

STUDIES ON DIAGNOSIS AND THERAPEUTICS OF CAMEL DERMAL MYCOSES

ऊंटों में त्वचीय फफुंद रोग का नैदानिक और
चिकित्सीय अध्ययन

VIKRAM SINGH DEWAL

B.V.Sc. & A.H.

THESIS

MASTER OF VETERINARY SCIENCE

(Veterinary Epidemiology and Preventive Medicine)



। पशुधनं नित्यं सर्वलोकोपकारकम् ।

2017

Department of Epidemiology and Preventive Veterinary Medicine
College of Veterinary and Animal Science, Bikaner
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Bikaner – 334001 (Rajasthan)

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THESIS

**Submitted to the
Rajasthan University of Veterinary and Animal Sciences, Bikaner
In partial fulfillment of the requirements for
the degree of**

**Master of Veterinary Science
(Veterinary Epidemiology and Preventive Medicine)**

FACULTY OF VETERINARY & ANIMAL SCIENCE

By

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B.V.Sc. & A.H.

2017

**Rajasthan University of Veterinary and Animal Sciences
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Place:

Vikram Singh Dewal

Date:

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LIST OF ABBREVIATIONS

%	Per cent
Gm	Gram
Hb	Haemoglobin
PCV	Packed cell volume
TLC	Total leukocytes count
TES	Total erythrocytes count
RBC	Red blood cell
DLC	Differential leucocytes count
ml	Milliliter
@	At the rate
i.e.	That is
S.No.	Serial Number
<i>et al.</i>	et alii (and co-workers)
mg/dl	milligram per deciliter
Cu.mm	cubic millimeter
N	Neutrophil
L	Lymphocyte
B	Basophil
M	Monocyte
E	Eosinophil
PLT	Platelets
AST	Aspartate aminotransferase
ALT	Alanine aminotranferase
ALKP	Alkaline phosphatase
U/L	Units per liter
Mmol/l	millimole per liter
NRCC	National research centre on camel
EDTA	Ethylene diamine tetra acetic acid
SDCA	Sabouraud's dextrose chloramphenicol agar
Cu	Copper
Co	Cobalt
Zn	Zinc
Se	Selenium

1. INTRODUCTION

Camel belongs to genus *Camelus* in the family camelidae under the suborder Tylopoda and order Artiodactyla. There are two types of camels, the one humped or dromedary camel (*Camelus dromedarius*), and the two humped or bactrian camel (*Camelus bactrianus*). *Camelus dromedaries* means “running camel”. *Camelus* is latin word for camel and Dromeus is Greek word for runner. *Camelus dromedrarius* also has many common names such as camel, one-humped camel, dromedary camel and Arabian camel.

The developing countries have 98.1 per cent of the total world camel population. India has third highest position with a population of 1.52 million camels (FAO, 2004). Indian camel population is mostly confined to the North Western part of the country, specially in the state of Rajasthan. According to State Animal Husbandry 19th livestock census of 2012, the camel population in Rajasthan is 325713, out of which, Bikaner possesses 46209 camels. Camel is the state animal of the Rajasthan.

Camel is an important component of the desert ecosystem where the flora of usually marginal land can meet the need of human food and energy. Recurring drought in arid and semiarid areas has decimated many livestock species. However, dromedary is still surviving in large numbers due to its outstanding tolerance to the rugged climate of high temperature, water deprivation, endurance for hunger and feed scarcity. As far as utility of this ungulate in arid desert tract is concerned, it is referred, as excellent means of carrying loads, transportation, agriculture and defence services in condition where other animals are scared and failure. Besides these, it's hide, hair and even milk are important products which contribute significantly towards the rural economy

The skin surface acts as anatomic and physiologic barrier between the animal and environment. During life time, skin has its own functions and even after death the utility of skin remains. The diseases of camels are closely related to their natural environment and the type of husbandry. Camelids like other livestock are exposed to a range of skin affections. Bacteria, viruses, parasites and fungi cause the skin affections. Amongst skin infections, fungal infections are more prevalent than the bacterial infections. Young animals are more prone to infection than adults, which could be related to the development of a stronger immunity in older animals due to the multiplicity of contacts with the fungus rather than an intrinsic role of age (Descamps *et al*, 2003; Moriello *et al*, 2003).

The specific requirements, enzyme system, genes responsible for pathogenicity of dermatophyte species, the various defence mechanisms of the hosts may also affect incidence and severity of dermatophytosis among host species and the particular distribution of lesions on the host. Gitao *et al* (1998) reported higher infection rates of camel dermatophilosis from Kenya, Sudan and Saudi Arabia in the wet season as compared to the dry season. Camel calves had higher infection rates than adult camels. Identification and characterization of camel skin fungi along with type of the lesions has been described in India (Tuteja *et al*, 2010 a; Tuteja *et al*, 2010 b; Tuteja *et al*, 2012; Tuteja *et al*, 2013 a; Tuteja *et al*, 2013 b; Tuteja *et al*, 2014 a; Tuteja *et al*, 2014 b).

Camel is the most suitable mammal for use in extreme climatic conditions (Wilson, 1984; Yagil, 1985). The skin infections causing contagious skin necrosis, dermatitis, wounds, abscesses or similar lesions pose the constant problem in camel. These infections are chronic. Though the diseases are not always fatal but an indirect great economical loss is incurred due to reduction in the working efficiency of

the animals. At many occasions the skin lesions spread rapidly over the body surface and it is very difficult to manage these lesions. Consequently, the camel becomes useless for any purpose. The inability to work or death of the animal results in severe monetary loss which shatters the socio-economic status of camel owner. The people most closely associated with the camel in Rajasthan are the Raika's. The Raika camel keepers have developed their own ways to treat diseases in camels, based on folk believes, traditional knowledge, skills, methods and practices. This traditional knowledge is handed down orally from generation to generation and it may disappear because of rapidly incoming socio-economic, environmental and technological changes.

Several published reports from different countries (Mohamed and Hussein, 1996; Agab, 1998; Nomanda, 1998 and Muhammad *et al*, 2005) have described the traditional practices used by the camel owners. Camel owners living in remote areas of the desert remained cut off from the comparatively progressive areas. The local environment specially the range land flora governs the needs of the resource poor farmers. So the local therapies specially the plant based therapies varied considerably from one region to other. Many of the present day remedies were borrowed from folk medicine, so there is paramount need to document and validate this indigenous system of treatment and collate with the scientifically validated knowledge, so as to have better understanding the rationale behind such practices.

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern pharmaceutical drugs. Plants generally produce many secondary metabolites, which constitute an important source of bactericides, fungicides, pesticides and many pharmaceutical drugs.

Looking to the severity of dermal mycoses in this area, the present study was undertaken with following objectives:

1. To find out the fungal flora causing dermal mycoses in camels.
2. To find out relationship between serum minerals responsible for maintaining skin integrity with dermal mycoses in camels.
3. To find out therapeutic efficacy of herbal drug formulation for the treatment of dermal mycoses in camels.

2. REVIEW OF LITERATURE

The relevant literature related to proposed study is reviewed under the following headings-

2.1 Causes of dermal mycoses in camel:

Georg, (1954) proposed a classification for dermatophytes based on their habitat. Based on a large survey of skin samples from animals and humans, dermatophytes were divided into three groups: 1. zoophylic - those found mainly in animals, but transmitted to other animal or to humans; 2. anthropophylic - those found mainly in humans and transmitted amongst humans, but very seldom to animals; 3. geophylic - dermatophytes found mainly in soil that infect both humans and animals.

Pal, (1956) identified routine filamentous fungi from affected calf skin were *Aspergillus* and *Penicillium* spp. various species of *Aspergillus* identified were *A. terreus*, *A. versicolor* and *A. niger*. Aspergillosis has been reported from calf skin.

Chittawar and Rao, (1982) reported *Microsporum canis* in dogs. *M.canis* was the main causative agent of canine dermatitis of mycotic origin in central India.

Pal and Singh, (1983) reported dermatophytosis in cattle. These skin infections were caused by *Microsporum canis* in cattle.

Thakur *et al.* (1983) reported *Trichophyton verrucosum* as a primary causative agent of dermatomycosis in water buffalo in Punjab.

Kuttin *et al.* (1986) surveyed for ringworm in camels and reported more than 25% of young animals suffering from *T.*

verrucosum infection, and less than 0.5% of the camels having *T. mentagrophytes*.

Abdel-Gewad, (1989) observed that frequency of occurrence of fungi in 120 claw samples of buffaloes and cows from different localities at Assiut governorate. The most common genera were *Chrysosporium*, *Aspergillus* and *Scopulariopsis*. Also other dermatophytes were isolated such as *Trichophyton terrestre*, *T. rubrum*, *Histoplasma capsulatum*, *Phialophora gougerotii* and *Microsporum distortum*.

Abdurahman and Bornstein, (1991) reported in Somalia that the most common dermatophytes found in camels are *Trichophyton mentagrophytes* and *Microsporum gypseum*, mixed infection of skin pathogens (eg. *Trichophyton sp.* and *Sarcoptic scabiei*) are common, making a correct diagnosis and treatment difficult.

Lewis *et al.* (1991) reported that *Microsporum* and *Trichophyton* are most frequently found dermatophytes in animals while the *Epidermatophyton* causes problems mainly in humans.

Rezabek *et al.* (1993) diagnosed histoplasmosis nine horses during 1986-1990. The infection with *Histoplasma capsulatum* caused granulomatous placentitis and abortion in one mare in the 7th month of gestation and three mares in the 10th month. Four newborn foals died from severe granulomatous pneumonia within a few days of birth; and a weanling thoroughbred developed granulomatous pneumonia and lymphadenitis at 5 months of age.

Mahmoud, (1993) reported sixteen species of dermatophytes belonging to nine genera of keratinophilic and cycloheximide resistant fungi from diseased camels. *Trichophyton*, *Microsporum* and

Chrysosporium were the most common genera identified. The younger individuals were more susceptible than adults.

Fadlelmula *et al.* (1993) conducted study on camel ringworm at Eastern Sudan. Ringworm was diagnosed in 217 out of 498 camels (43.5%). The peak incidence of the disease was found in autumn and winter. The disease was observed more frequently among young growing calves (1-2 years) than older animals but the prevalence among male and female animals was found to be similar. *Trichophyton verrucosum* was isolated in pure culture for the first time from camel ringworm in the Sudan.

Chandel and Kher, (1994) reported *H. capsulatum* from miliar necroses of the lungs in dromedary camel.

Gitao *et al.* (1998) reported *Dermatophilus congolensis* and *Microsporum gypseum* causing skin infections in camels of a dairy farm in Saudi Arabia.

Deshpande and Koppikar, (1999) isolated *Penicillium* from patients with keratitis, endophthalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, and urinary tract infections. Most *Penicillium* infections were encountered in immunosuppressed hosts. Corneal infections are usually post-traumatic.

Pitt, (2000) reported that *Penicillium* species produces a mycotoxin, ochratoxin-A, which is nephrotoxic and carcinogenic. The production of the toxin usually occurs in cereals at cold climate.

Kushwaha and Guarro, (2000) reported that *Trichophyton verrucosum* has been cited as the major agent encountered in cases of bovine, ovine and caprine ringworm. Other species such as *M. canis*,

M. gypseum, *T. mentagrophytes* and *T. equinum* have been isolated from some of these ruminants.

Lopez *et al.* (2000) recorded a case of cutaneous mucormycosis in a Spanish Thoroughbred mare. Animal was infected with *Absidia corymbifera*.

Al-Ani *et al.* (2002) reported that *Trichophyton* and *Microsporum* species are common important animal pathogens capable of causing dermatophytosis.

Guillot *et al.* (2004) reported eumycetoma in a 3-year-old male Siberian Husky dog in France. It was the first case in which *Cladophialophora bantiana* was identified as the causative agent of eumycetoma.

Al-Ani and Roberson, (2005) reported that *Epidermophyton*, *Microsporum*, *Trichophyton*, *Sporotrichum*, *Candida*, *Cladosporium*, *Coccidioides*, *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Aspergillus fumigatus* are responsible for causing various diseases in camelids. Fungi in the genera *Trichophyton* and *Sporotrichum* are most prevalent fungi in camelids in USA.

Ebrahimi *et al.* (2007) reported *Trichophyton verrucosum* and *Trichophyton tonsurans* from healthy skin coat of camels from Iran.

Wernery *et al.* (2007) cultured *Candida albicans* from skin scrapings of very young camel calves. The physical condition of dromedary calves was affected; they were smaller and weighed less than calves in the same age group.

Badali *et al.* (2008) reported that *Cladophialophora* is a genus of dark coloured yeast-like fungi which are remarkably frequently

encountered in human infections, ranging from mild cutaneous lesions to fatal encephalitis.

Chermette *et al.* (2008) reported that wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species *Microsporum gypseum*.

Khosravi *et al.* (2009) reported that *Cladosporium* and *Candida* species were the most frequent fungal isolates obtained from eye and nose of healthy dromedary camels in Iran.

Wisal and Salim, (2010) examined 106 skin scrapping sample from camels suspected to be infected with dermatophytes which comprises of 90 from Alshowak and 46 from Alobied. Out of which in 77 samples *Trichophyton verrucosum* was isolated, in 47 *Trichophyton mentagrophytes*, in 9 *Trichophyton schoenlenii* and *Trichophyton tonsurans* in 3 samples. Both female and male camels were found to be susceptible so all the camels less than 3 years old were, more susceptible to infection.

Tuteja *et al.* (2010b) found *cutaneous alternariosis* is an infectious agent mainly of camel calves of approximately one year of age in Rajasthan state of India. The disease occurs more frequently in semi-arid than arid region of the state.

Tuteja *et al.* (2010a) reported *Candida albicans* as the main etiological agent causing skin infections of the camel calves of less than one year of age.

Colin *et al.* (2013) discussed the various fungal agents which are pathogenic to human and animals. He was described morphology and microscopic character of different pathogenic fungi.

Tuteja *et al.* (2013a) observed that the species of common dermatophytes i.e. *Microsporum* and *Trichophyton* isolated from camel skin lesions included *M. audouinii*, *M. canis*, *M. nanum*, *M. ferrugineum*, *T. verrucosum*, *T. mentagrophytes*, *T. schoenleinii*, *T. equinum*, *T. concentricum*, *T. tonsurans*, *T. violaceum*, *T. soudanense* and *T. rubrum*. These fungi caused sporadic cases of skin infections in individually maintained camels as well as affecting many camels in the herds. These fungi create distinctive lesions of ring worm. The lesions of ring worm observed with *Trichophyton* spp. were comparatively dry, hard, crusty, granulomatous and larger in size.

Tuteja *et al.* (2013c) observed that *Aspergillus* as causative agents of opportunistic infections in man and animals. Among these *A. fumigatus* is the most commonly isolated species, followed by *A. flavus*, *A. niger*, *A. terreus*, and *A. versicolor* are isolated as opportunistic pathogens in camels

Tuteja *et al.* (2013b) reported skin infection in camel which caused by soil-born species *Absidia corymbifera*. *A. corymbifera* often cause food spoilage and only species known to cause disease in man and animals.

Tuteja *et al.* (2014b) reported that primarily the human pathogenic fungi *Epidermophyton floccosum* and *Scopulariopsis brevicaulis* also cause skin infections in camel.

Tuteja *et al.* (2014a) diagnosed dimorphic fungi viz. *Sporothrix schenckii*, *Coccidioides immitis / posadasii* and *Penicillium marneffeii* causing skin infections in camels.

Abdulaziz *et al.* (2015) carried out study at Qassim Region, Central of Saudi Arabia, to study dermatophytosis in a private farm of dromedary camels. Mycological examination of clinically diseased camels revealed the presence of *Trichophyton verrucosum* among them.

Rantala *et al.* (2015) reported endometritis case associated with *Cladophialophora bantiana* in a 15-year-old mare. The mare displayed infertility and uterine fluid accumulation with numerous black, hairy granules. Microscopically, the fluid revealed numerous septate, dark fungal hyphae and conidia in chains. Culture yielded *C. bantiana*.

Baghza *et al.* (2016) conducted study among 165 suspected camels in Dhamar area, Yemen. The infection was significantly higher among young animals of less than 12 months. The frequency of *Trichophyton* and *Microsporum* genera were 89.4 and 10.6 percent of the isolated genera, respectively. The identified species were *T. schoenlenii*, *T. verrucosum*, *T. mentagrophytes*, *T. tonsurans*, *M. audouinii* and *M. canis*. Almost half of the study animals were found infected with *T. schoenlenii*.

Brilhante *et al.* (2016) reported that coccidioidomycosis and histoplasmosis are systemic mycoses caused by the dimorphic fungi *Coccidioides spp.* and *Histoplasma capsulatum*, respectively, which affect humans and a variety of other animals, including equines.

Shokri and khosravi. (2016) found *Trichophyton verrucosum* in camels suspected of having dermatomycoses in Iran.

2.2 Relationship of serum minerals responsible for maintaining skin integrity with dermal mycoses:

Van den Broek and Stafford, (1988) reported the concentrations of zinc in serum, leucocytes and hair of normal dogs, dogs with zinc-responsive dermatosis, and the dogs with dermatitis not associated with zinc deficiency. The mean concentration of zinc in serum and hair in dogs with zinc-responsive dermatosis was significantly lower than in other dogs but the range of zinc concentrations overlapped that of the other dogs. The mean leucocyte zinc concentration was similar for each group of dogs. It is concluded that low zinc concentrations in serum and hair have only a corroborative value in the diagnosis of zinc-responsive dermatosis in dogs.

Thoday, (1989) reported that diet deficient in zinc cause the many skin diseases in dog including zinc-responsive dermatosis. The plasma zinc concentration below the mean of the reference range supports the diagnosis.

Al-Qudah *et al.* (1994) reported that dermatologic lesions in heifer caused by dermatophytosis are sometimes associated with selenium deficiency. There was no response observed to other treatments that had been used prior to the administration of selenium.

Krametter *et al.* (2005) reported in two cases of zinc deficiency in dairy goats from different flocks and not associated with a zinc-deficient diet. Hard, dry, hyperkeratotic skin, hair loss and pruritus especially prominent on the back, legs, udder, face and ears were the most common clinical signs. On initial examination, serum zinc concentrations were low in both goats. Although mild skin lesions persisted during the early stages of zinc supplementation, skin lesions completely resolved after prolonged oral zinc supplementation.

Withdrawal of zinc supplementation resulted in re-appearance of lesions in both animals.

Nisbet *et al.* (2006) reported the importance of zinc in calves with trichophyton infection. Serum zinc levels were lower in diseased calves than healthy ones.

