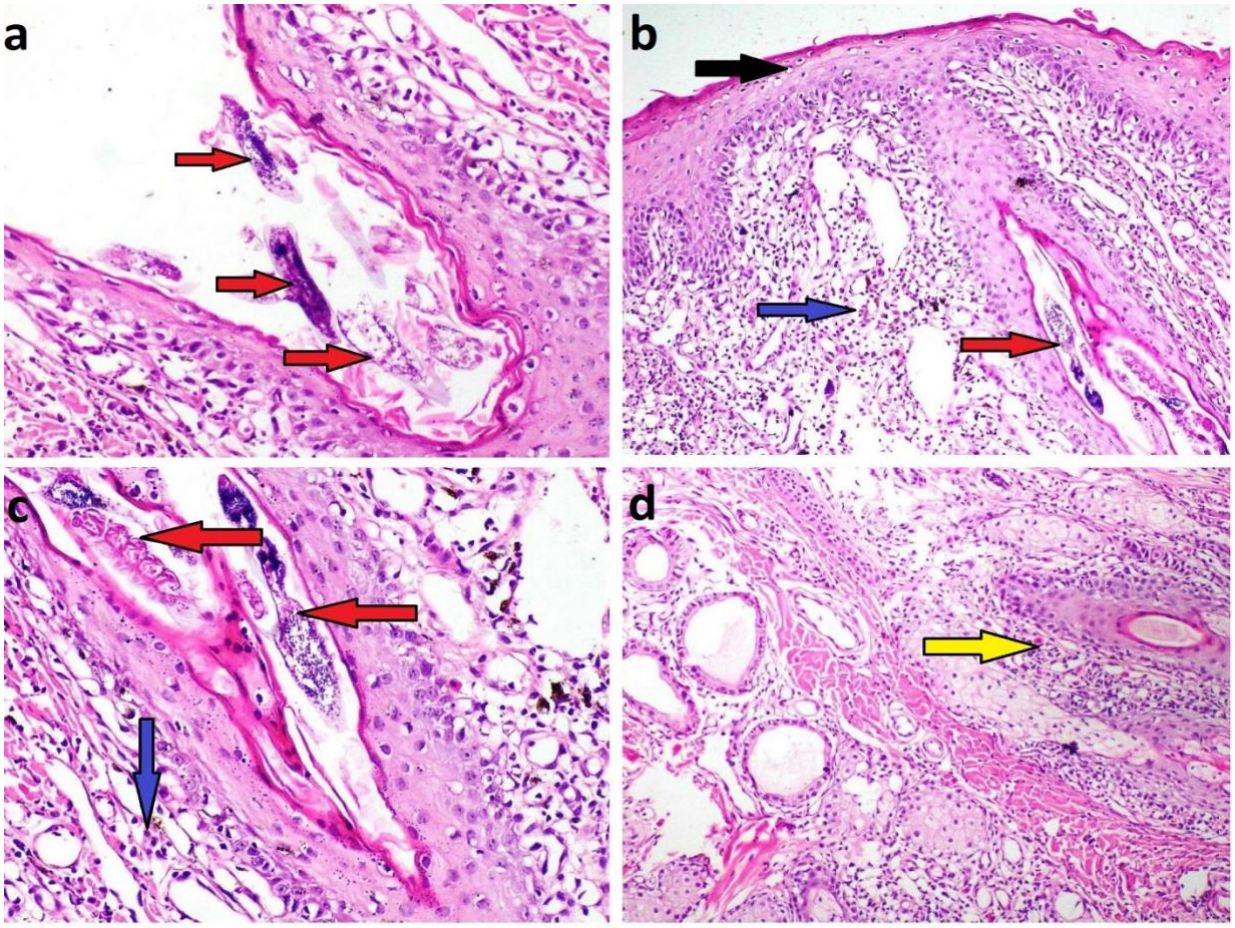

Fig. (6): Histopathology of a skin biopsy of demodicosis : (a) heavy infestation of the parasites (red arrows). (b&c) vacuolar degeneration of the epidermal prickle cells (black arrow), different stages of the parasite in the infundibulum (red arrow) and severe dermatitis (blue arrow). (d) Folliculitis and perifolliculitis (yellow arrow) (H & E , X 200 (a & c), X 100 (b & d).

