CLINICO-DIAGNOSTIC AND THERAPEUTIC STUDIES ON *MALASSEZIA* DERMATITIS IN DOGS

By

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CERTIFICATE

Dr. RAMANA VALLE has satisfactorily prosecuted the course of research and that the thesis entitled **"CLINICO-DIAGNOSTIC AND THERAPEUTIC STUDIES ON *MALASSEZIA* DERMATITIS IN DOGS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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This is to certify that the thesis entitled **"CLINICO-DIAGNOSTIC AND THERAPEUTIC STUDIES ON *MALASSEZIA* DERMATITIS IN DOGS"** submitted in partial fulfillment of the requirements for the degree of MASTER OF VETERINARY SCIENCE for Sri Venkateswara Veterinary University, Tirupati, is a record of the bonafied research work carried out by **Dr. RAMANA VALLE,** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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Dr. Ramana Valle. . . .

DECLARATION

I, **Dr. RAMANA VALLE** hereby declare that the thesis entitled **"CLINICO-DIAGNOSTIC AND THERAPEUTIC STUDIES ON *MALASSEZIA* DERMATITIS IN DOGS"** submitted to **SRI VENKATESWARA VETERINARY UNIVERSITY** for the Degree of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date: 07-03-2014 (Dr. RAMANA VALLE)

Name of the Author	**:**	**RAMANA VALLE**
Title of the Thesis	**:**	**"Clinico-diagnostic and therapeutic studies on *Malassezia* dermatitis in dogs"**
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ABSTRACT

On screening of 362 dogs with dermatological ailments during the period from March 2012 to August 2012, the prevalence of *Malassezia* dermatitis accounted for 10.22 per cent. The *Malassezia* dermatitis was more prevalent in adult dogs (51.35%) and less in puppies and dogs aged above 7 years (10.81%) with higher prevalence in males (64.86%) than females (35.14%). The condition was recorded higher in Labrador Retriever (43.24%) breed with greater incidence during May (37.83%) and June (27.02%) months. However no statistically significant difference ($P>0.05$) was noticed among different parameters studied regarding prevalence.

The principal clinical signs observed in affected dogs included pruritus, erythema, scaling, exudation, alopecia, offensive odour , hyperpigmentation and lichenification which were observed in 100%, 78.37%, 59.45%, 64.86%, 54.05%, 89.18, 48.64% and 43.24% of the affected dogs respectively. Ears (81.08%) were affected in majority of the dogs followed by other body parts.

Cytology revealed presence of *Malassezia* alone in 32.44% dogs and mixed infection with bacteria in 67.56% dogs. Mycological cultural examination of 37 samples that were positive for cytology revealed regular round colonies after 48 hours of incubation in Sabouraud's dextrose agar without the addition of oil denoted that *Malassezia pachydermatis* was lipid independent.

Malassezia dermatitis was associated with concurrent conditions like pyoderma (13.51%), hypothyroidism and renal failure (2.70%), demodicosis (10.81%), tick infestation (8.10%) and scabies (2.70%) in 40.54% of dogs.

In-vitro antifungal sensitivity test was done by using conventional antifungal agents and observed that 93.33% of the *Malassezia pachydermatis* isolates were sensitive to ketoconazole. Addition of *Cassia alata* leaf powder @ 10mg/ml to Sabouraud's dextrose agar plates inhibited the growth of *Malassezia pachydermatis*.

Alanine aminotransferase (ALT) and Gamma-glutamyltransferase (GGT) values in dogs affected with *Malassezia* dermatitis showed no significant difference ($P>0.05$) with the healthy dogs.

Therapeutic trails were undertaken based on the extent of clinical lesions and the animals were divided into four groups. Sixteen dogs with generalised *Malassezia* dermatitis were randomly allocated to two groups i.e Group I and Group II. Group I was treated with Tab. ketoconazole @ 10 mg/kg. b. wt. per orally and shampoo containing ketoconazole and chlorhexidine (Ketochlor®). Group II was treated with Tab. ketoconazole @ 10 mg/kg. b. wt. per orally and topical application of *Cassia alata* soap and *Cassia alata* ointment.

The efficacy of drugs used in Group I and Group II was assessed based upon clinical cure (pruritus score and clinical index score) and cytological examination. All the dogs in both the groups responded to the therapeutic regimen but with a difference in the onset of resolution of clinical signs and time taken for recovery. The time taken for recovery was 23.62±2.93 and 28.87±3.08 days respectively in Group I and Group II without any significant difference ($P>0.05$ between the two groups.). In both the groups adverse effects and significant ($P<0.05$) elevation of ALT levels was noticed after therapy.