Kojouri *et al.* (2009) reported in a humid area of Iran on 35 healthy and 35 infected cows that serum concentration of selenium and zinc in cattle with dermatophytosis were significantly lower than the healthy ones. In conclusion, it seems that zinc and selenium have a determinant role in immune status and the response of animal's immunity system to dermatophytosis.

Pasa and Kiral, (2009) observed that the serum zinc and vitamin A concentrations were found to be significantly lower in calves with dermatophytosis than those of healthy controls. Results shown that serum zinc and vitamin A concentrations were altered in calves with dermatophytosis.

Al-Qudah *et al.* (2010) reported the levels of trace minerals Zn, Cu, and Se in calves with dermatophytosis. The levels of Zn, Cu and Se were found significantly low in diseased calves than healthy calves.

Kuria *et al.* (2013) reported normal status of minerals in camels in north-eastern Kenya, which evaluated from the blood plasma. The average normal level of zink, copper, cobalt and selenium in blood plasma are 0.91, 0.82, 0.08 and 0.23, respectively in part per million.

2.3 Clinical signs:

Abdurahman and Bornstein, (1991) reported that ringworm usually occurs in young camels under three years of age and is characterised by localized thickening of skin. Lesions appear after as circumscribed, crusty and hairless, distributed over the head, neck, shoulders and limbs.

Manefield and Tinson, (1996) reported two clinical types of ringworm in camels. The first shows typical lesions that are gray-white in colour. These lesions are characterized by small, round alopecic areas, which may coalesce and mainly occur on the legs, neck and head of young animals. The second is a more generalized infection on head, neck, limbs and flanks whereby these lesions may initially be confused with mange.

Gitao *et al.* (1998) from Saudi Arabia observed that the lesions with mixed infection of *D. congolensis* and *M. gypseum* were discrete, circumscribed, crusty, hairless and were particularly found on the neck and fore legs in camel calves of less than one year of age, whereas other age group animals had extensive hair matting with crusty, hairless lesions, especially on the flanks.

Al-Ani *et al.* (2002) observed that calves aged 3 to 7 months developed clinical signs of ringworm. The skin of affected calves showed circular, circumscribed, grayish-white, crusty raised lesions. Focal pityriasis and alopecia were also observed. The lesions were most commonly found on the head, neck, dewlap and the chest area. In horses the lesions started as patches of raised hair and soreness. Several days later, the hair detached leaving bald, gray, shining areas approximately 3 cms in diameter.

Wisal and Salim, (2010) reported that ringworm occurs in camels less than 3 year age and is characterized by circumscribed crusty hairless lesion, 1-2 cm in diameter distributed over the head, neck, shoulder, limbs and flanks.

Tuteja *et al.* (2010a; 2012) reported that Skin candidiasis is an acute and contagious fungal skin infection of camel calves of less than one year of age, which causes morbidity in terms of reduced weight gain. Lesions of the disease are initially observed on the back near the hump, later on the lesions extend towards the abdomen and may cover the whole body. Lesions are initially round in shape and measure less than one centimeter in size which may enlarge to more than 10 cms in size and may co-alsace. The lesions are hard and fibrous crusts with papules accompanying alopecia. In prolonged cases it causes itching, uneasiness and may lead to bleeding and ulceration of skin and may result in weakness and debility of calves. When infection occurs in a herd it affects almost every young calf in that particular herd. Self cure has been observed in most of the cases.

Tuteja *et al.* (2010b) reported that the lesions of the *cutaneous alternariosis* were found on the body including the lips and udder.

Tuteja *et al.* (2013a) observed that *Microsporium spp.* has ability to degrade keratin and thus can reside on skin and it's appendages and remain non-invasive. These fungi can grow to create distinctive lesion associated with ring worm. These lesions are small disc-shaped marking and can occur anywhere on the body.

Abdulaziz *et al.* (2015) from Saudi Arabia reported clinical signs of ringworm caused by *Trichophyton verrucosum* were non-pruriginous dry circumscribed discrete, crusty hairless lesion distributed over the head, neck, shoulder, limbs and flanks.

2.4 Haematology:

Mwanzia and Mung'athia, (1997) observed that blood haematology and biochemical values were within the range in free ranging wildlife in outbreak of dermatophytosis in Kenya.

Rotstein *et al.* (1999) reported that hematology and serum chemistry analysis were found normal except for an elevated globulin fraction (8.6 mg/dl; range, 6.4-8.2 mg/dl) in free ranging panthers who suffered by dermatophytes infections in Florida.

Atakisi *et al.* (2006) examined liver function tests in dermatophytic cattle which showed increased enzymes activities in dermatophytic calves compared to those of healthy group. This increase was highly significant in the case of ADA, LDH, ALT, and AST. While dermatophytic calves showed positive results in microscopic examination, healthy ones were negative.

Mathur *et al.* (2011) found decrease in lymphocyte count and serum glucose whereas increase in hemoglobin, TLC, MCHC, neutrophil and eosinophil count in cases of dermatomycosis in camels.

Foutah *et al.* (2012) reported a significant reduction of erythrocytic count, haemoglobin content and packed cell volume, beside leukocytosis, neutrophilia, lymphocytosis and insignificant decrease in monocytes, eosinophils and basophiles were present in camels infected with ringworm. He could conclude that It could be concluded that ringworm in camels induce some adverse effect on haemato-biochemical parameters.

Sindha *et al.* (2015) found neutrophil Hb, PCV, and TEC significantly lower in cases of fungal skin infections in canines.

Nagarajan *et al.* (2016) observed that there is no any abrupt derivation in haemoglobin , PCV but differential cell count reveled mild elevation of eosinophils in Haryana breed of cattle in Tamilnadu which suffered from chronic mycotic dermatoses.

2.5 Therapeutics of camel dermal mycoses:

Ainsworth and Austwick, (1973) advocated use of Captan as fungicidal. Captan is a fungicide and its use causes lot of irritation in the affected camels.

Sharma and Dwivedi, (1990) prepared a herbal preparation containing onion (*Allium cepa*), garlic (*A. sativum*) and lemon (*Citrus limon*) extracts and powders of turmeric (*Curcuma longa*) and camphor in Karanj oil (*Pongamia glabra* [*P. pinnata*]). They used that prepration to treat ringworm due to *Trichophyton verrucosum* in 12 cattle and *T. verrucosum* and *Microsporum canis* in 21 dogs. Daily application of the drug preparation resulted in complete cure within 12-15 days of treatment. There was no sign of toxicity in animals treated with the preparation.

Schwartz and Dioli, (1992) found use of griseofulvin in camels. However this causes side effects such as nausea and diarrhoea.

Sharma *et al.* (1993) diagnosed ringworm in 13 calves, which were treated by daily application of an aqueous extract of garlic (*Allium sativum* Linn.) in petroleum jelly (1:10), and observed that complete resolution of all lesions was achieved in 12-14 days. The treatment induced initial signs of local irritation, followed by an inflammatory reaction.

Augusti K. T., (1996) reported that Onion and garlic contain many sulfur containing active principles mainly in the form of cysteine derivatives, viz. S-alkyl cysteine sulfoxides which decompose into a

variety of thiosulfinates and polysulfides by the action of an enzyme allinase on extraction. Decomposed products are volatile and present in the oils of onion and garlic. These possess antidiabetic, antibiotic, hypocholesterolaemic, fibrinolytic and various other biological actions.

Pattnaik *et al.* (1996) observed that five aromatic constituents of essential oils (cineole, citral, geraniol, linalool and menthol) were tested for antimicrobial activity against eighteen bacteria (including Gram-positive cocci and rods, and Gram-negative rods) and twelve fungi (three yeast-like and nine filamentous). Against fungi the citral and geraniol oils were found the most effective (inhibiting all twelve fungi), followed by linalool (inhibiting ten fungi), cineole and menthol (each of which inhibited seven fungi) compounds.

Paterson, S. (1997) used a combination of miconazole and chlorhexidine, which applied topically twice weekly was proved to be efficient for the treatment of dermatophytosis in horses

Joshi *et al.* (1998) reported a cysteine protease inhibitor exhibiting antifungal activity from pearl millet seeds which have been purified to homogeneity by ammonium sulphate precipitation and chromatographic procedures involving CM- sephadex and SP-sepharose cation exchange columns. The inhibitor exhibits potent antifungal activity against *Trichoderma reesei*, a dead wood fungus with minimum inhibitory dose to inhibit mycelial growth or spore germination is as low as 1 µg / ml (250 ng/disc). In addition to *Trichoderma reesei*, the antifungal activity is observed against some important phytopathogenic fungi, namely, *Claviceps*, *Helminthosporium*, *Curvularia*, *Alternaria* and *Fusarium species*.

Wernery and Kaaden, (2002) reported various hit and trial methods being adopted for treatment of dermal mycoses in camel. A variety of common fungicidal and fungistatic agents such as iodine, 5% sulphur in sesame oil, 5% salicylic acid, coal tar phenols (3.25%) with copper acetate (0.58%) and hydroxyquinolines may be applied topically as ringworm ointments onto the affected areas. Drugs used have included thiabendazole, flucytosine, and amphotericin B, but very little is known about their effect in camels.

Singh *et al.* (2004) observed that GC and GC-MS analysis of volatile oil obtained from *Piper nigrum* L resulted in the identification of 49 components accounting for 99.39% of the total amount, and the major components were β -caryophyllene (24.24%), limonene (16.88%), sabinene (13.01%), β -bisabolene (7.69%) and α -copaene (6.3%). The acetone extract of pepper showed the presence of 18 components accounting for 75.59% of the total amount. Piperine (33.53%), piperolein B (13.73%), piperamide (3.43%) and guineensine (3.23%) were the major components. The oil was found to be 100% effective in controlling the mycelial growth of *Fusarium graminearum* in inverted petriplate technique. The acetone extract retarded 100% mycelial growth of *Penicillium viridcatum* and *Aspergillus ochraceus* in food-poisoning technique. Volatile oil and acetone extract were identified as a better antioxidant for linseed oil, in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Hassan *et al.* (2004) reported that calves with ring worm (*Trichophyton verrucosum*) were employed to use the efficacy of herbal medical therapy by once rubbing Aloe Vera gel leaves topically daily on the affected skin lesions compared by traditional topically iodine ointment 10% twice application in a daily base for continuous twenty days. Three weeks treatment course and two weeks post- treatment, notable rapid, effective and curative result with subside of all skin

lesions and return to the accepted clinical picture were recorded in Aloe vera gel leaves treated calves comparing by iodine ointment treated ones.

Al-Ani and Roberson, (2005) reported that Amphotericin-B is the main stay of therapy for serious fungal infections and remains the most broad-spectrum antifungal agent available, but it may causes nephrotoxicosis. Prolong use of Griseofulvin is also helpful but causes diarrhoea in camels.

Erturk, (2006) studied therapeutic efficacy of eleven ethanolic extracts from spices of *Melissa officinalis*, *Mentha piperita*, *Laurus nobilis*, *Rhus coriaria*, *Dianthus coryophyllum*, *Piper nigrum*, *Capsicum annum*, *Juniperus oxycedrus*, *Erica arborea*, *Colutea arborescens*, and *Cuminum cyminum* collected from various regions of Turkey and local markets. These were assayed for the in vitro antibacterial activity against 3 Gram-positive and 2 Gram-negative bacteria, using agar dilution methods. In addition, their possible toxicity to *Candida albicans* and *Aspergillus niger* was determined, using both agar dilution and disc-diffusion methods.

Singh *et al.* (2006) studied the antifungal activities of coriander oil and its oleoresin against eight fungi using the inverted petri plate and food poison techniques. Using the inverted petri plate method, the essential oil was found to be highly active against *Curvularia palliscens*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Aspergillus terreus*. In the case of the oleoresin, more than 50% mycelial zone inhibition was obtained for the fungi *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus terreus*. Using the food poison technique, the essential oil showed 100% inhibition on the growth of *A. terreus*, *A. niger*, *F. graminearum* and *F. oxysporum*, whereas its oleoresin showed weaker fungitoxic activity, exhibiting 100% inhibition on the growth of *F. oxysporum* only.

Chaieb *et al.* (2007) reported that many essential oils are known to possess an antioxidant activity and antifungal properties and therefore they potentially act as antimycotic agents. Essential oil of clove (*Eugenia caryophyllata*) was isolated by hydrodistillation. It is observed that clove oil shows powerful antifungal activity.

Antoine-Moussiaux *et al.* (2007) in Niger area found that cow's milk butter being very effective in dermatophytosis.

Newbury *et al.* (2007) suggested that lime sulphur may be used in dogs and cats in combination with griseofulvin or itraconazole, but it has an offensive odour and may stain light-coloured hair.

Jeung and Choi, (2007) carried out study to investigate the potential of using plant oils derived from *Leptospermum petersonii* Bailey and *Syzygium aromaticum* L. Merr. Et Perry as natural antifungal agents. The antifungal effects of essential oils at dermatophytes *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Microsporum gypseum* were evaluated using the agar diffusion method.

Tuteja *et al.* (2011) standardized treatment for skin infection other than skin candidiasis. They used Sulphur in mustard (*Brassica* spp.) oil (1:10), for topical application at alternate days for 1-2 week.

Tuteja *et al.* (2012) standardized treatment protocol for skin candidiasis by incorporating the ethno veterinary knowledge used by the farmers. Therapeutic potential of three formulations consisting of 2% potassium iodide; 6% sulphur in mustard oil; and 6% sulphur and 3% salicylic acid in mustard oil were evaluated topically in naturally occurring cases of skin candidiasis in camel calves. All the three treatments were found effective with almost similar application schedule but with variable duration of treatment. This is long term

treatment schedule with a minimum of eight applications is effective only against skin candidiasis.

Bhikane *et al.* (2015) reported Vetomax spray- a polyherbal preparation to be effective in treatment of dermatomycosis in cattle.

3. MATERIALS & METHODS

The present study was conducted as per following headings:

- 3.1 Animals
- 3.2 Anamnesis and clinical examination
- 3.3 Screening criteria for camel to be included in study (Clinical picture/ photograph of the animal)
- 3.4 Collection of skin scrapings
- 3.5 Direct microscopic examination
- 3.6 Culture examination
- 3.7 Storage of fungal isolates
- 3.8 Collection of blood samples
- 3.9 Haematological examination
 - (i) Haemoglobin (HB) estimation
 - (ii) Determination of packed cell volume (PCV)
 - (iii) Total leucocyte count (TLC)
 - (iv) Total erythrocyte count (TEC)
 - (v) Differential leucocyte count (DLC)
- 3.10 Biochemical estimation
 - (i) Total serum protein

 - (ii) Alkaline phosphatase (ALKP)

(iii) Serum aspartate aminotransferase (SGOT)

(iv) Serum albumin

(v) Serum alanine aminotransferase (SGPT)

(vi) Serum globulin

3.11 Estimation of serum minerals (Zn, Co, Cu, Se)

(i) Digestion of serum samples by Microwave digestion system (MDS)

(ii) Estimation of digested serum minerals by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

3.12 Treatment trial of camel dermal mycosis

3.13 Statistical analysis

3.1 Animals:

Initially the animals were selected on the basis of gross lesions. Finally a total of 16 animals found positive for dermal mycoses by direct and cultural examination of skin scrapings (irrespective of sex, age and breed) at the village Motigarh, Tehsil Chatargarh, Bikaner district were taken for the study.

All these animals were photographed before start of the treatment, after each application of the drug and photo at one and two months of the start of the treatment.

3.2 Anamnesis and clinical examination:

A detailed history was recorded from every owner of the camel under study as follows

- (i) Animal's details: Breed, age, sex, duration of ownership, management practices and total animals kept by owner.
- (ii) Duration of skin problem along with treatment and control measures adopted by the owner.
- (iii) Previous history of illness, associated disease and use of camel.
- (iv) Feeding practices
- (v) Owner's observation: Details of earliest signs and symptoms, progress and nature of the disease.

A complete physical examination was carried out and status of all body systems was evaluated and noted.

3.3 Screening criteria for camel to be included in study:

The camels which showing peculiar skin lesion of mycoses along with other sign and symptoms of pruritis, alopecia were included in the study. In some of these animals, lesions were distinctive of ring worm. These lesions were small disc-shaped markings and occurred anywhere on the body. In few other animals, ring worm lesions which were comparatively dry, hard, crusty, granulomatous, larger in size, were also present. In camel calves the lesions which included small to large disk shaped marking present anywhere on the body, later these marking develop crusts, which can ooze and may turn bloody on scratching. In all these animals lesions were mostly white in appearance.

3.4 Collection of samples:

In affected camels with clear skin lesions, ointment or other local applications, if present were first removed with an alcohol wipe. Then using a blunt scalpel lesions were firmly scraped, particularly at the

advancing border. Most recent lesions were chosen for scraping. These samples were collected in sterile vials. Then, these samples were transferred to the laboratory in thermocol box packed with brine packs.

3.5 Direct microscopic examination:

It was performed by placing the scrapings on a glass slide with two or three drops of 20 % potassium hydroxide and placing a cover slip over it. The sample was warmed for five minutes over a flame and was then carefully examined microscopically for the presence of hyphae and/or arthroconidia.

3.6 Culture examination:

Samples were first mixed with Sabouraud's dextrose chloramphenicol broth and were incubated at 28° C for up to 24 hours. Then, these samples were inoculated onto Sabouraud's dextrose chloramphenicol agar (SDCA) plates and were incubated at 28 C for 3-4 weeks. In case the growth appeared to be of dimorphic fungi, another plate was subcultured and incubated at 37° C for up to 2-weeks for confirming the yeast stage of the isolate. These plates were examined daily for the growth of the fungi. The resultant growth was examined for the colony morphology. Microscopic examination was carried out using either lacto phenol cotton blue or calcoflour white stains using wet mount method (Halley and Standard, 1973).

3.7 Storage of isolates:

All these isolates were stored on SDCA slants for four months at refrigerator temperature and then were again subcultured at four month intervals, specially those isolates where identification of the isolate could not be made immediately.

3.8 Collection of blood samples:

For haematological examination blood samples from all these 16 cases of dermal mycoses were collected by jugular vein in sterile vacutainers having ethylene diamine tetra acetic acid (EDTA) disodium salt as an anticoagulant added at the rate of 1mg/ml of blood of as recommended by Jain (1986).

For biochemical studies, blood was simultaneously collected in another sterile vacutainers having no anticoagulant. These vacutainer tubes were kept in slanting position for one hour at 37⁰C. Blood clots of these slants were broken and tubes were centrifuged at 2,500 rpm for 30 min. The serum was harvested in small Pyrex tubes and was stored in the deep freeze at -20⁰C till analysis.

Blood samples were collected from all these cases before and after two months of the start of the treatment.

3.9 Haematological examination:

For haematological examination blood samples were analysed for haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and differential leucocyte count as per the method described by Jain (1986).