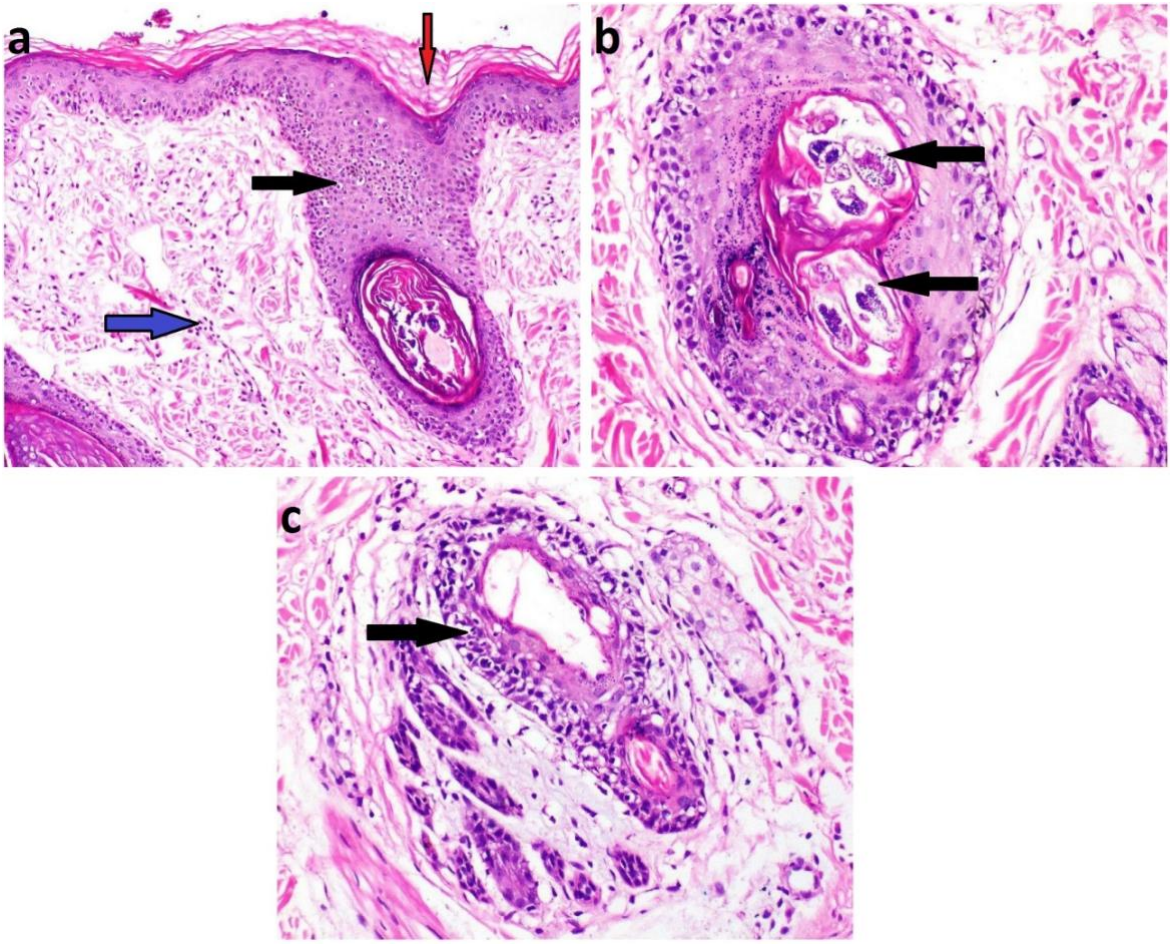

Fig. (7): Histopathology of skin biopsies of demodecosis : (a) laminar orthokeratotic hyperkeratosis (red arrow), acanthosis, vacuolated keratinocytes of the infundibula (black arrow) and moderate inflammatory infiltrate in the dermis (blue arrow). (b) mites in different stages of development in the hair follicles (black arrows). (c) folliculitis (black arrow) (H & E stain, X 100 (a) and X 200 (b & c).