Sixteen dogs with localised *Malassezia* dermatitis were randomly allocated to form two groups i.e. Group III and Group IV. Dogs in Group III were treated with topical application of ketoconazole cream and shampoo containing ketoconazole and chlorhexidine (Ketochlor®) while Group IV dogs were treated with topical application of *Cassia alata* soap and *Cassia alata* ointment. The per cent cure was 100% in both the groups with the average time taken for recovery as 17.50±2.29 and 22.75±2.29 days in Group III and Group IV respectively with no significant difference ($P>0.05$) between the two groups.

It is concluded that *Cassia alata* ointment and soap could treat *Malassezia* dermatitis with similar efficacy as that of antifungal agents and suggests that *Cassia alata* could be used as an alternative to synthetic antifungal agents as it is locally available and treatment is cost effective with no adverse effects even after prolonged application. Hence, *Cassia alata* could be incorporated into soap and ointment and made commercially available for the treatment of canine *Malassezia* dermatitis as the plant has antifungal and anti-inflammatory properties.

List of symbols and abbreviations

%	-	per cent or percentage
et. al.	-	and others
±	-	plus or minus
IU	-	international unit
ºC	-	degrees celsius
mg/kg	-	milligram per kilogram
b. wt	-	body weight
@	-	at the rate of
SID	-	once daily
BID	-	twice daily
TID	-	thrice daily
L	-	liter
ml	-	milliliter
M/S	-	messers
Gm	-	gram
Kg	-	kilogram
SPSS	-	statistical package for social sciences
<	-	less than
SE	-	standard error
n	-	number
No	-	number
viz	-	namely
SDA	-	Sabouraud’s dextrose agar
CIS	-	clinical index score
i.e.	-	that is
/	-	per

CHAPTER – I

1. INTRODUCTION

The popularity of having dog as a pet is increasing over the years with the fast changing socio-economic scenario in our country. The strong bonding between dog owners and their pet dogs leads to concern about the health and well being of later. India being a tropical country, the climatic conditions favour the onset of disease conditions. Skin problems are one among those that are widely prevalent in this country. Though skin affections are not usually fatal, these are aesthetically disagreeable to the owner and cause discomfort both to the dog and the owner.

Malassezia dermatitis is a relatively common skin infection in dogs caused by *Malassezia pachydermatis* which is a lipophilic non-mycelial yeast, a commensal of healthy canine skin mucosa. The yeast may become a pathogen whenever alterations in the skin surface microclimate or host defence occurs. The proliferation of yeast is suspected to be promoted by excessive sebum production or disruption of epidermal barrier, accumulation of moisture, concurrent dermatitis, atopy and bacterial skin infections (Patterson and Frank, 2002). The colonization of yeast in pet carnivores acts as a source of infection for immune compromised humans and thus public health importance of this organism cannot be undermined (Kumar *et. al*., 2002b).

Malassezia dermatitis in dogs may be localised or generalised and is clinically characterized by intense pruritus, alopecia, hyperpigmentation, lichenification and increased skin thickness. It could be secondary to other primary diseases like endocrinopathies (hypothyroidism, hyperadrenocorticism and diabetes mellitus), keratinisation disorders, immunologic dysfunctions and skin neoplasms (Mircean *et. al*., 2010).

At times the condition is ignored as it is perceived to be a non life threatening condition. However, when the condition becomes chronic and severe the skin's ability to serve as a barrier is compromised and other cutaneous infections might create problems.

The availability of suitable antifungal drugs is limited and could be affordable only by economically sound owners. Treatment of fungal infections is hampered by problems of solubility, stability and absorption of the existing drugs. Azoles such as ketoconazole, clotrimazole, fluconazole and itraconazole are antifungal agents available in clinical practice and ketoconazole has become the agent of choice for many fungal infections (Fernandez *et. al.*, 1998).

However the drugs that are currently used in the treatment of *Malassezia* dermatitis are expensive and mostly unaffordable by people with low socio economic status. They have varied therapeutic spectrum and cause side effects on long term use besides cost factor.

Due to increasing resistance of yeast against conventional drugs as well as the observable side effects only limited number of antifungal agents are commonly used in veterinary practice. The yeast infection whether superficial or systemic often pose substantial management problems and are still a major concern. Therefore it is required to develop a more economic antifungal agent with least side effects.

Traditional medicine is the oldest method of curing diseases and infections, for this various plants have been used in different parts of the world. Recently some higher plants have attracted the attention of microbiologists in search of some phytochemicals for antimicrobial action. Plants with antifungal properties could provide a niche for herbal formulation against *Malassezia* dermatitis in dogs with possible better efficacy and affordability (Damodaran and Venkataraman, 1994).

Cassia alata or *Senna alata* belongs to the family Caesalpinaceae is a pantropical ornamental shrub, distributed from tropical America to India. It is commonly known as ringworm bush or Candle stick plant (Sanskrit name: Dadrughna). The plant is widely used in India, Australia, West African countries and other tropical regions and is an official drug in the herbal pharmacopeia (Sule *et. al*., 2011).