(i) Haemoglobin (Hb) estimation:

Hemoglobin was determined by Sahli-Hellige haemoglobinometer. Blood was drawn in Sahli's pipette up to 20 cubic millimeter mark. It was then transferred to haemoglobinometer tube containing 4-5 drops of 0.1N hydrochloric acid and mixed well. The tube was then kept for 5 minutes for the haemoglobin to change into acid haematin. The fluid was diluted with distilled water drop by drop and mixing after each drop until it matched to the colour of the standard comparison tubes. The haemoglobinometer tube was read to give the amount of haemoglobin in g/dl of the blood.

(ii) Packed cell volume (PCV):

For determination of packed cell volume, microhaematocrit method was adopted. Non-heparinised capillary tubes were filled with blood up to three-fourth of total length. The blood adhered over the end of capillary tubes was wiped off with the help of a moist filter paper. The opposite ends of tubes were sealed over the spirit lamp by rotating between the thumb and the index finger for 2-3 seconds over the flame near its base. After perfect sealing of the end, the tubes were centrifuged for 5 minutes at 12,000 rpm in microhaematocrit centrifuge machine.

After centrifugation, packed cell volume was determined with the help of a special microhaematocrit reader scale. The bottom of the red column of capillary tube was adjusted with the zero line and the plasma level was matched with the hundred lines and top of red column excluding buffy layer was read in per cent.

(iii) Total erythrocyte count (TEC):

The RBC pipette was filled up to 0.5 marks with the blood. The diluting fluid (Hayem's fluid) was drawn up to 101 marks. After shaking the pipette for the three minutes, the fluid in its stem was discarded. The counting chambers of the haemocytometer were carefully charged with the diluted blood after placing cover slip. It was ensured that blood cells were evenly distributed over the counting chamber and overloading was avoided. The red blood corpuscles present in the four corner small squares and one small central square of the large central square were counted under high power of the microscope.

Calculations

Numbers of red blood cells per cubic millimeter were calculated after multiplying the number of cells counted by 10,000 according to the following formula:

$$\text{Total erythrocytes} = \text{Cells counted} \times 200 \times 10 \times 5 \text{ per cubic mm}$$

Where:

200 stands for dilution

10 stands for depth in mm

5 stands for the $1/5^{\text{th}}$ of square millimeter counted

(iv) Total leucocytes count (TLC):

The WBC pipette was filled up to 0.5 marks with blood and the WBC diluting fluid was drawn up to 11 marks. After shaking the pipette for three minutes, the fluid in its stem was discarded. Counting chamber of the haemocytometer was carefully charged with diluted blood after placing cover slip. The cells were counted under low power objective of the microscope in the large four corner squares of the haemocytometer.

Calculations

The numbers of leucocytes in one cubic millimeter of blood were calculated by multiplying the total leucocytes counted by factor 50, according to the following formula:

Total leucocytes per cubic mm = 4 cells counted x 20 x 10

Where:

20 stands for dilution.

10 stands for depth in mm.

4 stands for the number of square millimeters counted.

(v) Differential leucocytes count (DLC):

1. The dried blood film was flooded with methyl alcohol for 10 min.
2. The slides were washed gently with tap water to remove alcohol.
3. Fixed slide was stained in working dilution of Geimsa's stain (1:10) for 30 minutes.
4. The slide was washed with tap water and dried in air.
5. A drop of cedar wood oil was placed on the smear and slide was examined under oil immersion lens of microscope for the differential leucocyte count.

Neutrophils, eosinophils, basophils, lymphocytes and monocytes were differentiated and expressed into percentage.

3.10 Biochemical estimation

Biochemical analysis of serum samples was done to estimate serum total protein, alkaline phosphatase (ALKP), serum aspartate aminotransferase (SGOT), serum albumin, serum alanine aminotransferase (SGPT), serum globulin. These were determined by the Vet Test Chemistry Analyzer using kit supplied by Idexx laboratories, as per the manufacturer's subscribed procedure.

Procedure of vet test chemistry analyzer using Idexx test kits.

First of all power was connected and the analyzer was turned on, after 30 minutes power switching, the on screen instructions-species information, patient number etc. were entered. Selected vet test chemistry slides were inserted when instructed by analyzer. Samples were kept at room temperature before feeding and were inserted as per manufacturer's manual in vet test analyzer using automatic pipettor, connected with the analyzer. All the necessary instructions given by analyzer were followed. The results were displayed on screen as well as in the form of printed slip after automatic analysis process of about 5 to 6 minutes, depending on the chemistries.

3.11 Estimation of serum minerals (Zn, Co, Cu, Se)

Minerals (Zn, Co, Cu, Se) present in serum of camels affected from dermal mycoses were estimated by Microwave digestion system (MDS) and ICP. The samples of before treatment and after recovery both are estimated by the same procedure as per the manufacturer's subscribed procedure.

(i) Digestion of serum samples by MDS

Digestion of serum samples was done by Microwave digestion system model MDS-10 of SINEO Microwave Chemistry Technology Co. Ltd. China.

For digestion 0.5 ml of serum sample was taken in the digestion vessel to this 7 ml of conc. HNO₃ was added. The instrument was warmed for 10-15 minutes, then prepared sample vessels were placed inside the cavity of the instrument. Then, the automated programme for serum digestion was created, which allows digestion in three stages by controlling temperature at 130°, 150° and 160° C for 10 minutes. After digestion cooling starts automatically. Finally the digested sample was diluted to 100 ml with distilled water.

(ii) Mineral Estimation by ICP

Estimation of minerals in digested serum samples was done by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) as per the manufacturer's instructions (Thermo Scientific Ltd. USA). Instrument was allowed to warm up for 10-15 minutes and then the required sample details were feeded in the instrument and the diluted sample was subjected to estimation as per the described procedure by following instrument instructions.

3.12 Treatment trial of camel dermal mycosis

Treatment of the dermal mycoses cases was done with herbal drug formulation developed by I.C.A.R.-National Research Centre on Camel, Bikaner (Provisional Patent Filed). First all the skin debris present over the lesions were scrapped by using scrub-pads. Then the drug lotion was applied topically on whole of the body surface after mixing in tap water in the ratio of 1:1 as per the defined topical

application schedule, with a total of three applications on 0, 3 and 7 days.

3.13 Statistical analysis

The data obtained in research work were statistically analyzed by Paired T test and compared as per the standard procedures suggested by Snedecor and Cochran (1994).



Fig. 1 Photograph showing of Microwave digestion system (MDS)

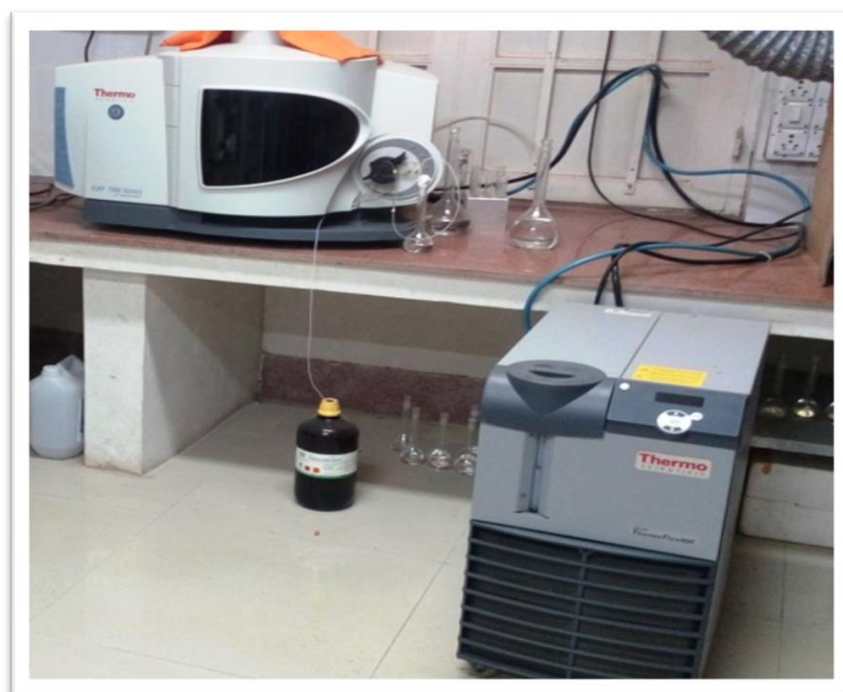


Fig. 2 Photograph showing Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)



Fig. 3. Photograph showing application of herbal formulation

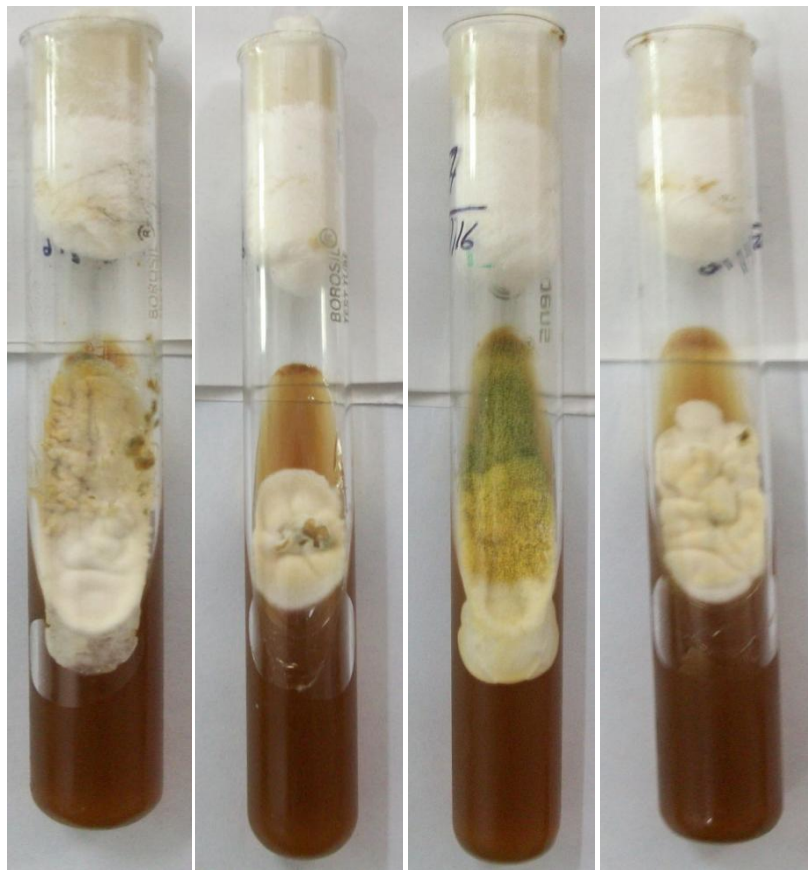


Fig. 4 Photograph showing culture of fungi in SDCA slant for storage

4. RESULTS & DISCUSSION

Looking into the gravity of dermal mycoses in camel in the arid region of the Rajasthan state of India, an effort has been made to draw and evaluates a picture of diagnostic and haemato-biochemical parameters of camel suffering from dermal mycoses and evaluate the efficacy of a new formulation developed for the treatment of dermal mycoses especially for camels which is still under the patenting process.

The present study entitled “Studies on diagnosis and therapeutics of camel dermal mycoses” was conducted on sixteen clinical cases of camels suffering from dermal mycoses in the village Motigarh, tehsil Chatargarh and district Bikaner. Differential diagnosis of other skin infections from dermal mycoses was made on the basis of characteristic diagnostic lesions, direct microscopy and culture examination of skin scrapings.

All the fungal isolates were identified to species level to know the etiological agents responsible for causing dermal mycoses in camels. Antioxidant minerals known for maintaining the skin integrity were analyzed to know their role in causation of the dermal mycoses and hematological parameters were studied to know the alterations occurring if any in clinical cases of dermal mycoses in camels.

4.1 Clinical manifestations

In the present study main clinical manifestations were alopecia, itching, thickening of skin and emaciation. Gross appearance of lesions were dry hard rounded nodular small disk shape crusty granulomatus lesions whitish to gray in colour. The lesions were most commonly found on legs, neck, axillaries, chest, abdomen, tail, head and lips. Similar findings were observed by Abdurahman and Bornstein, (1991); Manefield and Tinson, (1996); Tuteja *et al.* (2010a; 2012) and Abdulaziz *et al.* (2015). The causative agent of dermal mycoses in camels along with clinical lesions and involvement of body parts of the camels are shown in the Table 1 and in photographs Fig. 5 -12.

Table 1: Isolation and identification of causative agents along with main clinical manifestations.

Sr. No.	Fungal Genus	Fungal species	Age of animal	No of animal	Gross morphology of the lesions	Body parts involved	Associated symptoms recorded
1	<i>Trichophyton</i>	<i>T. rubrum</i>	21 yr.	1	Dry, hard, crusty, granulomatus larger lesions	Legs, axillaries, tail abdomen and neck	Alopecia, itching
		<i>T. verrucosum</i>	2-3 yr.	1			
		<i>T. schoenleinii</i>	1-2 yr.	1			
2	<i>Microsporum</i>	<i>M. canis.</i>	15 yr.	1	Small disc-shaped marking associated with ring worm	Thigh, abdomen and axillaries	Alopecia, itching
3	<i>Histoplasma</i>	<i>H. capsulatum</i>	~1yr	2	Small nodular lesions, whitish to gray in colour	Neck, axillaries and legs	Alopecia, itching and emaciation
4	<i>Candida</i>	<i>C. albicans</i>	~1yr	2	Lesions were rounded hard and fibrous crusts, papules	Chest, legs and abdomen	Alopecia, itching and emaciation
5	<i>Cladophialophora</i>	<i>C. bantiana</i>	<1 yr	1	Large crusty whitish lesions spread mainly at head and axillaries	Head, lips and axillaries	Alopecia, itching and thickening of skin
6	<i>Absidia</i>	<i>A. corgmbifera</i>	<1 yr	1	Granulomatous nodular lesions, whitish in colour	Chest, legs and axillaries	Alopecia, itching and emaciation
7	<i>Coccidiodes</i>	<i>C. immitis</i>	15 yr.	1	Nodular lesion from 1-2 cm, whitish scar formation	Abdomen and axillaries	Alopecia and emaciation
8	<i>Penicillium</i>	<i>Penicillium spp</i>	7 yr.	1	Acne like skin papules and large hard whitish lesion	Head, thigh, axillaries	Alopecia, itching
		<i>Penicillium spp</i>	12 yr.	1			
9	<i>Aspergillus</i>	<i>A. terreus</i>	1-2 yr	1	Erythematous to violacaeous nodules, plaques and papules	Neck, pad, knee, neck and axillaries	Alopecia, itching
		<i>A. niger</i>	20 yr.	1			
		<i>A. versicolor</i>	2 yr.	1			



Fig. 5 Photograph showing lesions of *Trichophyton schoenleinii* on chest

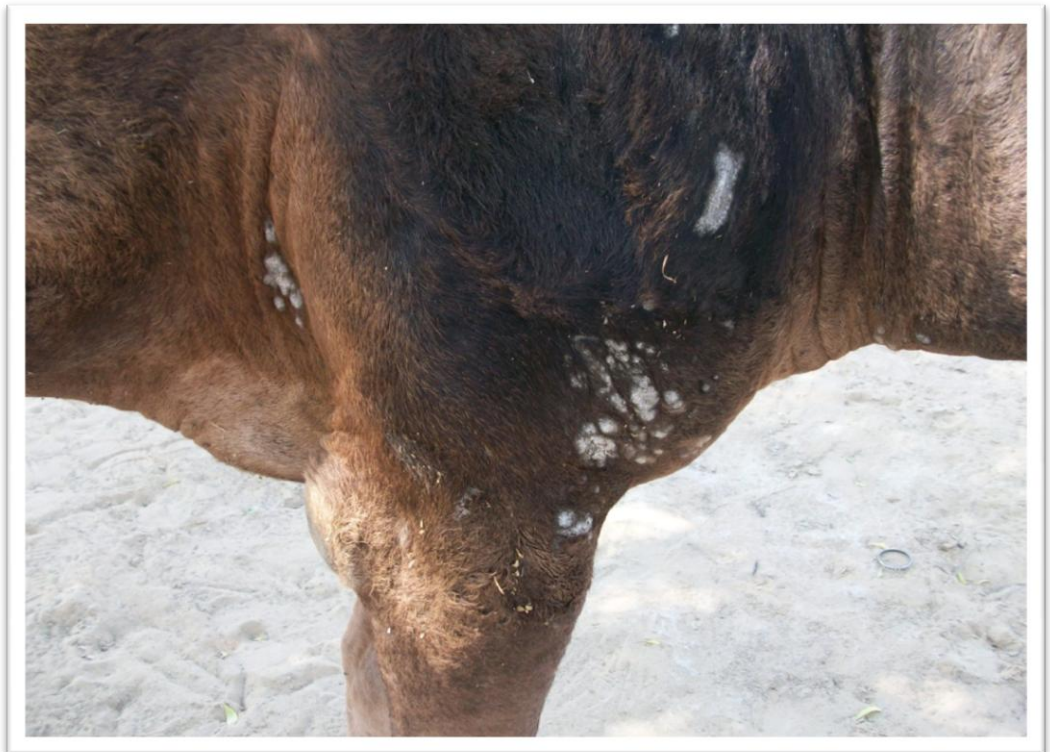


Fig. 6 Photograph showing lesions of *Absidia corymbifera* on leg



Fig. 7 Photograph showing lesions of *Histoplasma capsulatum* on neck



Fig. 8 Photograph showing lesions of *Cladophialophora bantiana* on head



Fig. 9 Photograph showing lesions of *Trichophyton verrucosum* on chest



Fig. 10 Photograph showing lesions of *Candida albicans* on legs

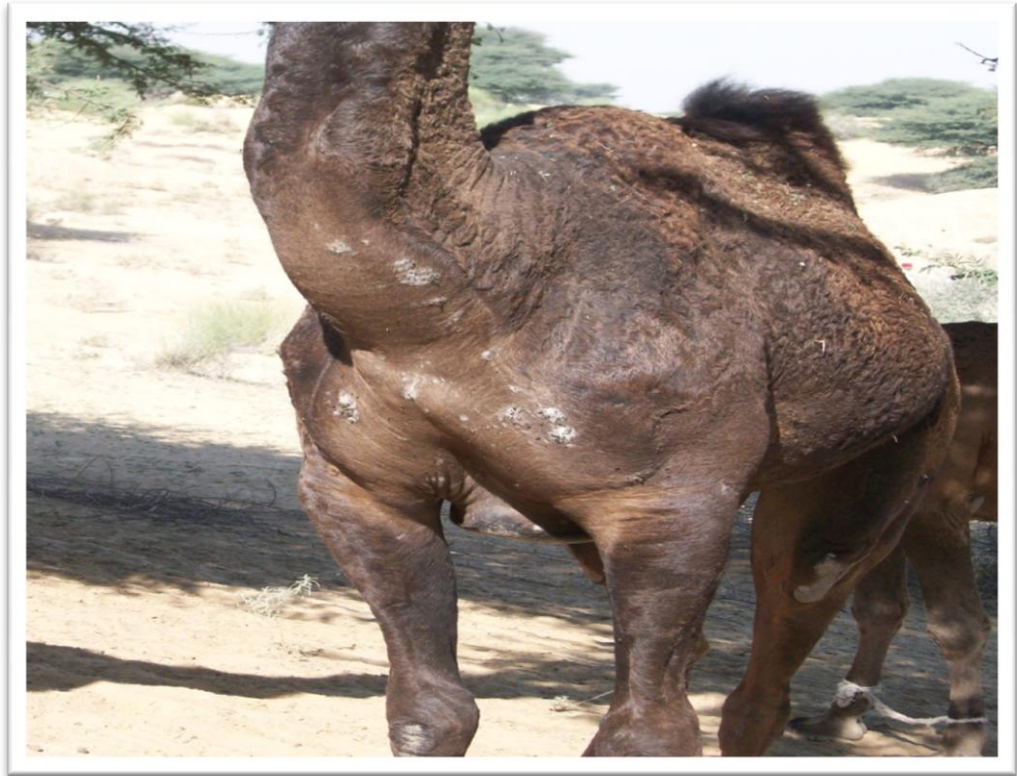


Fig. 11 Photograph showing lesions of *Trichophyton rubrum* on chest



Fig. 12 Photograph showing lesions of *Candida albicans* on legs

4.2 Identification of fungal agent by culture examination

Identified fungal agents from dermal mycoses affected camels are as follows.