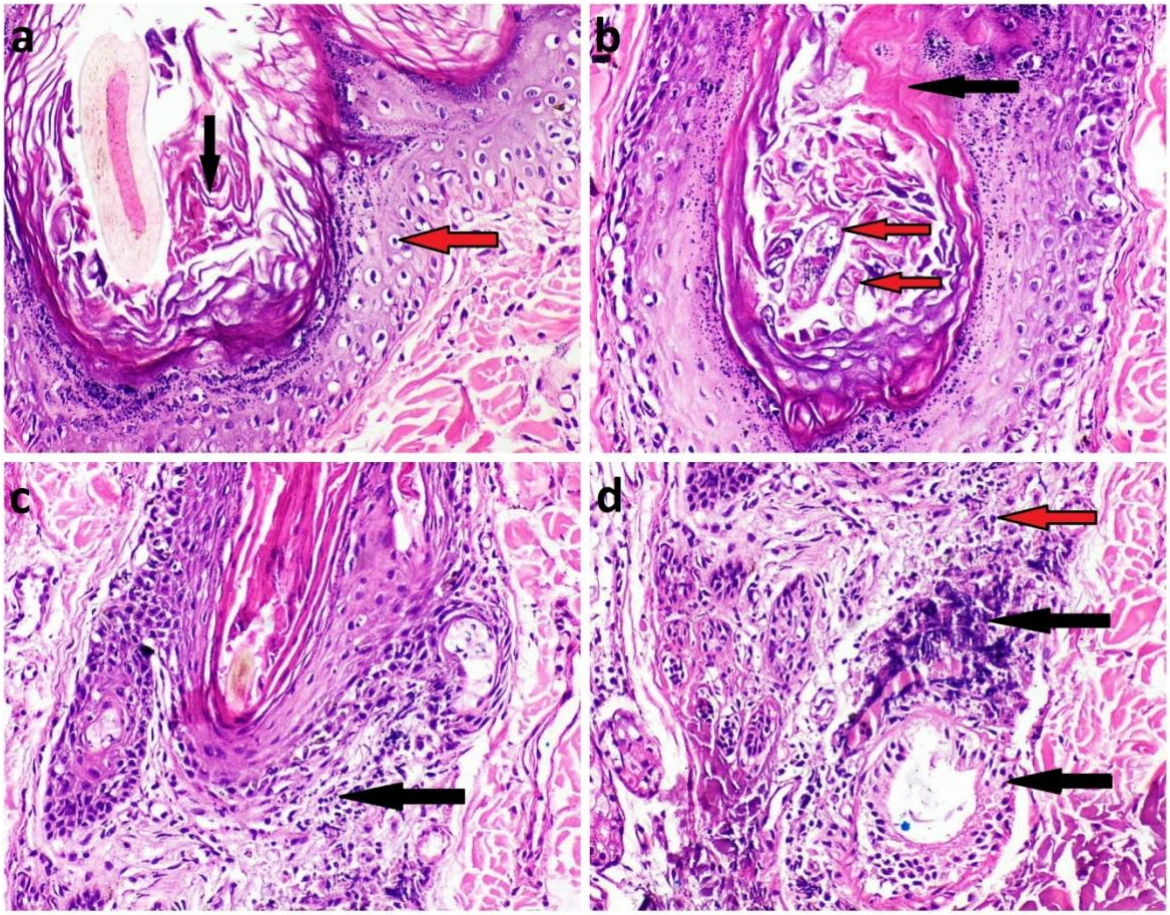

Fig. (8): Histopathology of skin biopsies of demodecosis :(a) orthokeratotic hyperkeratosis (black arrow), and vacuolar degeneration of epidermal cells (red arrow). (b) hyperkeratosis (black arrow) and different stages of development of the mite (black arrows). (c) folliculitis (black arrow). (d) necrosis of adnexa (black arrow) and inflammatory cells infiltration in the dermis (red arrow) (H & E stain, X 200).