Table.2 Relative frequency of different fungal organism

S.No.	Fungal organism	No. of Isolates
1.	<i>Trichophyton</i>	3
2.	<i>Aspergillus</i>	3
3.	<i>Histoplasma</i>	2
4.	<i>Candida</i>	2
5.	<i>Penicillum</i>	2
6.	<i>Microsporum</i>	1
7.	<i>Coccidiodes</i>	1
8.	<i>Absidia</i>	1
9.	<i>Clodophialophora</i>	1
10.	Total	16

In present study various fungal isolates identified by culture examination were *Trichophyton* (3/16), *Aspergillus* (3/16), *Histoplasma* (2/16), *Penicillum* (2/16) *Candida* (2/16), *Microsporam*(1/16), *Clodophialophora*(1/16), *Absidia*(1/16), and *Coccidiodes* (1/16).

Table 3. Age and severity wise distribution of fungal isolates

S.No.	Age group	No. of infected animals	Severity of lesions	
			Moderate	Severe
1.	<1 year	6	2	4
2.	1-2 year	3	1	2
3.	>2 year	7	4	3

A total of 16 camels of different age group infected with dermal mycoses were divided into two groups on the basis of severity of lesions. The moderate group of animal include apparently healthy animals with less number of lesions on body, while in severe group include emaciated animals with more number of lesions were presents on various part of the body.

In the present study, dermal mycoses cases were investigated in the post rainy season. Dermatophytes grow best in warm and humid environments. Therefore rainy season coupled with temperature of the desert is most conducive for the growth of dermatophytes. Dermatophytes are keratinophilic (keratin digesting) fungi which are common inhabitants of the soil, where they possess the hairs and skin cells shed by animals, as well as all types of keratin products that fall from animals and humans during the natural and continuous cycle of skin and coat shedding. The group of keratinophilic fungi is very large, but only three genera *Microsporum*, *Trichophyton* and *Epidermophyton* are known to cause dermatophytosis and infect the keratinized tissues; hair, skin and nails (Aly *et al*, 2000; Aman *et al*, 2001; Elewski, 2000; Nweze, 2001; Roldan *et al*, 2000; Rubio-Calvo *et al*, 2001) and likewise skin, hair and claws in all the domestic animals worldwide. These organisms are called dermatophytes. *Microsporum* and *Trichophyton* are most frequently found in animals while the *Epidermatophyton* causes problems mainly in humans (Lewis *et al*, 1991). The particular ability of these fungi to be transmissible to animals, as well as to humans, signifies that they are important veterinary and human pathogens worldwide (Chretien and Garagusi, 1990).

In the present study, major dermatophytes involved in causation of dermatophytosis in camel were *Trichophyton* and *Microsporum*. Species of these dermatophytes identified were *M. canis*, *T. verrucosum*, *T. schoenleini* and *T. rubrum* (Table1)

1 *Trichophyton*

In the present study, three species of trichophyton were observed namely *Trichophyton rubrum*, *Trichophyton verrucosum* and *Trichophyton schoenleinii*.

Some of the *Trichophyton* spp. are cosmopolitan while others have a limited geographic distribution. Trichophyton has the ability to invade keratinized tissues and possess several enzymes such as acid proteinases, elastase, keratinases and other proteinases which act as virulence factors (Weitzman and Summerbell, 1995).

(i) *Trichophyton rubrum*:

In present investigation *Trichophyton rubrum* was isolated from one case (case.no.9). Macroscopically the culture show a violet to red-violet glabrous surface with radial furrows in front view of plate and a dark red brown in colour at reverse. Microscopic examination of fungal culture showed few pyriform lateral microconidia, pencil shaped macroconidia, arthroconidia produced from hyphae. Morphological character of colony and microscopic finding are similar to *Trichophyton rubrum* as described by Colin *et al.* (2013).

T. rubrum infection have been reported from cattle (Chatterjee and Sen Gupta, 1979; Sharma *et al.*, 1979; Sarkar *et al.*, 1985; Mitra, 1998) and dog (Yamada *et al.*, 1991; Ranganathan *et al.*, 1998);

Sahai and Mishra (2011) reported *T. mentagrophytes*, *T. tonsurans*, *T. verrucosum*, *T. schoenleinii* and *T. rubrum* from human cases from central India. Singal *et al.*, (2001) reported *T. violaceum*, *T. schoenleinii*, *T. tonsurans*, *T. verrucosum* and *T. mentagrophytes* from human cases from North India.



Fig.13 Photograph showing colony of *Trichophyton rubrum* in front and reverse view of plate

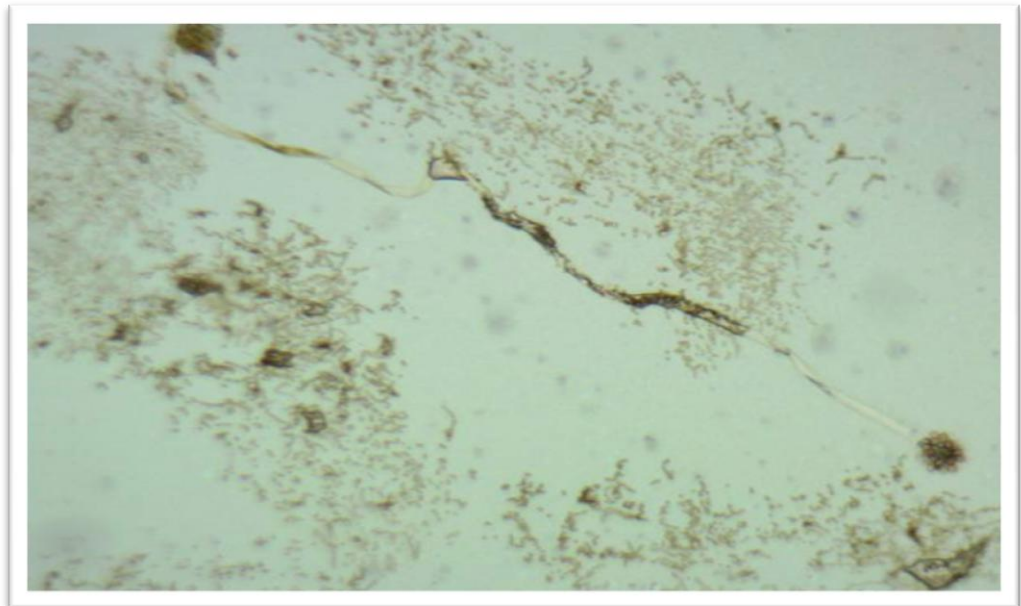


Fig.14 Microphotograph showing arthroconidia of *Trichophyton rubrum*

(ii) *Trichophyton verrucosum*:

In present study *Trichophyton verrucosum* was isolated from one case (case no. 12). On SDCA, colonies were small, button-or-disk-shaped, white to cream coloured, with a suede-like to velvety surface, a raised centre with some submerged growth in front view of plate. In reverse view, pigment vary from non-pigmented to yellow. Microscopic examination of fungal culture showed broad, irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains. The tips of some hyphae are broad and club-shaped and occasionally divided. Macroconidia are only rarely produced, but some present, has a characteristic tail or string bean shape. Morphological character of colony and microscopic finding are similar to *Trichophyton verrucosum* as described by Colin *et al.* (2013).

Infection with *T. schoenleinii* has been reported in camels (Chatterjee *et al.*, 1978). Ebrahimi *et al.* (2007) reported *T. verrucosum* and *T. tonsurans* from healthy skin coat of camels from Iran. Survey of ring worm in camels, showed over 25 per cent of young animals suffered from *T. verrucosum* infection and less than 0.5 per cent with *T. mentagrophytes* (Kuttin *et al.*, 1986). *T. verrucosum* was the primary causal agent in young camels and *T. mentagrophytes* in older camels (Mahmoud, 1993). The peak incidence of disease was autumn and winter, with incidence was highest in young growing calves (1-2 years). Lesions were observed primarily on head, neck and shoulder with frequent extension to the flanks and limbs. *T. verrucosum* was isolated as the primary causal organism (Fadlelmula *et al.*, 1993). *T. verrucosum* and *T. mentagrophytes var mentagrophytes* was the common cause of dermatophytosis in alpacas and llamas. Spores of *T. verrucosum* and *T. mentagrophytes* may remain viable for up to 4 - 5 years in hair and cellular debris scraped off the animal and left attached to barn walls, fence posts, trees and other fixtures, blankets, leads, grooming apparatus *etc.*

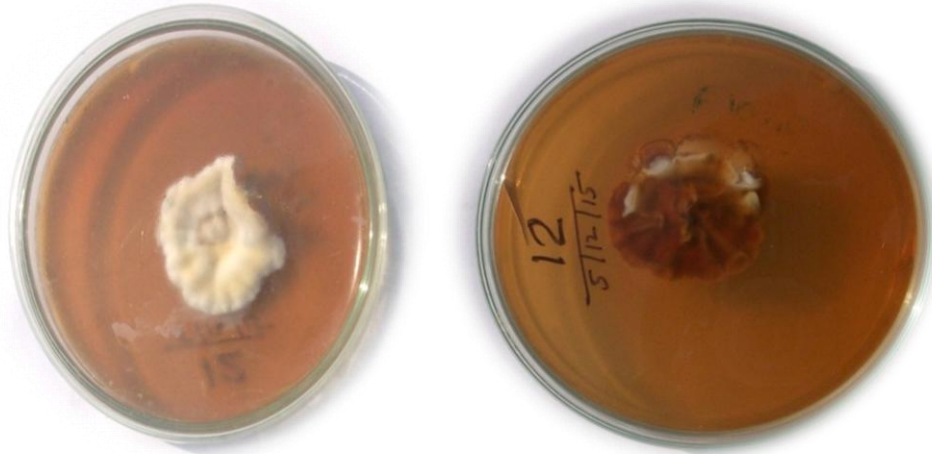


Fig.15 Photograph showing colony of *Trichophyton verrucosum*.in front and reverse view of plate

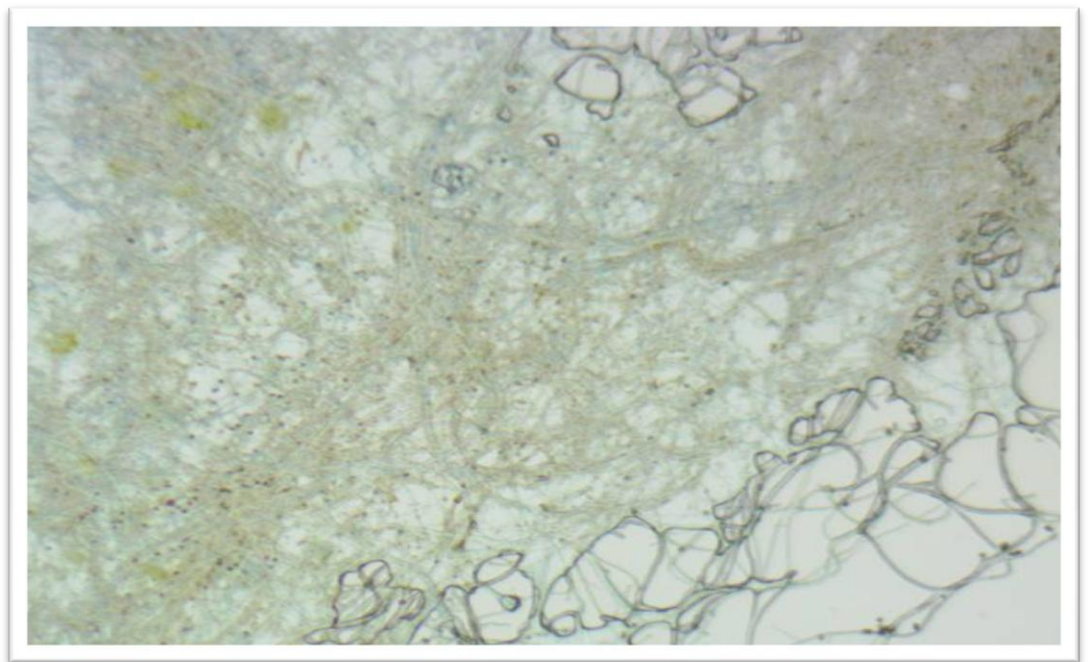


Fig.16 Microphotograph showing hyphae and macroconidia of *Trichophyton verrucosum*

T. verrucosum infection have been reported from cattle (Malik *et al.*, 1984; Sarkar *et al.*, 1985; Pal, 1987; Quinn *et al.*, 1994), buffalo (Thakur *et al.*, 1983; Pal and Singh, 1983), sheep (Chatterjee and Sen Gupta, 1979; Thakur *et al.*, 1983), goat (Gupta *et al.*, 1970; Chatterjee and Sen Gupta, 1979; Pal and Singh, 1983) and dog (Chatterjee and Sen Gupta, 1979)

(iii) *Trichophyton schoenleinii* :

In present study *Trichophyton schoenleinii* was isolated from one case (case no. 13). On SDCA, colonies were slow growing, waxy or suede-like with a deeply folded honey-comb-like thallus and some sub-surface growth. The thallus was cream coloured to yellow to orange brown in front view of plate. Cultures are difficult to maintain in their typical convoluted form and rapidly become flat and downy. There was no pigmentation in reverse view.

Microscopic examination of fungal culture showed no macroconidia and microconidia in routine cultures; however numerous chlamydoconidia were present. Characteristic antler hyphae with swollen nail head tips present. Morphological character of colony and microscopic finding are similar to *Trichophyton schoenleinii* as described by Maertens and Marr (2007) and Versalovic *et al.* (2011).

Ringworm is more common in tropical than temperate climates particularly in countries having hot and humid climatic condition (Pascoe, 1976). Calves at weaning time are highly susceptible to ringworm infection (Al-Ani *et al.*, 2002). This may be in part due to their weak immunity and the high pH of the skin (Radostits *et al.* 1997). Susceptibility to dermatophytosis depends also on the general health status of animals. Young animals are more infected than adults, which could be related to the development of a stronger immunity in older animals due to the multiplicity of contacts with the fungus rather than an intrinsic role of age. For example, the strong immune response elicited by *T. verrucosum* may explain the common occurrence of ringworm in calves and young cattle and its rarity in adults.



Fig.17 Photograph showing colony of *Trichophyton schoenleinii* in front and reverse view of plate

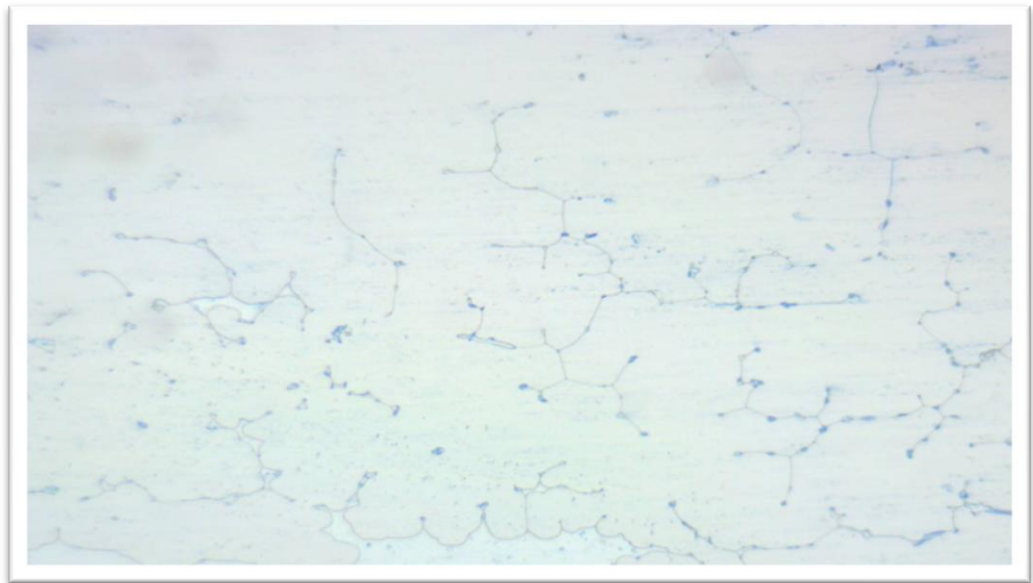


Fig.18 Microphotograph showing hyphae with swollen nail head tips of *Trichophyton schoenleinii*

2. *Microsporium canis* :

In present investigation *Microsporium canis* was isolated from one case (case no. 14). Macroscopically colonies on SDCA were flat, spreading, white to cream-coloured with a dense cottony surface which may show some radial grooves in front view of plate. Colonies usually have a bright golden yellow to brownish yellow pigment in reverse view. Microscopic examination of fungal culture showed that macroconidia were typically spindle-shaped with 5-15 cells, verrucose, thick-walled and often have a terminal knob. A few pyriform to clavate microconidia were also present.

Morphological character of colony and microscopic finding are similar to *Microsporium canis* as described by Colin *et al.* (2013).