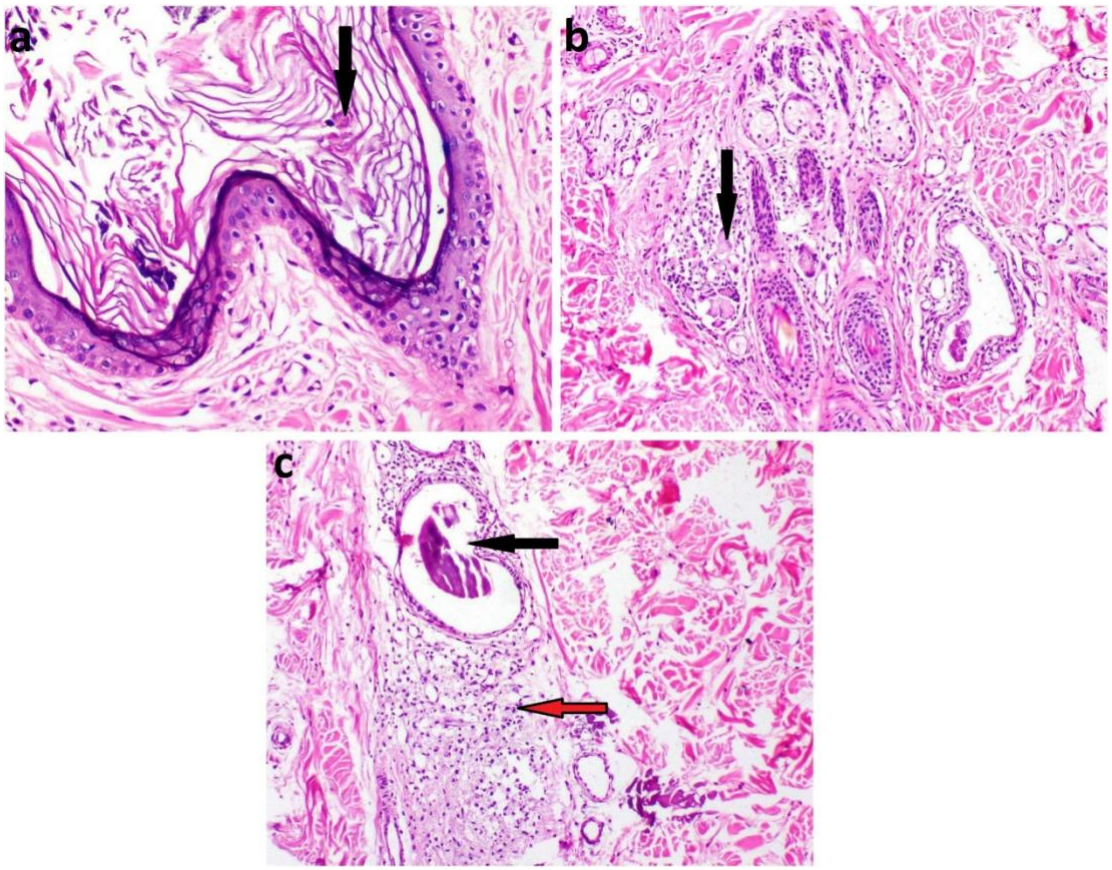

Fig. (9): Histopathology of skin biopsies of demodecosis : (a) laminar orthokeratotic hyperkeratosis (black arrow). (b) necrosis of the follicles (black arrow) associated with dermal inflammatory cells infiltration. (c) cystic dilatation of sweet gland with intraluminal cast (black arrow) and periglandular massive inflammatory cells infiltration (red arrow) (H & E stain, X

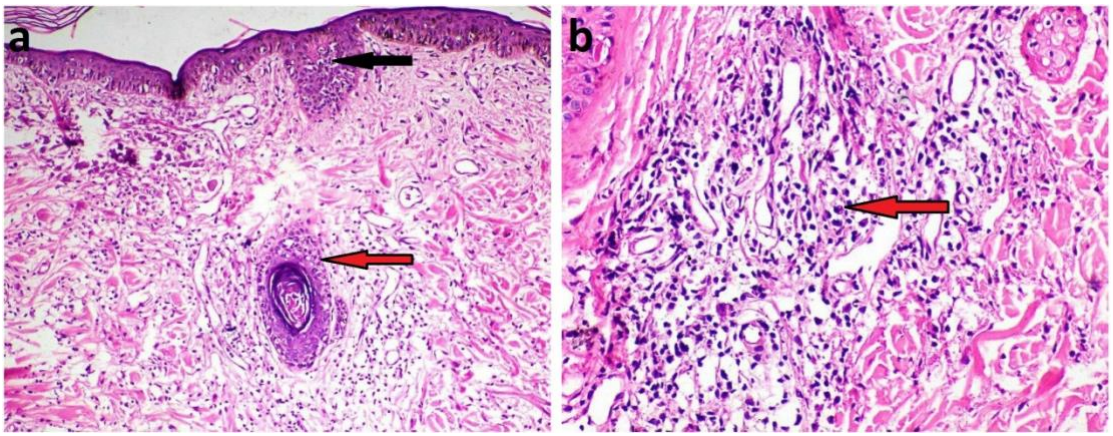

Fig. (10): Histopathology of skin biopsies of demodecosis: showing (a) vacuolated epidermal cells (black arrow) and keratinocytes of the follicle (red arrow) associated with perifolliculitis. (b) dermatitis exhibited by perivascular infiltration with lymphocytes and macrophages (red arrow) (H & E stain, X 100 (a) and X 200 (b).

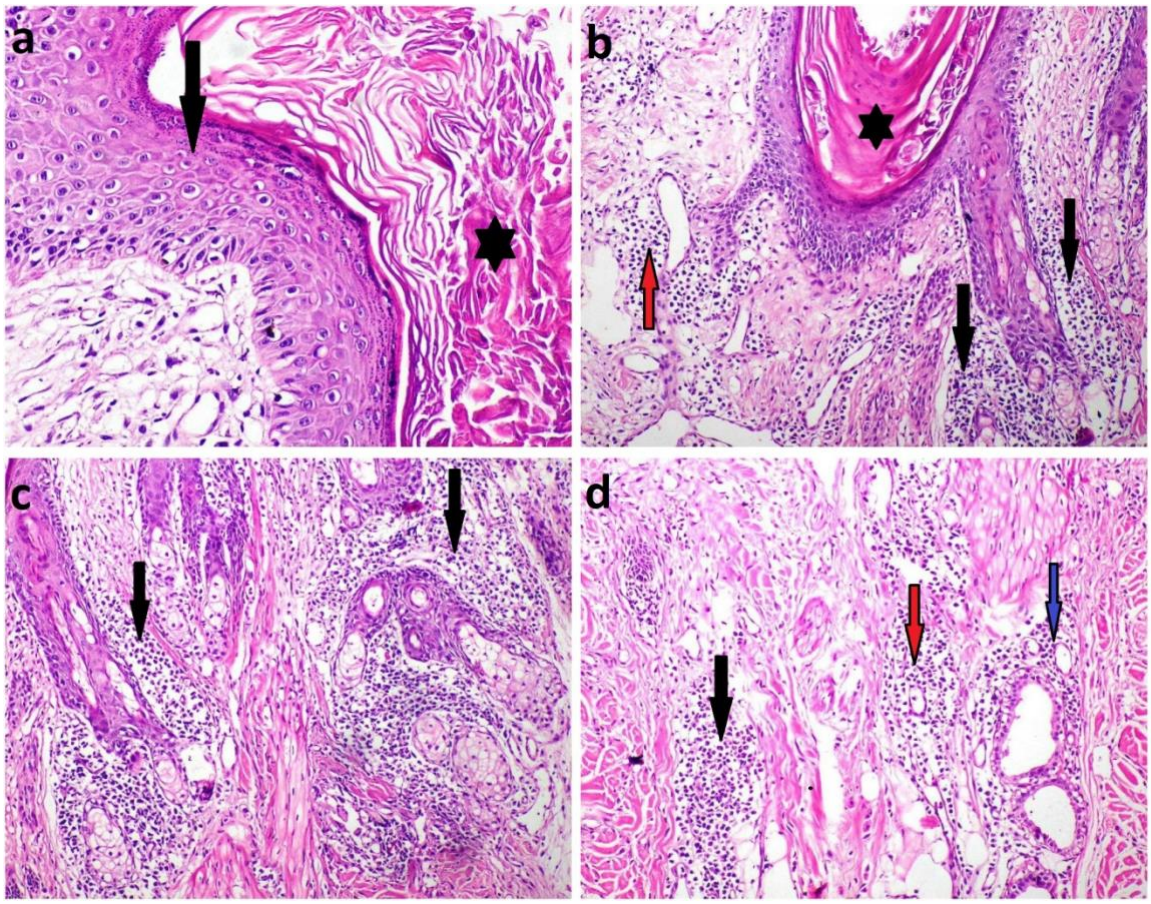

Fig. (11): Histopathology of skin biopsies of demodecosis :(a) orthokeratotic hyperkeratosis (asterisk), and acanthosis of epidermal cells (black arrow). (b) hyperkeratosis (asterisk), perifollicultis (black arrows) and perivasculitis (red arrow). (c) marked dermatitis and perifolliculitis (black arrow). (d) necrosis of adnexa (black arrow), periglandular (blue arrow) and perivascular (red arrow) inflammatory cells infiltration (H & E stain, X 200 (a) and X 100 (b, c & d).

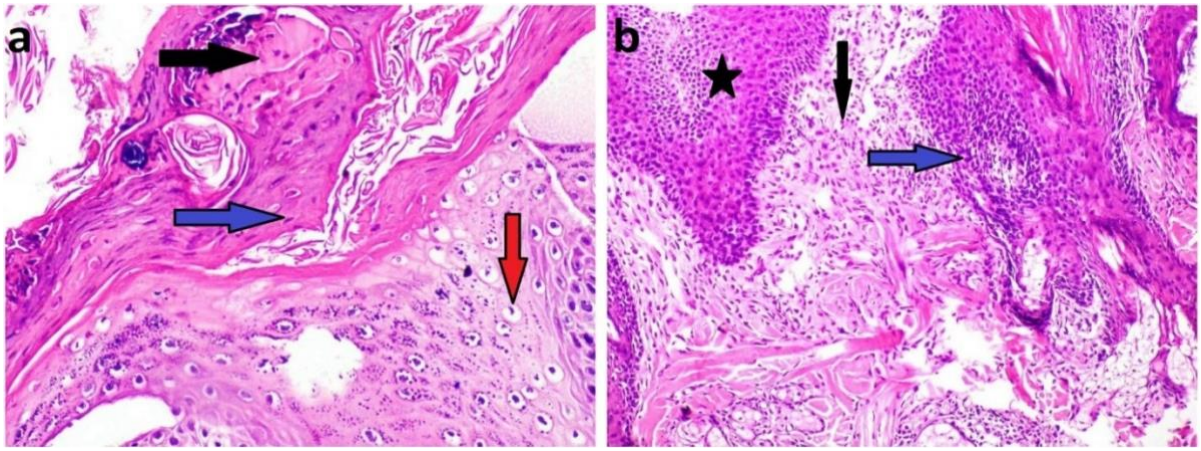

Fig. (12): Histopathology of skin biopsies of demodecosis :(a) crust formation (black arrow) with parakeratotic hyperkeratosis (blue arrow) and vacuolated epidermal cells (red arrow). (b) acanthosis (asterisk), severe dermatitis (black arrow), folliculitis and perifolliculitis (blue arrow). (H & E stain, X 200 (a) and X 100 (b)).