Most of the *Microsporium* species are widely distributed in the world while some have restricted geographic distribution. *Microsporium* has the ability to degrade keratin and thus can reside on skin and its appendages and remains non-invasive. The keratinases, proteinases and elastases of the fungus may act as virulence factors. Infection with *M. canis* has been reported in camel (Tuteja *et al.*, 2013a) cattle (Pal and Singh, 1983) and dog (Khan *et al.*, 1982; Chittawar and Rao, 1982; Malik, *et al.*, 1984). Infection with *M. gypseum* has been reported in camels (Gitao *et al.*, 1998).

In camels *Microsporium* and *Trichophyton* fungi were found, causing sporadic cases of skin infections in individually maintained camels.

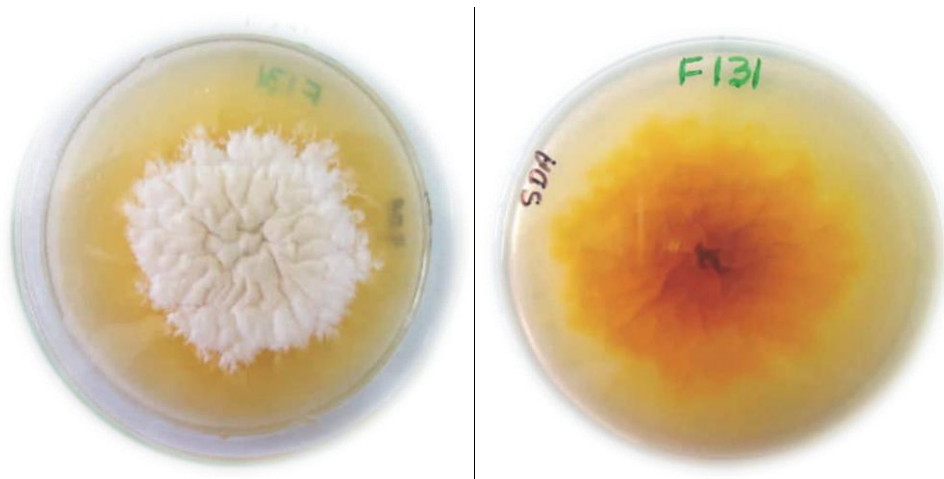


Fig. 19 Photograph showing colony of *Microsporium canis* in front and reverse view of plate

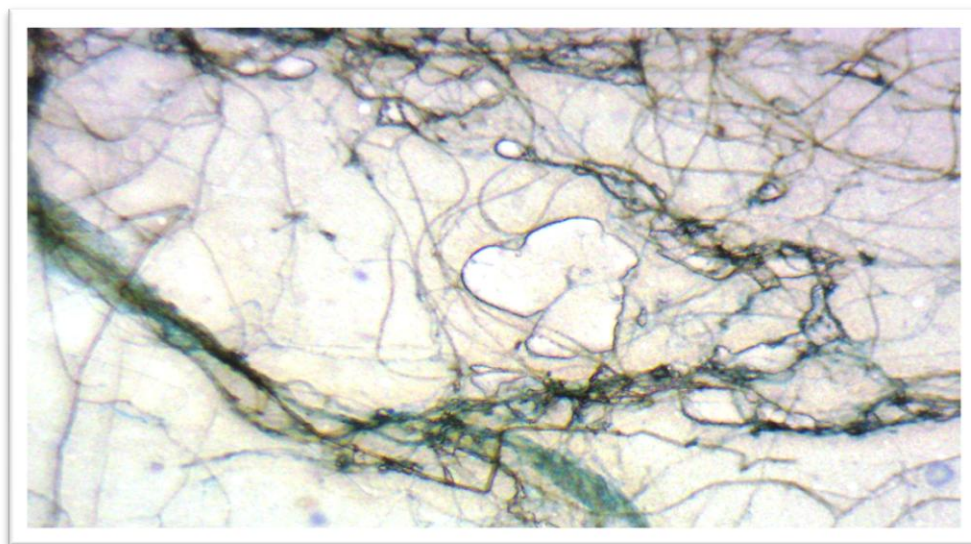


Fig.20 Microphotograph showing macroconidium, intercalary chlamydoconidium of *M. canis*

As transmission of ringworm occurs through direct contact with infected animals or indirectly from contaminated fomites, all circumstances that favour those contacts act as predisposing factors. It explains a higher occurrence of ringworm in animals which are confined in catteries, kennels, stables, cowsheds or intensive breeding units. In addition, the high resistance of the dermatophyte conidia for months or years in the environment explains why the use of material that is shared between animals for grooming, harnessing or transportation favors the contamination. Interestingly, the prevalence of equine ringworm was higher in training farms than among breeding horses and a majority of lesions were located on the girth area (Pascoe, 1976). For geophilic dermatophytes, the soil is the reservoir in which the fungi multiply. Thus, the risk of contamination is higher for animals with outdoor contacts. It was interesting to know that *Histoplasma*, *Candida*, *Cladophialophora* and *Absidia* were infecting very young camels of approximately one year or lesser in age (Table.1). This may be in part due to their weak immunity and the high pH of the skin (Radostits *et al.* 1997).

3. *Histoplasma capsulatum*:

In present investigation, *Histoplasma capsulatum* was isolated from two cases (case no. 1 and 6). *Histoplasma capsulatum* exhibits thermal dimorphism growing in living tissue or in culture at 37°C as a budding yeast-like fungus and in soil or culture at temperatures below 30°C as a mould. Colonies on SDCA were flat, spreading, irregular white or buff-brown, suede-like to cottony in front view of plate and pale yellow-brown in reverse view. Microscopic morphology shows the presence of characteristic large, rounded, single-celled, tuberculate macroconidia formed on short, hyaline, undifferentiated conidiophores. Small, round to pyriform microconidia, borne on short branches or directly on the sides of the hyphae may also be present.

Morphological character of colony and microscopic finding are similar to *Histoplasma capsulatum* as described by Colin *et al.* (2013).

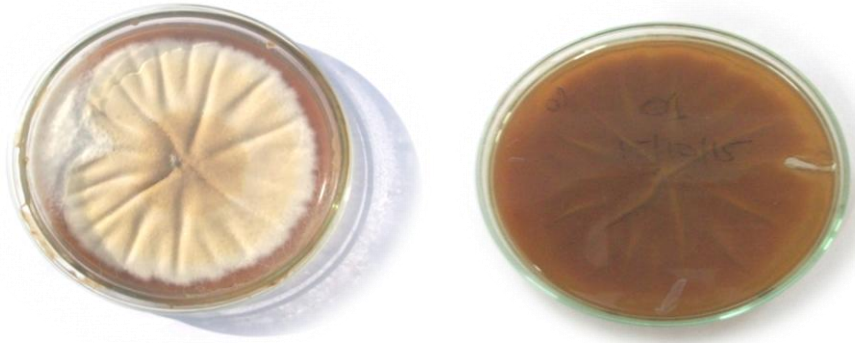


Fig. 21 Photograph showing colony of *Histoplasma capsulatum* in front and reverse view of plate

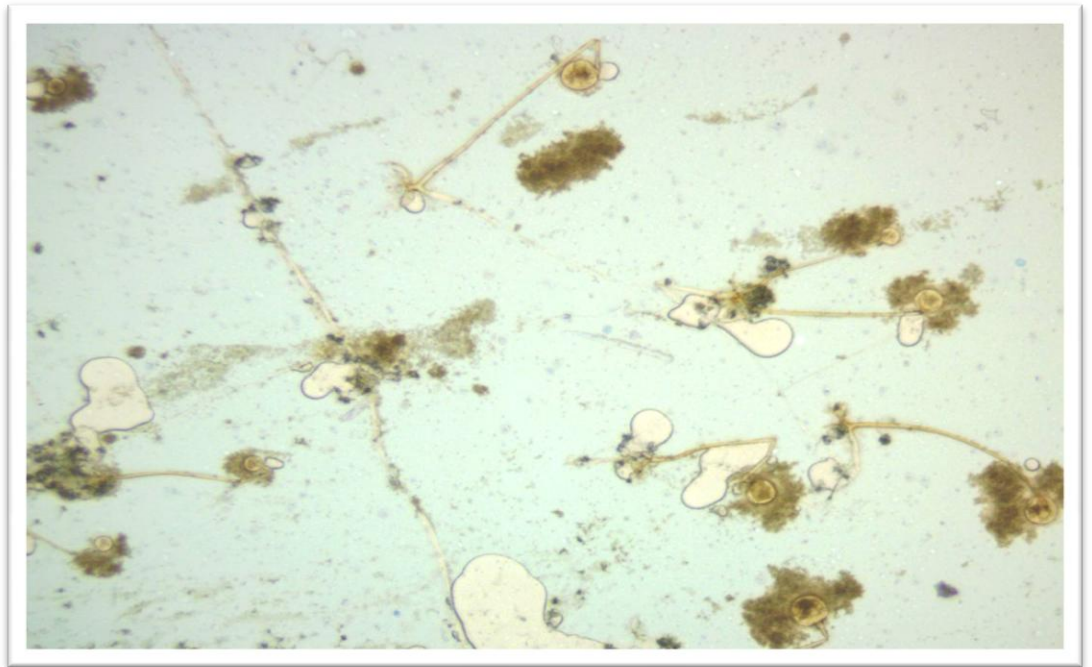


Fig. 22 Microphotograph showing Large, rounded, single-celled, capsulated macroconidia and small microconidia of *H. capsulatum*

Histoplasma capsulatum and *Coccidioides immitis* the dimorphic fungi had been isolated from camel skin infections (Table.1). *H. capsulatum* had been reported to cause miliar necroses of the lungs in dromedary camel (Chandel and Kher, 1994). Tuteja *et al* (2014) found *C. immitis* causing skin infection in dromedary camel.

Histoplasmosis and Coccidioidomycosis are systemic mycoses caused by these dimorphic fungi *Coccidioides* spp. and *H. capsulatum*, respectively, which affect humans and a variety of other animals, including equines (Brilhante *et al.* 2016). These fungi can cause disease even in immunocompetent individuals. The dimorphism presented by these pathogens is considered one of the most relevant virulence factors in the pathogenesis of these fungi. The establishment of these pathogens in the host directly depends on the conversion of the filamentous phase to the parasitic yeast phase (Nemecek *et al.* 2006).

4. *Candida albicans* :

In the present investigation, *Candida albicans* was isolated from two cases (case no. 15 and 16). Skin candidiasis is an acute and contagious fungal infection of camel calves of less than one year of age. The infection affects almost every young calf in that particular herd. It is also called '*thikria*' by camel keepers.

Macroscopically colonies on SDCA were appearing glistening and smooth with white cream to brown colour in front view of plate. Microscopically branched pseudohyphae with dense verticils of blastoconidia. Spherical chlamydospores, mostly terminal, often on a slightly swollen subtending cell, are formed near the edge of the cover slip.

Morphological character of colony and microscopic finding are similar to *Candida albicans* as described by Colin *et al.* (2013).

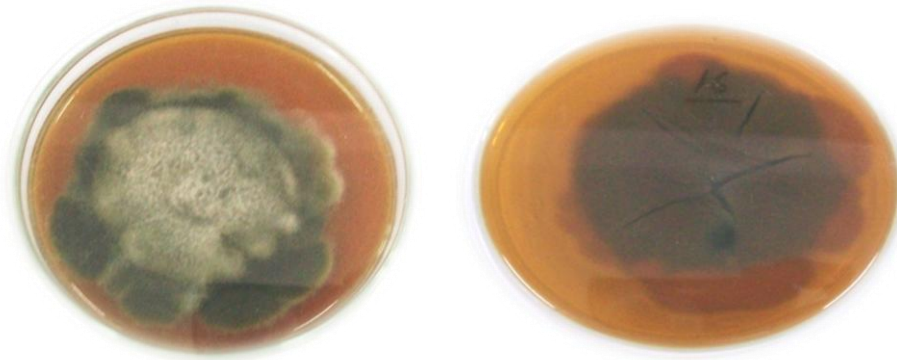


Fig. 23 Photograph showing colony of *Candida albicans* in front and reverse view of plate

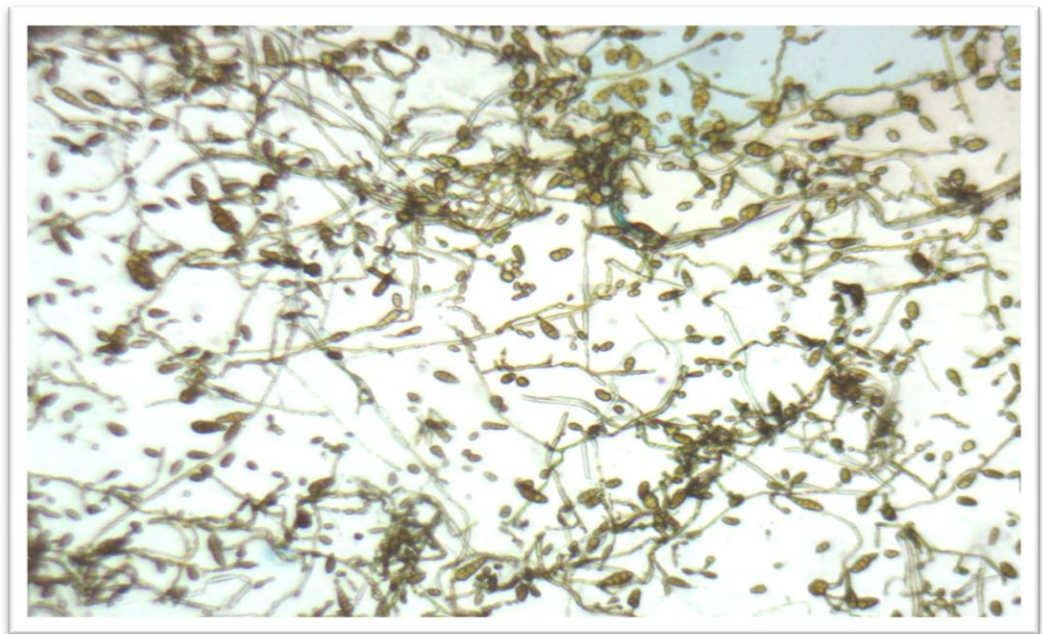


Fig. 24 Microphotograph showing chlamydospores at tip of hyphae of *Candida albicans*

Wernery *et al* (2007) repeatedly cultured *Candida albicans* from skin scrapings of very young camel calves. The physical condition of dromedary calves was affected; they were smaller and weighed less than calves in the same age group. Similar occurrence of skin candidiasis in young camel calves has been reported from India with almost similar signs and symptoms (Tuteja *et al.*, 2010a; 2012).

5. *Cladophialophora bantiana*:

In the present investigation *Cladophialophora bantiane* was isolated from one case (case no. 7). Macroscopally the colony of the *Clodophialophora bantiana* was heaped, often irregularly folded in center with powdery to felt like texture. Colour of the culture was pale olive-green in front and olive black on reverse.

Microscopic examination of fungal culture showed the conidia were pale enlogated with truncated or pointed ends formed in long rarely branched chains that usually intact on mounting. Morphological character of colony and microscopic finding are similar to *Clodophialophora bantiana* as described by Colin *et al.* (2013).

Cladophialophora bantiana caused equine endometritis in a 15-year-old mare (Rantala *et al.*, 2015) and Eumycetoma in a 3-year-old male dog (Guillot *et al.*, 2004).

Cladophialophora bantiana frequently encountered in human infections, ranging from mild cutaneous lesions to fatal encephalitis (Badali *et al.*, 2008)

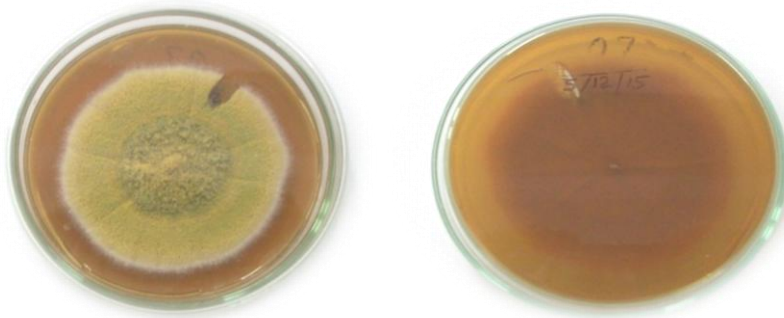


Fig. 25 Photograph showing colony of *Cladophialophora bantiana* in front and reverse view of plate

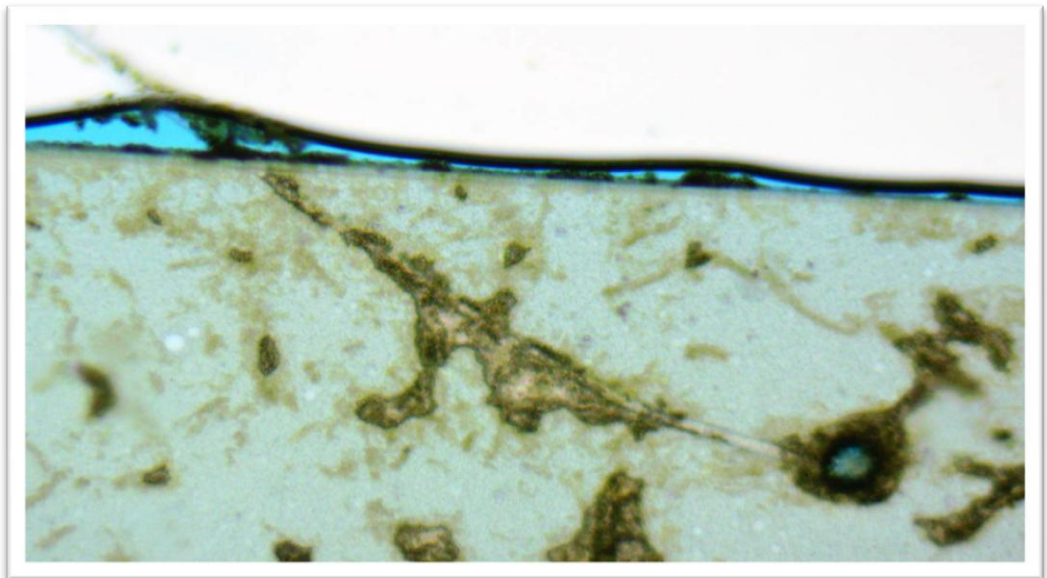


Fig. 26 Microphotograph showing elongated conidia *Cladophialophora bantiana*

6. *Absidia corymbifera*:

In the present investigation *Absidia corymbifera* was isolated from one case (case no. 4). *Absidia* are filamentous fungi that are cosmopolitan and ubiquitous in nature as common environmental contaminants. They often cause food spoilage. *Absidia* currently contains 21 species mostly they are soil-borne. *Absidia corymbifera* is the only species known to cause disease in man and animals. *Absidia* is characterized by a differentiation of the hyphae into arched stolons bearing more or less verticillate sporangiophores at the internode and rhizoids formed at the point of contact with the substrate. This feature separates species of *Absidia* from the genus *Rhizopus* where the sporangia arise from the nodes and are therefore found opposite the rhizoids. *A. corymbifera* grows rapidly.

Macroscopically the culture showed flat, woolly to cottony and olive grey colony in front view of plate, which were mature within 4 days.

Microscopic examination of fungal culture showed wide non septate hyphae. The sporangiophores were branched and arise in groups of 2-5 at the internodes. Sporangiophores carried pyriform, relatively small sporangia. Morphological character of colony and microscopic finding are similar to *Absidia corymbifera* as described by Colin *et al.* (2013).

A. corymbifera causing skin infection in camel has also been reported by Tuteja *et al.* (2013). *A. corymbifera* do cause skin infection in horses (Lopez *et al.*, 2000). It is very rarely observed in immunocompetent host (Hagensee *et al.*, 1994; Ribes *et al.*, 2000).

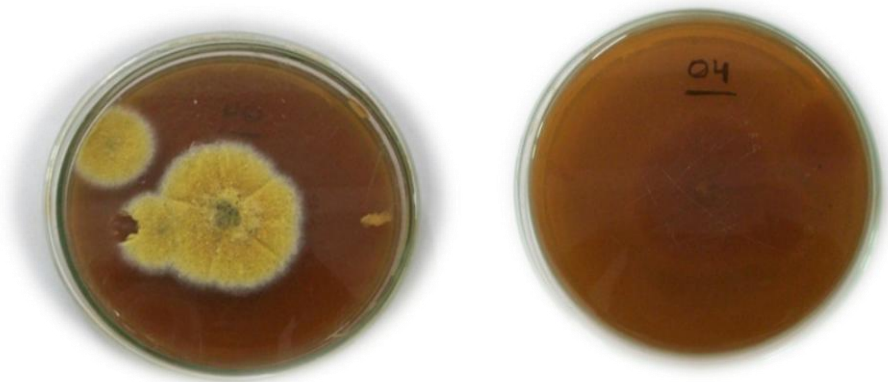


Fig. 27 Photograph showing colony of *Absidia corymbifera* in front and reverse view of plate.

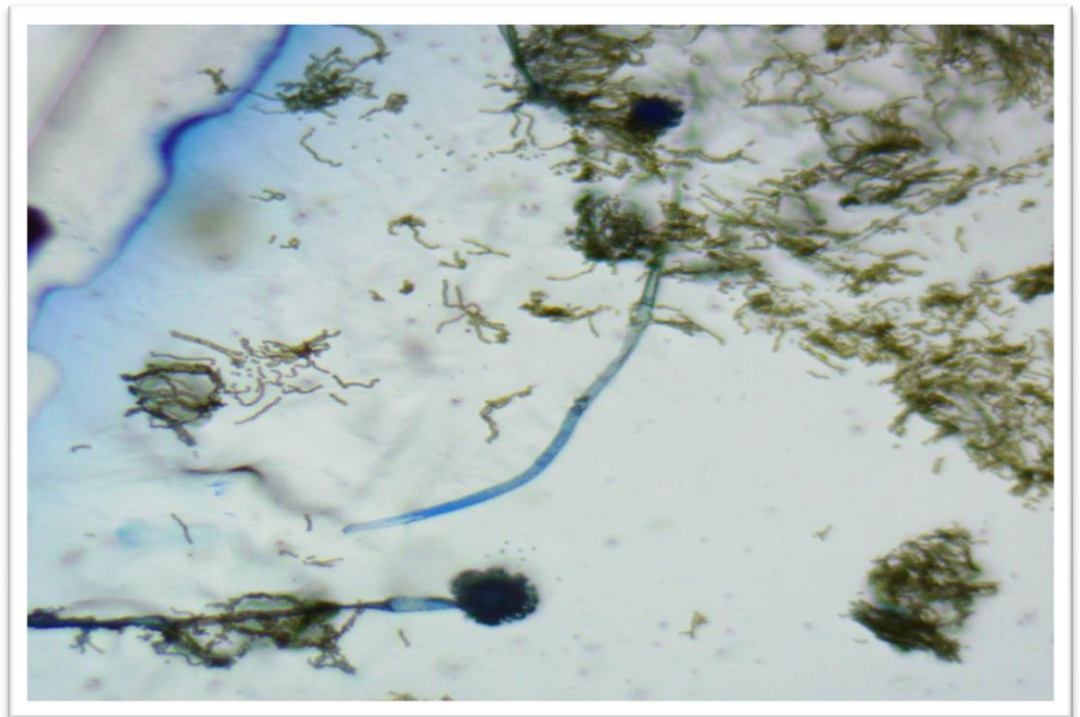


Fig. 28 Microphotograph showing sporangium filled with sporangio spores *Absidia corymbifera*

7. *Coccidioides immitis*:

In present investigation *Coccidioides immitis* was isolated from one case (case no. 8). *Coccidioides* are thermally dimorphic fungi found in soil particularly at warm and dry areas with low rain fall, high summer temperatures and low altitude. Macroscopically colonies were moist, glabrous, membranous, white and grayish initially, later producing white and cottony aerial mycelium in front view of plate, with age, colonies become tan to brown in colour.

Microscopic examination of fungal culture showed that morphology varies depending upon the incubation temperature. at 25°C, hyphae and arthroconidia were produced. Arthroconidia were thick-walled, barrel shaped. These arthroconidia alternate with empty disjuncture cells. At 37°C, large, round, thick-walled spherules filled with endospores were observed. Morphological character of colony and microscopic finding are similar to *Coccidioides immitis* as described by Colin *et al.* (2013).

Coccidioides spp. are associated with arid and semi arid regions, which have an average temperature above 30°C, recurrent droughts, and poor and sparse xerophytic vegetation (Paixão *et al.* 2004). These fungi are the soil saprophytes; contact of injured skin with material contaminated by the mycelial form of the pathogen is the main route of infection (Ameni, 2006). In equines, the cases of coccidioides described traditionally are severe and often fatal (Higgins and Pusterla, 2006). The clinical presentations include cutaneous lesions (Drutz and Huppert, 1983).

Coccidioidomycosis is one of the true systemic mycoses (Galgiani, 1999). Inhalation of the dry arthroconidia of *C. immitis*, which are carried by dust storms, initiates the infection. Hematogenous spread of the organism results in infection of skin, bones, joints, lymph nodes, adrenal glands and central nervous system (Ampel *et al.*, 1986; Bayer and Guze, 1979; Blair and Logan, 2001).



Fig. 29 Photograph showing colony of *Coccidioides immitis* in front and reverse view of plate

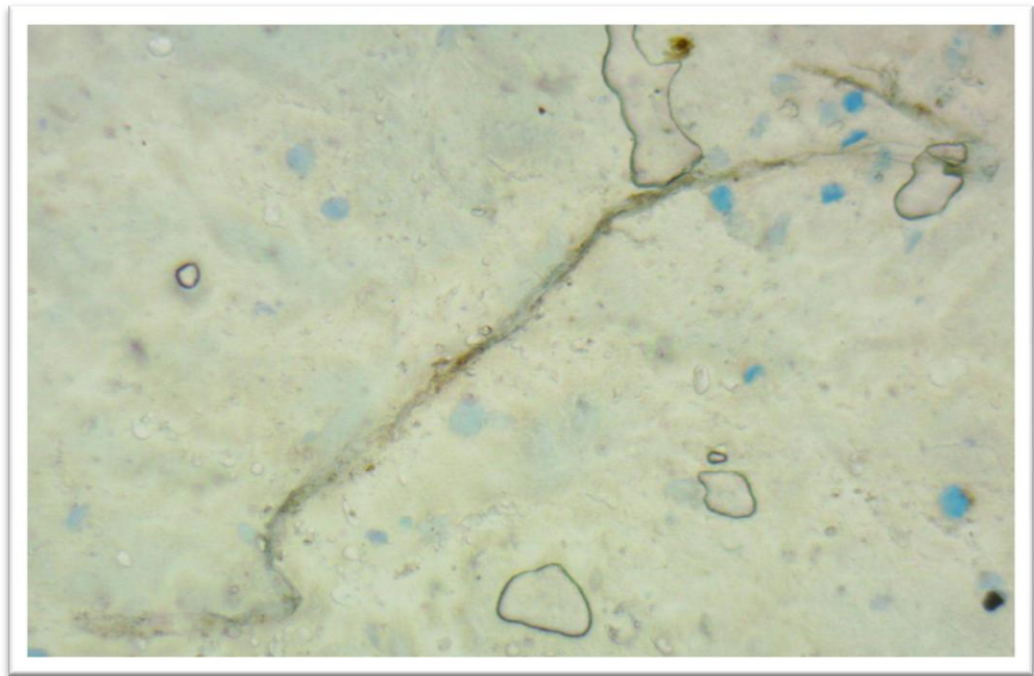


Fig. 30 Microphotograph showing arthrospore formation in the hyphae of *Coccidioides immitis*

8. *Penicillium spp.:*

In present investigation *penicillium spp* was isolated from two cases (case no. 10 and 11). The colonies were rapid growing, flat, filamentous, velvety, woolly or cottony. Colours of the colonies were initially white and become blue green, gray, olive gray to dark black in front view of plate. Reverse view of colony is usually pale to yellowish. Microscopic examination of fungal culture showed septate hyaline hyphae, simple or branched conidiophores, metulae, phialides and conidia are observed. Metulae are secondary branches that form on conidiophores.

Morphological character of colony and microscopic finding are similar to *penicillium spp* as described by Colin *et al.* (2013); Maertens and Marr (2007) and Versalovic *et al.* (2011).

Penicillium species are known to produce mycotoxins (Pitt *et al*, 2000). *Penicillium* has been isolated from patients with keratitis (Deshpande and Koppikar, 1999), endophtalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, and urinary tract infections. Most *Penicillium* infections are encountered in immunosuppressed hosts. Corneal infections are usually post-traumatic (Deshpande and Koppikar, 1999). In addition to its infectious potential, *P. verrucosum* produces a mycotoxin, ochratoxin-A, which is nephrotoxic and carcinogenic. The production of the toxin usually occurs in cereals at cold climate. (Pitt, 2000).

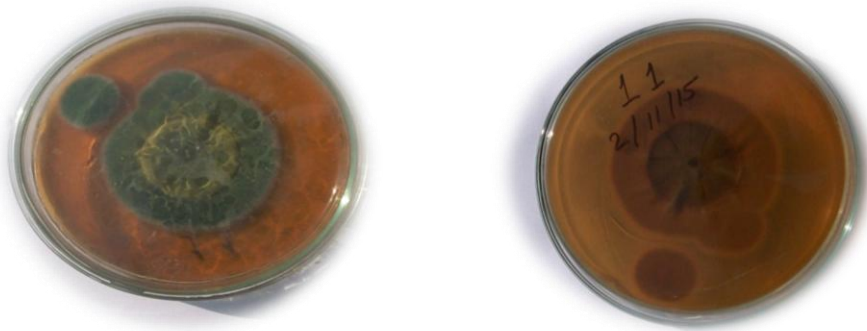


Fig. 31 Photograph showing colony of *penicillium spp* in front and reverse of plate

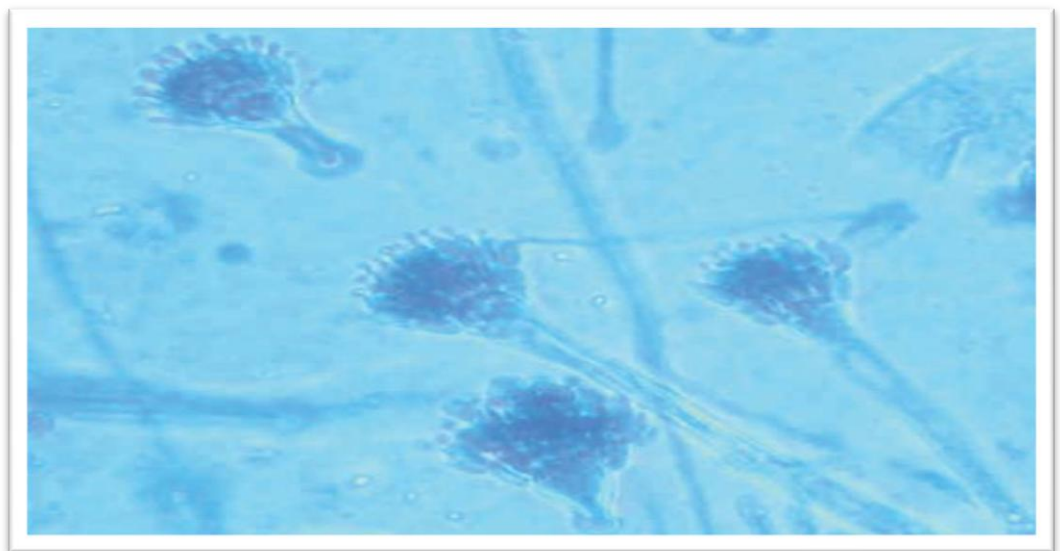


Fig. 32 Microphotograph showing hyphae, conidiospore and phialides of *penicillium spp*

9. ***Aspergillus*:**

In present study filamentous fungi identified were *Aspergillus* and *Penicillium* species. Various species of *Aspergillus* identified were *A. terreus*, *A. versicolor* and *A. niger*. Aspergillosis has been reported in calf affecting the skin (Pal, 1956). The occurrence of fungi in lymph nodes of domestic buffaloes (*Bubalis bubalis*) subjected to slaughter was investigated. *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus* were isolated (Pal and Ragi, 1989). *A. terreus*, *A. nidulans* and *A. flavus* were isolated from the respiratory tract of buffaloes, goats and sheep (Singh and Singh, 1970). *A. niger* has also been associated with dermatitis (Pal *et al*, 1987). *A. terreus* was isolated from a mycetoma case in a dog (Pal and Verma, 1987). Pal *et al* (2007) reported a case of otitis in camel due to *A. flavus*.

(i) ***Aspergillus terreus***

In present investigation *Aspergillus terreus* was isolated from one case (case no. 2). *Aspergillus* is a filamentous and ubiquitous fungus. The major macroscopic features remarkable in species identification are the growth rate, colour of the colony and thermo tolerance. *Aspergillus* colonies were downy to powdery. The surface colour may vary depending on the species. The reverse was uncoloured to pale yellow in most isolates.

Macroscopically colour of the colony was cinnamon to brown in front view of plate and in reverse view colour is white to brown. Microscopic examination of fungal culture showed conidiophore were short, smooth, colourless, biserial phialides and vesicle is round with compact columnar head. Conidia are rounded, 2 μ m in diameter and smooth.

Other *Aspergillus* spp. with metulae, but the columnar appearance of the spring heads and the small conidia should help to differentiate this species complex from the others. Morphological character of colony and microscopic finding are similar to *Aspergillus terreus* as described by Colin *et al*. (2013).

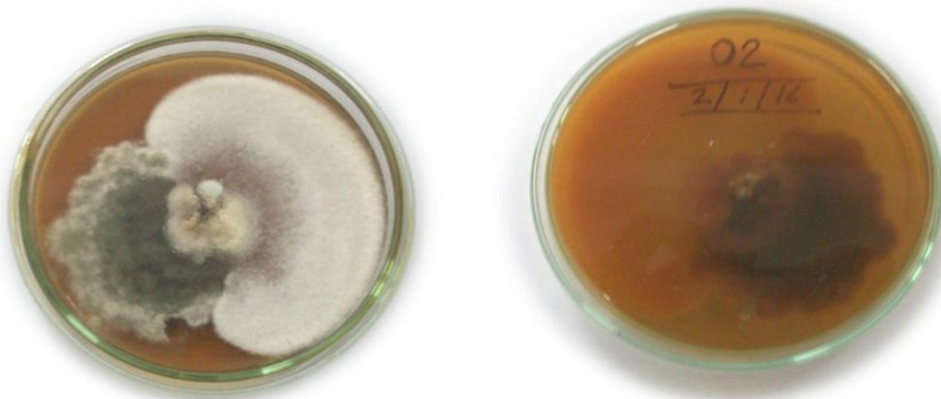


Fig. 33 Photograph showing colony of *Aspergillus terreus* in front and reverse view of plate

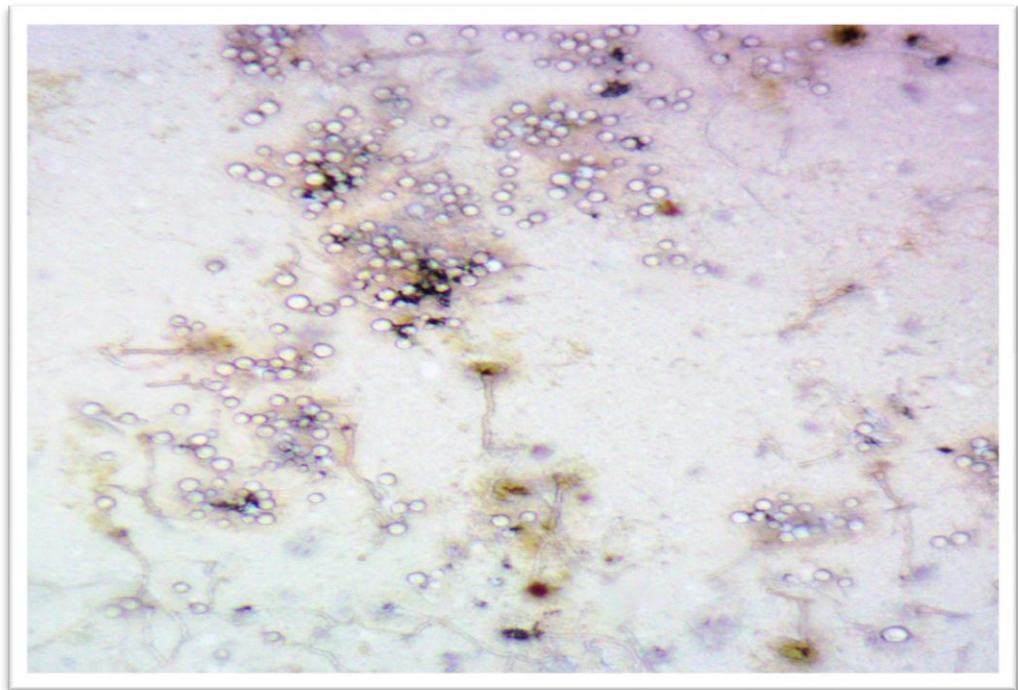


Fig. 34 Microphotograph showing rounded Conidia of *Aspergillus terreus*

Aspergillus is a filamentous and ubiquitous fungus. *Aspergillus* includes over 185 species. Some of these species has been reported as causative agents of opportunistic infections in man and animals. Among these *A. fumigatus* is the most commonly isolated species, followed by *A. flavus*, *A. niger*, *A. terreus*, and *A. versicolor* are isolated as opportunistic pathogens in camels (Tuteja *et al.* 2013c)

Aspergillus is well-known to play a role in three different clinical settings in man by opportunistic infections, allergic states and toxicoses. Immunosuppression is the major factor predisposing to development of opportunistic infections (Ho and Yuen, 2000). These infections may present in a wide spectrum, varying from local involvement to dissemination and as a whole called aspergillosis. Almost any organ or system in the human body may be involved like cutaneous aspergillosis (Arikan *et al.*, 1998), osteomyelitis (Flynn *et al.*, 1990), pulmonary aspergillosis (Gefter, 1992), fungemia (Duthie and Denning, 1995), rhinosinusitis (Gillespie and O'Malley, 2000), otitis (Gordon and Giddings, 1994; Harley *et al.*, 1995), endocarditis (Gumbo *et al.*, 2000), onychomycosis (Gupta and Summerbell, 1999), myocarditis (Rouby *et al.*, 1998), meningitis (Mikolich *et al.*, 1996), sinusitis (Drakos *et al.*, 1993).