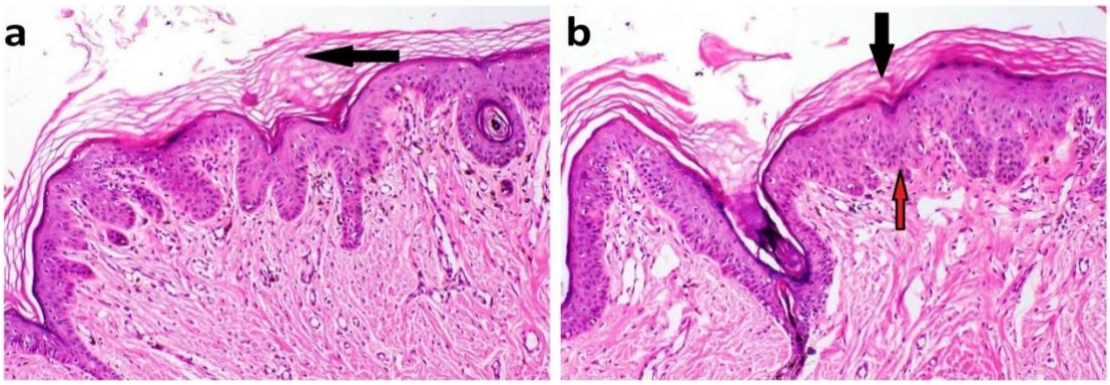

Fig. (13): Histopathology of skin biopsies of dermatophytosis: (a & b) laminar orthokeratotic hyperkeratosis (black arrow) and acanthosis (red arrow). (H & E, X 100 and 200 respectively).

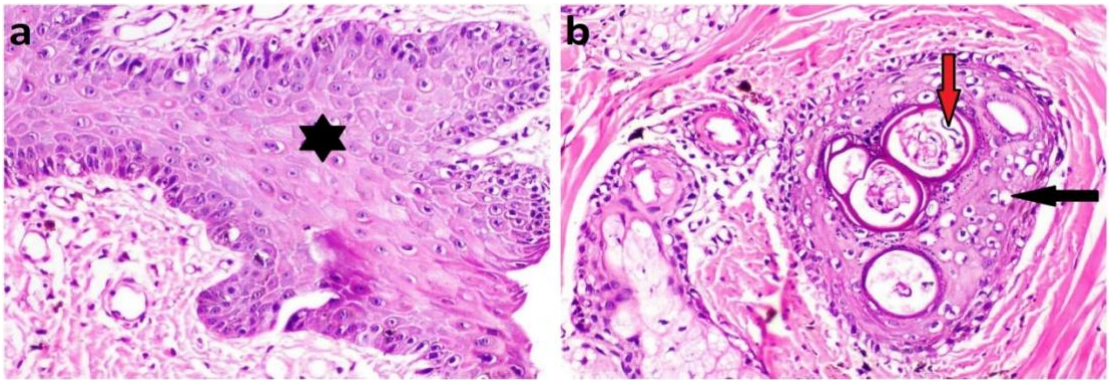

Fig. (14): Histopathology of skin biopsies of dermatophytosis :(a) acanthosis of prickle cells of the epidermis (asterisk) (b) vacuolar degeneration of keratinocytes (black arrow) with presence of fungal hyphae (red arrow). (H & E stain, X 200).

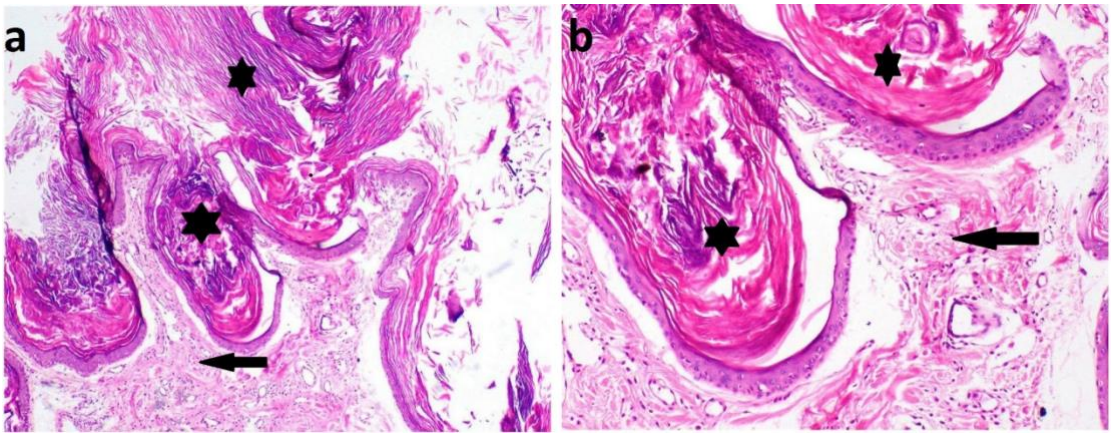

Fig. (15): Histopathology of skin biopsies of dermatophytosis :(a & b) marked hyperkeratosis (asterisk) and slight dermal edema associated with few inflammatory cells infiltration (black arrow). (H & E, X 40 and 100 respectively).