(ii) *Aspergillus niger*:

In present investigation *Aspergillus niger* was isolated from one case (case no. 3). Macroscopically topography are flat, colour of the colony was black and reverse colour was white to yellow with granular texture., Microscopically conidiophore are thick walled long, smooth, colourless or brown, biserial phialides and vesicle is round with radiate head. Morphological character of colony and microscopic finding are similar to *Aspergillus niger* as described by Colin *et al.* (2013).



Fig. 35 Photograph showing colony of *Aspergillus niger* in front and reverse view of plate

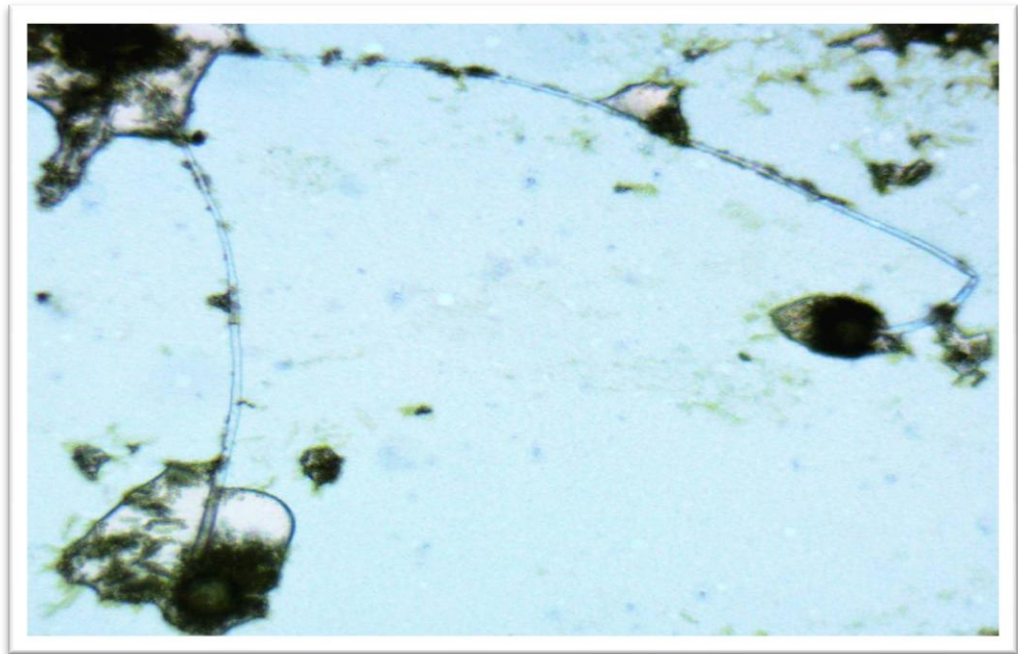


Fig. 36 Microphotograph showing round radiate head vesicle of *Aspergillus niger*

Cutaneous aspergillosis may be primary or secondary. Primary cutaneous aspergillosis refers to cases in which the initial infection begins in the skin due to direct inoculation, whereas secondary cutaneous aspergillosis is due to haematogenous seeding or spread from contiguous structures. The clinical manifestations of primary cutaneous aspergillosis were variable and non-specific. Typically, the lesions begin as erythematous, violaceous indurated plaques which progress to necrotic ulcers with central eschar formation (Prystowsky *et al*, 1976; Magid *et al*, 1988). Less commonly, hemorrhagic vesicles or bullae, subcutaneous nodules, granulomas, pustular lesions and vegetating plaques are observed (Jones *et al*, 1986; Van Burik *et al*, 1998; Isaac, 1996; Miele *et al*, 2002). Secondary cutaneous aspergillosis refers to cases in which skin involvement arising due to hematogenous seeding is a well recognized feature of disseminated aspergillosis and usually occurs in the setting of significant immunological impairment. Typically there are multiple skin lesions in anatomically unrelated sites. A variety of clinical appearances similar to primary cutaneous aspergillosis are described, including erythematous to violaceaeous nodules, plaques and papules with necrotic ulcerative and suppurative tendencies (Findlay *et al*, 1971; Gercovich *et al*, 1975).

(iii) *Aspergillus versicolor*:

In present investigation, *Aspergillus versicolor* was isolated from one case (case no. 5). Macroscopically colour of the colony was white at the beginning, turns to yellow, tan, pale green or pink in front view of plate and in reverse view colour was white to yellow or purplish red. Microscopic examination of fungal culture showed conidiophores were long, smooth, colourless, biseriate phialides and vesicle is round with loosely radiate head. Morphological character of colony and microscopic finding are similar to *Aspergillus versicolor* as described by Colin *et al*. (2013).



Fig. 37 Photograph showing colony of *Aspergillus versicolor* in front and reverse view of plate

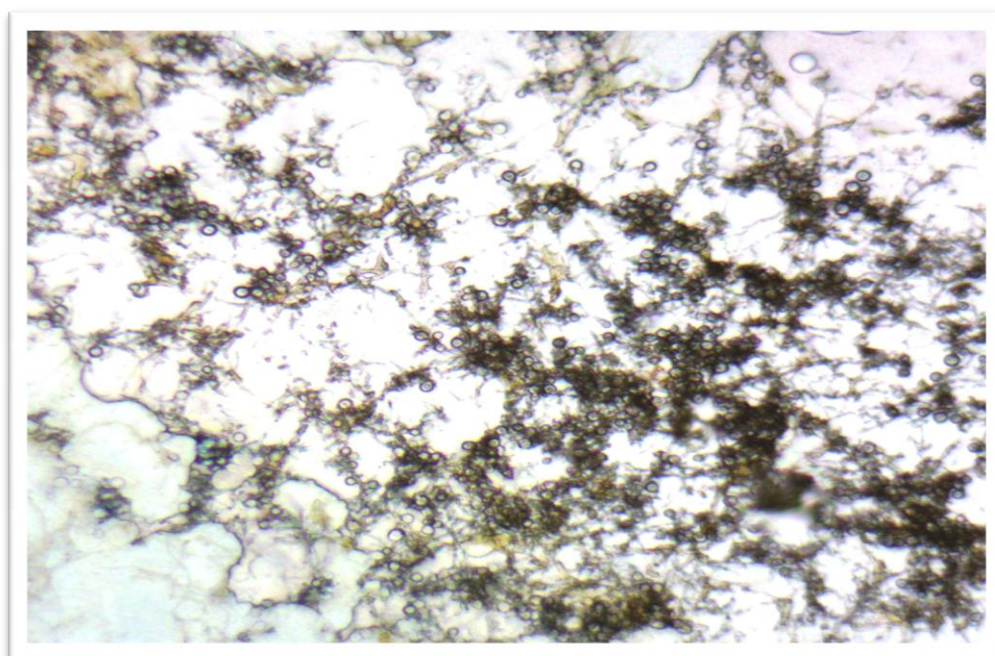


Fig. 38 Microphotograph showing spores and hyphae of *Aspergillus versicolor*.

4.3 Haemato-biochemical parameters:

The mean \pm SE values of TEC, haemoglobin, PCV, TLC and Differential leukocyte count of dermal mycoses cases of camels, before and after treatment is presented in Table 4.

Table 4: Mean \pm SE blood picture of camels before and after treatment

Parameter	Pre- treatment	Post- treatment
Erythrogram		
RBCs ($10^6/ \text{mm}^3$)	9.9 \pm 0.33	10.0625 \pm 0.25
HB (gm %)	9.0625 \pm 0.31	9.2375 \pm 0.27
PCV (%)	30.5625 \pm 1.10	30.9375 \pm 0.95
TLC ($10^3/\text{mm}^3$)	12.4375 \pm 0.87	11.75 \pm 0.30
Differential leukocytic count ($10^3/\text{mm}^3$)		
Neutrophils (%)	64.125 \pm 1.96	62.1875 \pm 1.35*
Monocytes (%)	1.8125 \pm 0.19	1.9375 \pm 0.11
Lymphocyte (%)	30.188 \pm 1.73	31.75 \pm 1.29*
Esinophil (%)	3.874 \pm 0.56	4.125 \pm 0.54

* Mean differ significantly ($P \leq 0.05$).

Analysis of haematological values by Parired T test, revealed that neutrophil and lymphocytic count varied significantly. Whereas total erythrocyte count, Hemoglobin concentration, packed cell volume, total leucocytes count, monocytic count and eosinophilic count pre and post treatment did not varied significantly (Table.3) ($P \leq 0.05$). Significant variation in neutrophils and lymphocytes only may be due to the fact that secondary bacterial infections might have invaded the discontinued integrity of the skin caused by the fungal lesions. Foutah *et al.* (2012) reported a significant reduction of erythrocyte count,

haemoglobin content and packed cell volume. Beside this, leukocytosis, neurophilia, lymphocytosis and insignificant decrease in monocytes and eosinophils were observed in camels infected with ringworm. They were of the opinion that ringworm in camels induces some adverse effect on haemato-biochemical parameters. Mathur *et al.* (2011) observed that variations in most of the haematological parameters in dematomyoses in camels occurred within the normal physiological range.

Biochemical estimation:

Mean \pm SE values of liver enzymes and protein profile of dermal mycoses cases of camels, before and after treatment is presented in the Table 5.

Table 5: Mean Liver enzymes, protein profile of camels before and after treatment.

Parameter	Pre- treatment	Post- treatment
Liver enzymes		
AST(U/L) SGOT	35.8125 \pm 4.34	34.0625 \pm 2.57
ALT(U/L) SGPT	22.875 \pm 1.21	19.1875 \pm 1.92*
ALKP(U/L)	69.4375 \pm 16.2	54.25 \pm 11.3*
Protein profile		
T.P.(gm/dl)	6.8625 \pm 0.32	6.8125 \pm 0.18
Albumin (gm/dl)	3.65 \pm 0.20	3.75625 \pm 0.12
Globulin (gm/dl)	3.21875 \pm 0.16	2.9875 \pm 0.089
A/G (ratio)	1.134	1.2578

* Mean differ significantly (P \leq 0.05).

After recovery the values of ALKP and ALT decreased significantly as compared to values recorded before treatment while AST decreased insignificantly. Foutah *et al.* (2012) reported significant

Increase in ALT and ALKP in camels suffering with ringworm as compared to control.

Insignificant reduction in serum total protein, albumin beside insignificant increase in globulin was observed in the present study (Table.5). It has also been observed by Foutah *et al.* (2012) and Gorakh *et al.* (2006), in camels suffering with ringworm.

4.4 Estimation of serum minerals (Zn, Co, Cu, Se)

Mean \pm SE values of serum minerals profiles of dermal mycoses cases of camels before and after treatment is presented in the Table 6.

Table 6: Mean \pm SE value of serum minerals of camels before and after treatment (in PPB)

Treatment	Cu	Co	Se	Zn
Pre-treatment	1377.74 \pm 465	58.669 \pm 21.13	619.825 \pm 61.01	2082.06 \pm 97
Post-treatment	1480.46 \pm 331	73.375 \pm 26.6	758.9875 \pm 55.2*	2594.469 \pm 695

Mean* differ significantly ($P \leq 0.05$)

In the present study statistically significant increase ($P \leq 0.05$) in serum Selenium (Se) status was observed in recovered camels as compared to pre treatment infected. Insignificant increase in Copper (Cu), Cobalt (Co) and Zinc (Zn) was recorded in camels after recovery compared to pre treatment values.

Similarly significant decrease in blood Se concentration has been reported in cattle infected with dermatophytes. Insignificant decrease in Zn concentration was observed in infected camels, whereas in cattle significant decrease of this mineral has recorded due to infection with dermatophytes (Al-Qudah et al., 2010; Kojouri et al., 2009). Insignificant decrease in Co and Cu concentration occurs in infected camels. These are similar to the findings of Al-Qudah et al. (2010) and Kojouri et al. (2009) in cattle.

4.5 Treatment trial:

In all 16 camels were subjected to the therapeutic efficacy of herbal drug which has been developed at NRCC, Bikaner. The herbal medicine was applied locally at three stages at 0 day, 3rd day and on 7th day. After the 2 months of first application of drug, camels were again examined for any symptom of dermal mycoses and presence of lesions on body surface.

In all the recovered camels a total three application were required for the total recovery. The recoveries in these camels were manifested by subside clinical symptoms like itching, alopecia, crusty circular lesion. It was observed that after treatment there was no scar presented over the skin and it becomes soft and glossy, thereby improving the appearance of animal.

Efficacy of treatment by complete recovery along with age group and severity of lesions are presented in the Table 7.

Table 7: Efficacy of herbal drug for the treatment of dermal mycoses in camel.

Age group of the animals	Total no. of animals treated	Severity of the lesions		Persistence of the lesions (In months)	Efficacy of treatment		
		Moderate	Severe		Grossly complete recovery from lesions 15 th day	Mycological recovery 15 th day	Regrowth of hair at the end of the treatment (Apparently normal) 60 days
≤1 yr.	6	2	4	1-2	6/6	6/6	6/6
1-2 yr.	3	1	2	1-12	3/3	3/3	3/3
>2 yr.	7	4	3	1-12	7/7	7/7	7/7

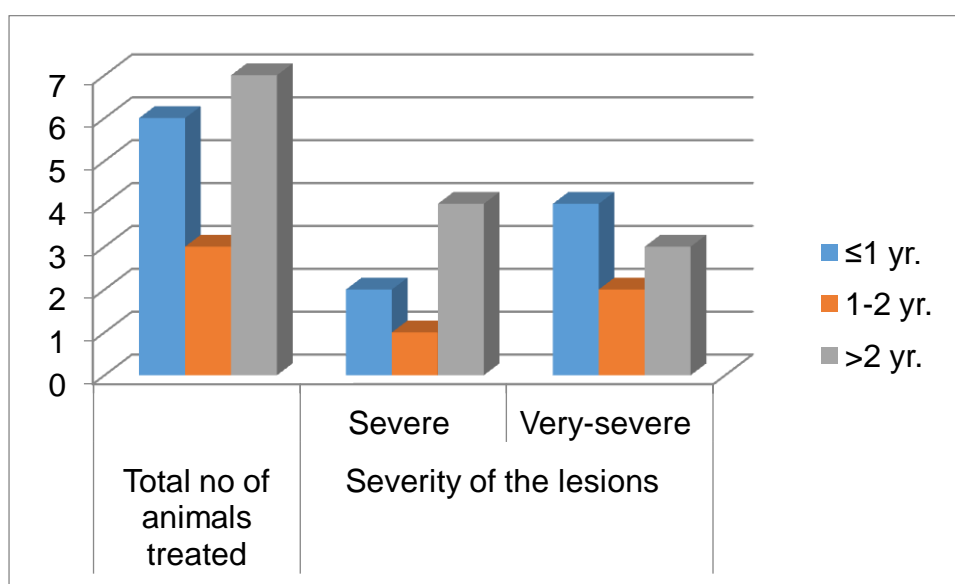


Fig. 39 Bar diagram showing severity of lesions with relation of age

In the present study, the herbal formulation under trial was found to possess broad spectrum of activity. Various ethno veterinary practices are being used for the treatment of dermal mycoses in camels. These practices include various hit and trial methods, some of them having side effects like irritation, nausea and diarrhoea. Moreover these treatments either may result in failure or do not provide broad spectrum of activity against variety of fungi causing skin infections in camels. A variety of common fungicidal and fungistatic agents such as iodine, 5% sulphur in sesame oil, 5% salicylic acid, coal tar phenols (3.25%) with copper acetate (0.58%) and hydroxyquinolines may be applied topically as ringworm ointments onto the affected areas (Wernery and Kaaden, 2002). Captan is a fungicide for ornamental plants. The use of Captan has been advocated (Ainsworth and Austwick, 1973) but lot of irritation occurs in the affected camels. Treatment of dermatophytoses with griseofulvin is very effective in cattle (Coetzer *et al.* 1994), but it causes side effects in camels such as nausea and diarrhea and is therefore not recommended (Schwartz and Dioli, 1992). Drugs used have included thiabendazole, flucytosine, and amphotericin B, but very little is known about their effect on camelids (Wernery and Kaaden, 2002). Tuteja *et al.* (2012) reported treatment of skin candidiasis in camel calves with three formulations consisting of 2% potassium iodide; 6% sulphur in mustard oil; and 6% sulphur and 3% salicylic acid in mustard oil application topically in naturally occurring cases in camel calves. All the three treatments were found effective with almost similar application schedule but with variable duration of treatment. This is long term treatment schedule with a minimum of eight applications is effective only against skin candidiasis (Tuteja *et al.* 2012).



Fig. 40 Photograph showing of *Histoplasma capsulatum* infected camel calf Before treatment



Fig. 41 Photograph showing of *Histoplasma capsulatum* infected camel calf after treatment



Fig. 42 Photograph showing of *Absidia corymbifera* infected camel calf before treatment

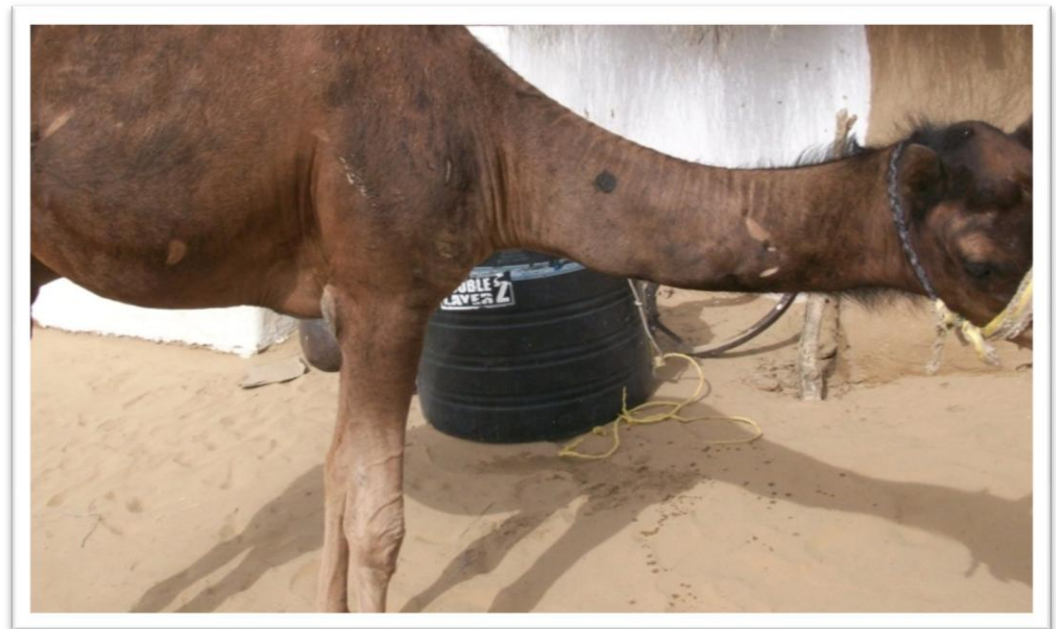


Fig. 43 Photograph showing of *Absidia corymbifera* infected camel calf after treatment



Fig. 44 Photograph showing of *Candida albicans* infected camel calf before treatment

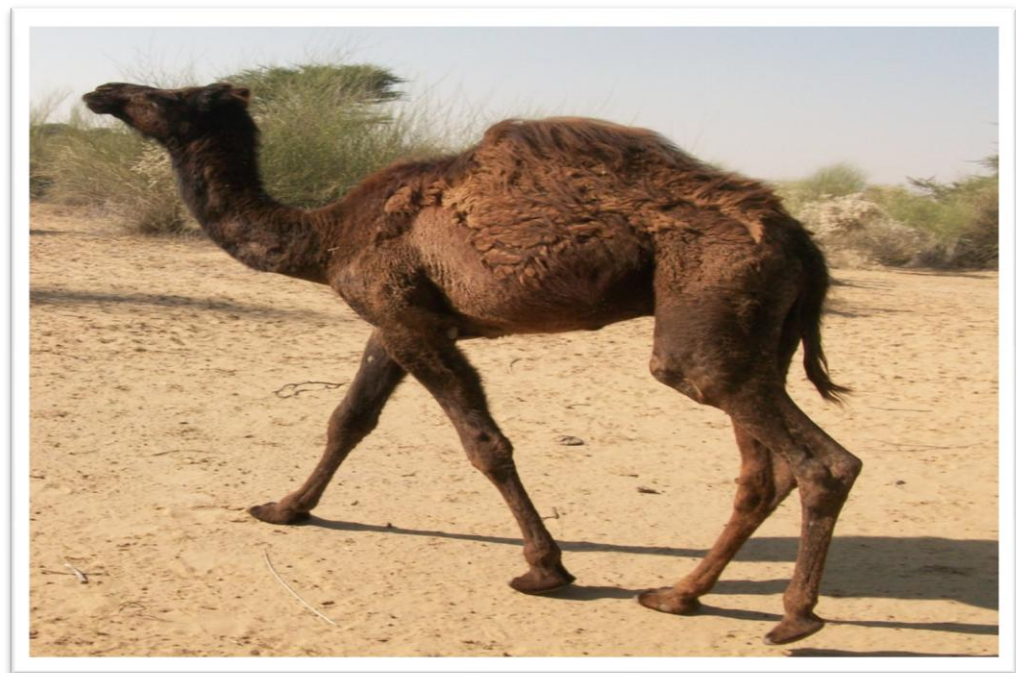


Fig. 45 Photograph showing of *Candida albicans* infected camel calf after treatment



Fig. 46 Photograph showing of *Trichophyton verrucosum* infected camel before treatment



Fig. 47 Photograph showing of *Trichophyton verrucosum* infected camel after treatment

5. SUMMARY AND CONCLUSION

The study was conducted during the year 2015-2016 in the area adjacent to Bikaner, Rajasthan. A total of 16 dermal mycoses infected camels (of either sex or different age group) were included in the present study. The disease affected most of the animals during post rainy season and the young ones were most commonly affected. The major clinical manifestations were itching, alopecia, crusty circular lesions specially concentrated over neck, chest, axillaries, legs and abdomen. Skin scrapings and blood samples were collected from all the affected subjects aseptically. Microscopic examination, haemato-biochemical examination and culture examination were performed before and after treatment. An effort was made to draw a picture of clinico-diagnostic and haemato-biochemical parameters of camels suffering from dermal mycoses and evaluate the efficacy of herbal formulation.

Culture examination of skin scrapping samples from dermal mycoses suffered camel revealed the growth of various fungal agents which were identified as *Trichophyton rubrum*, *T. verrucosum*, *T. schoenleinii*, *Microsporum canis*, *Candida albicans*, *Clodophialophora bantiana*, *Absidia corgmbifera*, *Coccidiodes immitis*, *penicillum spp.*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus versicolor* and a previously unidentified species *Histoplasma capsulatum* which was reported systematically not in topically in camel.

Haemato-biochemical results revealed significant decrease in neutrophil and increase in lymphocytic count. Whereas total erythrocyte count, hemoglobin concentration, packed cell volume, total leucocytes count, monocytic count and eosinophilic count did not varied significantly pre and post treatment. After recovery significant

reduction in ALKP and ALT were recorded compared to the values before treatment. Estimation of Serum minerals values in the present study revealed that serum Se status after recovery increased significantly as compared to pre treatment.

This novel study was intended to test the therapeutic efficacy of herbal drug which has been developed at NRCC Bikaner. The camels were examined for any symptom of dermal mycoses and presence of lesions on body surface. The infected camels subjected for application of the herbal preparation. Application of drug was carried out with a predesigned protocol on days 0, 3rd and 7th, respectively. Post treatment observations suggested that three applications were sufficient for the complete recovery. The recoveries in these camels were manifested by subsidence of clinical symptoms like itching, alopecia, crusty circular lesion. It was observed that after treatment the skin become soft and glossy and there was no scar present over the skin thereby improving the appearance of animal.

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**Studies on Diagnosis and Therapeutics of Camel
Dermal Mycoses
M.V.Sc. Thesis**

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ABSTRACT

The study was conducted during the year 2015-2016 in the area adjacent to Bikaner, an arid desert region of Rajasthan. A total of 16 camels of either sex or different age group infected with dermal mycoses were included in the present study. The major clinical manifestations were itching, alopecia, crusty circular lesions over different body parts. Microscopic examination, haemato-biochemical examination and culture examination were performed before and after treatment to evaluate the efficacy of herbal formulation.

Culture examination of samples from dermal mycoses suffered camel revealed various fungal agents including a previously unidentified species *Histoplasma capsulatum*, which was reported systematically not topically in camel. Haematogram picture depicted a significant decrease in neutrophil and increase in lymphocytic count. Whereas rest of hematological parameters did not varied significantly. After recovery significant reduction in ALKP and ALT were recorded while AST, serum total protein, albumin and globulin varied non significantly. Post treatment serum Se values increased significantly as compared to pre treatment. A non significant increase in Cu, Co and Zn were recorded in study group. The infected camels from dermal mycoses showed complete recovery with 3 applications of drug. The excellent therapeutic efficacy of herbal drug with its economic importance driven a force towards the use of herbal product and rise a new hope in the context of the present chemotherapeutic era.

ऊटों में त्वचीय फफुंद रोग का नैदानिक और चिकित्सीय अध्ययन

स्नातकोत्तर शोध ग्रन्थ

जनापादकीय रोग विज्ञान एवं निवारक पशु औषध विज्ञान विभाग
पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय
राजस्थान पशु चिकित्सा एवं पशु विज्ञान विश्वविद्यालय
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यह अध्ययन वर्ष 2015-16 के दौरान राजस्थान के शुष्क रेगिस्थानी क्षेत्र, बीकानेर क्षेत्र के निकट आयोजित किया गया, इस अध्ययन में कुल 16 त्वचीय फफुंद रोग से संक्रमित ऊटों को शामिल किया गया, जो कि विभिन्न आयुवर्ग तथा लिंग से संबंधित थे। जिनकी प्रमुख नैदानिक अभिव्यक्तियाँ खुजली, खालित्य तथा शरीर के विभिन्न हिस्सों पर क्रस्टीपरिपत्र धाव थे। हर्बल औषधि के प्रभावकारिता के मूल्यांकन के लिए उपचार से पूर्व तथा उपचार के उपरान्त सूक्ष्म परीक्षण एवं रक्तीय-जैव रासायनिक परीक्षण किया गया।

त्वचीय फफुंद रोग से पीड़ित ऊटों से लिये गये नमूनों के कल्चर परीक्षण से विभिन्न फंगस ऐजेटो का पता चला तथा साथ ही हिस्टोप्लाज्मा केप्सूलेटम नामक फंगल ऐजेंट का पता लगाया गया जोकि त्वचीय रूप से ऊटों में अज्ञात था। रक्तीय परीक्षण से न्यूट्रोफिस में महत्वपूर्ण कमी तथा लिम्फोसाइटिक गणना में महत्वपूर्ण वृद्धि पायी गई। अन्य रक्तीय मापदण्डों में गैर महत्वपूर्ण विविधता देखी गई। उपचार के उपरान्त ए.एल.के.पी. तथा ए.एल.टी. में महत्वपूर्ण कमी पायी गई जबकि कुल सीरम प्रोटीन, ए एस टी, एल्बुमिन और ग्लोब्युलिन में गैर महत्वपूर्ण विविधता दर्ज किये गये। उपचार पूर्व की अपेक्षा उपचार के उपरान्त सीरम सेलेनियम में महत्वपूर्ण वृद्धि दर्ज की गई साथ में जिंक, कोपर तथा कोबाल्ट में गैर महत्वपूर्ण वृद्धि दर्ज की गई। वर्तमान अध्ययन में त्वचीय फफुंद रोग से संक्रमित ऊटों में तीन अनुप्रयोगों के उपरान्त पूर्ण त्वचीय स्वस्थता दर्ज की गई। हर्बल दवा की उत्कृष्ट चिकित्सीय प्रभाविता साथ ही आर्थिक महत्वता के कारण हर्बल उत्पादों के उपयोग को महत्वपूर्ण बल मिला है तथा आज के किमोथैरेपी युग में एक नई आशा दी है।

Table 1. Questionnaires (history sheet) for diagnosis and treatment of dermal mycoses in camel

Department of Epidemiology and Preventive Veterinary Medicine

College of Veterinary and Animal Science, Bikaner

Rajasthan University of Veterinary and Animal Science, Bikaner

Case History / Questionnaire

Sample/Case no.: ----- Date: -----

Sr. No.	Question	Answer
1	Animal owner name	
2	Address	
3	Mob. No.	
4	Breed of camel	
5	Age	
6	Sex	
7	Body weight (approx)	
8	General condition	
9	Previous history of illness	
10	Associated disease	
11	Use of camel	
12	Total no. camels kept by the owner	

13	Beside camel other animals kept	
14	Animal reared on open/ close house	
15	Feeding practices	
16	Control measure adopt by owner	
17	Duration of skin problem	

Before first application

Photographs Blood samples Skin scrapping

Loss of hair Presence of scar Presence of itching

Medicine application schedule

1. First application (0 day) Date: _____

2. Second application (4th day) Date: _____

3. Third application (7th day) Date: _____

After 2 month of first application

Photographs Blood samples Skin scrapping

Loss of hair Presence of scar Presence of itching

Table 2. Values of haematology in dermal mycoses affected camel before treatment

S.N.	HB gm %	PCV %	TLC 10 ³ /um	TEC 10 ⁶ / um	D L C %	N %	M %	L %	E %
1	8.4	28	10	9		55	2	40	3
2	8.2	28	12	10		62	2	34	2
3	10.2	35	12	11		66	1	30	3
4	10.4	37	13	10. 2		64	2	30	4
5	9	29	24	10		56	1	35	8
6	8.6	28	13	9		58	3	37	2
7	10	33	11	10. 4		62	1	33	4
8	11	35	15	12		72	1	22	5
9	6	21	8	6.4		71	3	23	3
10	10	35	10	12		68	2	28	2
11	9.8	32	12	10. 4		70	1	27	2
12	10.2	36	13	10		65	3	30	2
13	8.2	26	10	9		62	2	33	3
14	8	28	12	9		80	1	16	3
15	9	31	12	10		47	2	42	9
16	8	27	12	10		68	2	23	7
Me an± SE	9.0625 ±0.31	30.5625 ±1.10	12.4375 ±0.87	9.9 ±0. 33		64.12 5±1.9 6	1.812 5±0.1 9	30.188 ±1.73	3.874 ±0.56

Table 3. Values of haematology in dermal mycoses affected camel after treatment

S.N.	HB gm %	PCV %	TLC 10 ³ /um	TEC 10 ⁶ /um	DL C %	N %	M %	L %	E %
1	9	28	11	10		60	2	36	2
2	9.2	34	12	9		58	2	38	2
3	10.2	34	12	10		59	1	36	4
4	11	36	12	10		60	2	34	4
5	8	26	14	11		54	3	37	6
6	8.4	29	12	9		58	2	37	3
7	9	32	11	10		58	2	35	5
8	11.4	36	13	11		66	2	26	6
9	8	24	10	8		68	2	27	3
10	11	34	10	12		62	2	33	3
11	8.6	33	12	10		68	1	29	2
12	9	34	13	11		68	2	28	2
13	8.6	28	11	10		64	2	30	4
14	8.4	26	12	10		72	2	23	3
15	8.6	32	13	9		54	2	36	8
16	9.4	29	10	11		66	2	23	9
Mea n± SE	9.2375± 0.27	30.9375 ±0.95	11.75± 0.30	10.0625 ±0.25		62.18 75±1. 35	1.937 5± 0.11	31.7 5 ±1.2 9	4.125 ±0.54

Table 4: Values of biochemistry in dermal mycoses affected camel before treatment

S.N.	T. P. (g/dl)	ALKP (lu/l)	AST (sgot) IU/l	GLOB g/dl	ALB g/l	ALT(sgpt) lu/l
1	3.9	30	0	2.7	1.2	26
2	6.2	54	59	2.8	3.5	21
3	7.6	34	23	3.6	4	18
4	7	58	26	3.2	3.8	21
5	7.2	65	46	3.4	3.8	20
6	6.7	95	33	2.8	3.9	24
7	5.6	64	36	2.8	2.8	26
8	7	21	24	3.3	3.7	18
9	7.7	28	56	3.6	4.1	26
10	5.9	29	16	2.7	3.2	26
11	6.4	40	22	2.9	3.5	22
12	7.2	26	46	3.4	3.8	24
13	6.5	36	28	2.8	3.7	36
14	7.5	78	42	3.1	4.4	24
15	7.3	212	58	3.1	4.2	16
16	10.1	241	58	5.3	4.8	18
Mean	6.8625	69.4375±16.2	35.8125±4	3.21875±0.	3.65±0.2	22.875±1.
± SE	± 0.32		.34	16	0	21

Table 5: Values of biochemistry in dermal mycoses affected camel after treatment

S.N.	T. P. (g/dl)	ALKP (lu/l)	AST(sgot) IU/l	GLOB g/dl	ALB g/l	ALT(sgpt) lu/l
1	4.8	30	18	2.3	2.5	10
2	6.8	36	46	3.2	3.6	35
3	7	24	26	3.4	3.6	14
4	6.4	54	33	2.7	3.6	21
5	7	48	56	2.9	4.1	18
6	7.2	46	40	3.2	4	11
7	7	46	30	3.2	3.8	22
8	7.2	28	32	2.8	4.4	11
9	7.6	32	30	3.2	4.4	23
10	6	28	20	2.4	3.6	17
11	7.8	32	26	2.6	4.2	24
12	6.4	38	28	2.8	3.6	13
13	6.8	28	32	3.1	3.7	32
14	7.8	64	48	3.5	4.3	28
15	6.8	160	38	3.3	3.5	12
16	6.4	174	42	3.2	3.2	16
Mea	6.8125±0.	54.25±11	34.0625±2.	2.9875±0.0	3.75625±0.	19.1875±1.
n	18	.3	57	89	12	92

Table 6 : Values of serum minerals in dermal mycoses affected camel before treatment

S. No.	Co (ppb)	Cu (ppb)	Zn (ppb)	Se (ppb)
1	220.2	1080.1	209.2	636
2	120.2	7761.1	1107.8	719.6
3	113	2558.8	1176	1025.8
4	86.2	1298.8	123.8	770.6
5	5.4	318.8	433.12	335
6	22.7	520.6	65.4	219
7	29.8	1880.4	1337.4	944.8
8	5	1853.4	15226	728.2
9	11.3	870	1289.1	381.6
10	8.4	161.8	18.8	507.6
11	12.2	151	345.1	567.3
12	276.6	1708.2	1290.2	1067.4
13	27.7	387.7	2537	471.8
14	0	1201.9	7284.5	523.3
15	0	156.7	412.1	575.7
16	0	134.6	457.5	443.5
Mean± SE	58.669±21.13	1377.74±465	2082.06±97	619.825±61.01

Table 8: Values of serum minerals in dermal mycoses affected camel after treatment

S. No.	Co (ppb)	Cu (ppb)	Zn (ppb)	Se (ppb)
1	329	946.4	468.4	947
2	112.4	4868.4	913.4	928.6
3	4.6	4374.4	1000.8	1296.6
4	0	1201.5	7290.6	535.2
5	0	685.4	6673.5	729.4
6	268.4	717	479	402
7	0	1109.3	4467	734.6
8	0	2086.2	4690.4	935.8
9	15.2	1153.6	1396.6	601.6
10	0	593.4	356.8	537.8
11	97.4	618.6	525.8	675.6
12	156.6	1896.4	1147	1001.4
13	168.4	879	2879	690.8
14	0	1712.8	8342.4	651.6
15	9	548.6	343.8	658.6
16	13	296.4	537	817.2
Mean	73.375±26.6	1480.46±331	2594.469±695	758.9875±55.2