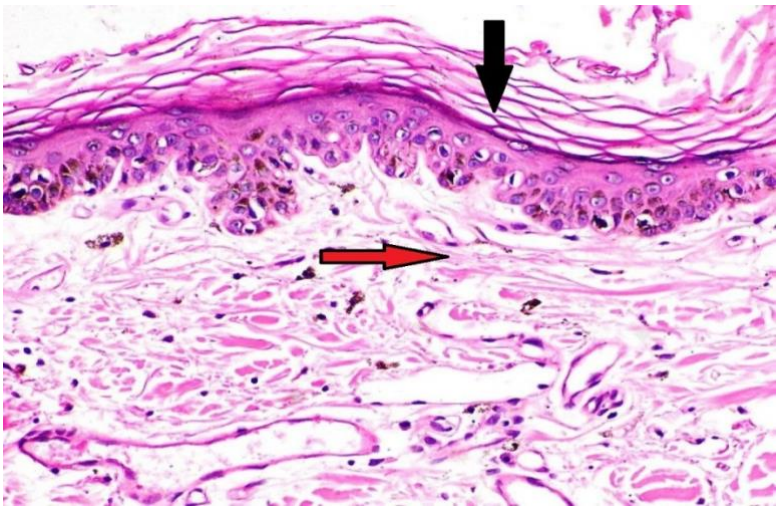

Fig. (16): Histopathology of skin biopsies of dermatophytosis : laminar orthokeratotic hyperkeratosis (black arrow) and dermal edema (red arrow) (H & E, X 200).

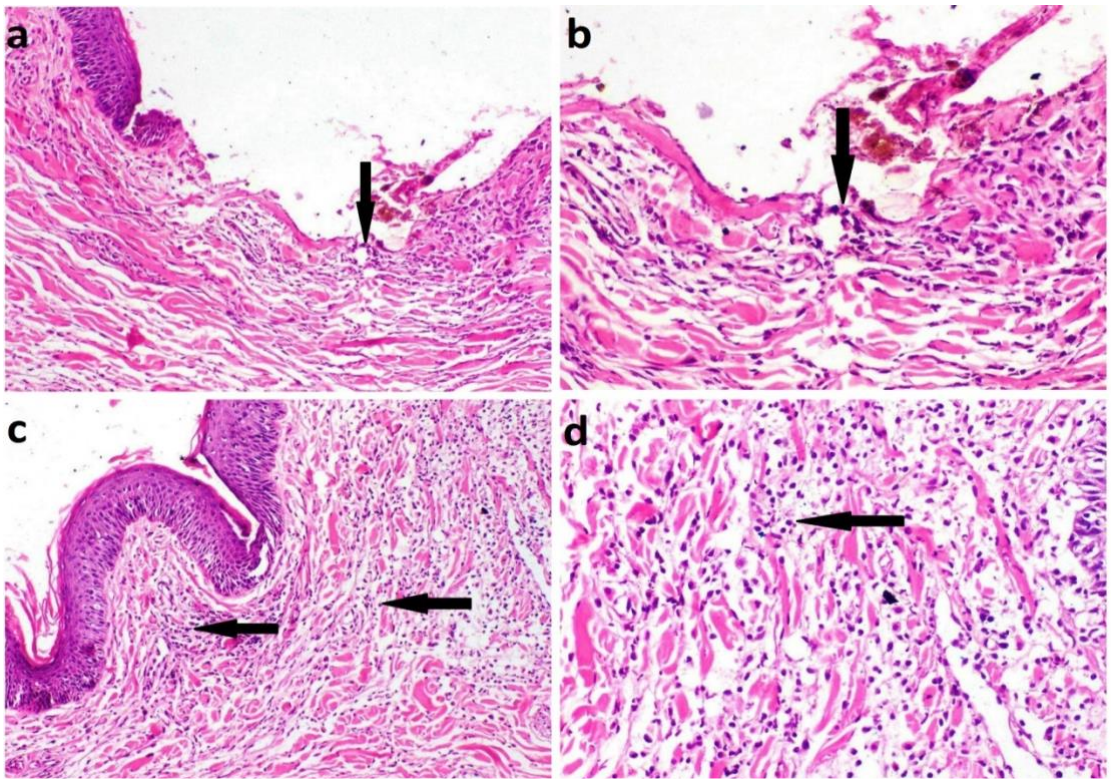

Fig. (17): Histopathology of skin biopsies of demodecosis : (a & b) focal extensive area of epidermal necrosis and ulceration associated with inflammatory cells infiltration (arrow). (c & d) severe dermatitis exhibited by extensive inflammatory infiltrate in the dermis (arrow). (H & E, X 100 (a & c), X 200 (b & d).

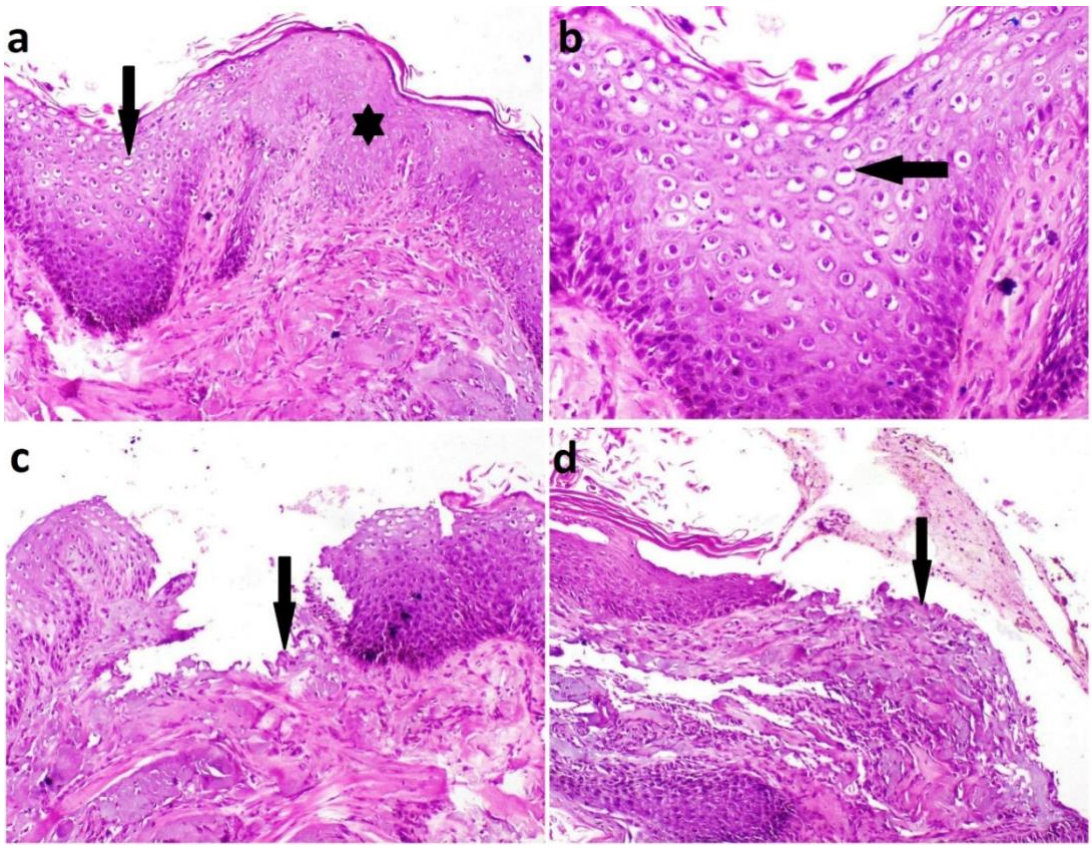

Fig. (18): Histopathology of skin biopsies of canine atopic dermatitis: (a & b) acanthosis (asterisk) and spongiosis (arrow) of prickle cell layer. (c & d) focal necrosis and ulceration of the epidermis (H & E stain, X 100 (a, c & d) and X200 (b).

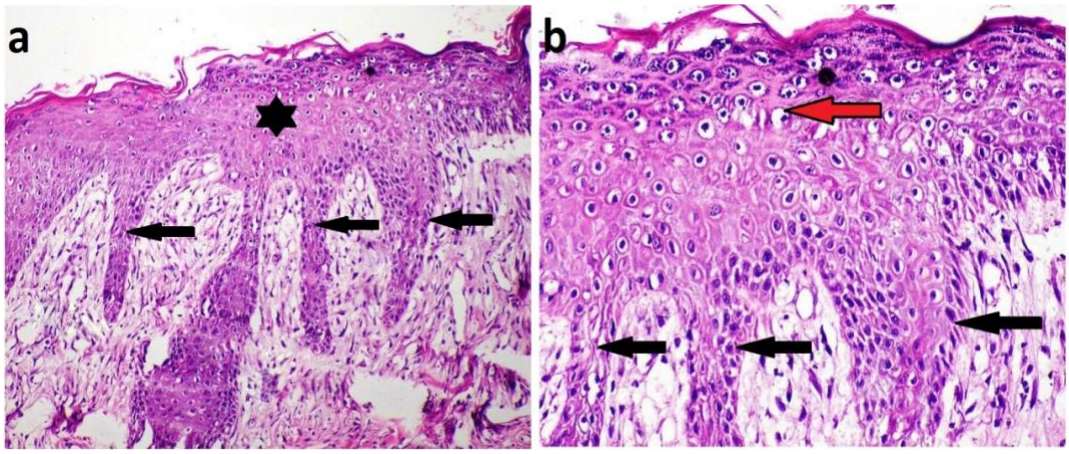

Fig. (19): Histopathology of skin biopsies of mixed infection (a & b) acanthosis of epidermal prickle cell layer (asterisk) with papillary projections in the dermis (black arrows) and connective tissue core. Note spongiosis of epidermal cells (red arrow) (H & E stain, X 100 (a) and X 200 (b).