The plant is well known as a relatively cheap alternative drug to the standard expensive antifungal drugs due to its acceptable potency in managing skin infections of humans. Leaves of this plant have been used as antifungal agents in folklore medicine. Developing an affordable and acceptable drug for the treatment of *Malassezia* dermatitis in dogs is exploitable from this medically important plant. The incorporation of *Cassia alata* extracts and plant materials into soaps and ointments to treat dermatological ailments is one of the major ways to utilize the medicinal plants (Oladele *et. al*., 2010). Hence the present study has been undertaken with the following objectives

1. To study the prevalence of *Malassezia* dermatitis in dogs in and around Vijayawada and Gannavaram.
2. To study the symptomatology in *Malassezia* dermatitis.
3. To determine the therapeutic efficacy of *Cassia alata* against *Malassezia* dermatitis in dogs.
4. To compare the therapeutic efficacy of *Cassia alata* with conventional antifungal drug.

CHAPTER - II

2. REVIEW OF LITERATURE

Pertinent literature on the prevalence, symptomatology, diagnosis and treatment of *Malassezia* dermatitis is reviewed under the following headings.

2.1 EPIDEMIOLOGY

Malassezia pachydermatis is frequently found on wild and domestic carnivores, and rarely on humans (Guillot and Bond, 1999).

Pier *et. al*. (2000) reported that *Malassezia* yeasts were first isolated by Gustafson in 1955, from the external ear canal of healthy and in dogs with otitis externa.

The genus *Malassezia* belongs to the family *Cryptococcaceae*, order *Cryptococcales*, class of *Blastomycetes*, division *Deuteromycotina*. The reproduction of *Malassezia* yeasts is asexual, with production of blastoconidia through budding, forming a round, ovoid or cylindrical cell which separates from the mother cell (Nobre *et. al*., 2001).

David *et. al*. (2003) reported that *Malassezia pachydermatis* proliferate only by enteroblastic budding, with the bud arising from a broad base by monopolar budding.

Malassezia was first reported from scales in lesions of pityriasis (Tinea versicolor) by Eichstedt in 1846 and later quoted by Chen and Hill (2005). *Malassezia* yeasts are commensal skin organisms of warm blooded vertebrates and are considered to be opportunistic pathogen that causes human and animal skin disorders (Cafarchia *et. al*., 2005a).

Eidi *et. al*. (2011) documented 13 species in the genus *Malassezia,* of which 12 are lipid dependent (*M. furfur, M. sympodialis, M. globosa, M. obtusa, M. sloofiae, M. restricta, M. nana, M. dermatis, M. japonica, M. yamatoensis, M. caprae and M. equina*) and one non-lipid dependent species (*M. pachydermatis*).

Crosaz *et. al*. (2013) reported that a total of 14 *Malassezia* species were available, out of which 13 were lipid dependent and require specific nutrient in the medium. *Malassezia pachydermatis* was the only lipophilic yeast that may be isolated in regular media like Sabourauds's dextrose agar.

2.1.1 Prevalence

Feijo *et. al*. (1998) examined dogs for the presence of *Malassezia pachydermatis* and recorded an incidence of 11.40% (4/35).

Morris (1999) reported that *Malassezia* yeasts colonize in high numbers in sparsely haired, and moist areas of the skin and mucosa of normal dogs than in densely haired dogs with dry areas of skin.

Nobre *et. al*. (2001) recorded an incidence of *Malassezia pachydermatis* as 76.50% of otitic dogs.

Kumar *et. al*. (2002a), in their study on incidence of *Malassezia pachydermatis*, recorded *Malassezia* organisms from 82.18 % of otitic dogs by smear cytology and cultural examination.

Cafarchia *et. al*. (2005b) isolated *M. pachydermatis* species of 61.10% dogs with skin lesions.

Girao *et. al*. (2006) reported that *M. pachydermatis* was positive by both cytological and cultural samples obtained from 84 dogs (57.53%) out of 146 dogs with otitis.

Jain *et. al*. (2007) opined that *Malassezia pachydermatis* organisms are normal inhabitants of the skin and are commonly found in the ear canal, anal sacs, muzzle, lips, axillae, interdigital areas, rectum and vagina of dogs.

Nardoni *et. al*. (2007) reported that the frequency of isolation of *Malassezia* yeasts was higher from interdigital areas (70.70%) followed by ears (63.40%), nail folds (35.70%), mouth (33.30%), groin (30.90%), conjunctiva and axillae (23.80%), perineum and anus (19.00%) and peri-anal glands (9.50%).

Khosravi *et. al*. (2008) in a study on isolation of *Malassezia* species reported that the prevalence of *M. pachydermatis* was 83.30% and 70.27% in healthy ears and otitis externa respectively.

In a study conducted by Brito *et. al*. (2009) *M. pachydermatis* was isolated from 207 (92.80%) out of the 223 animals tested for yeasts. The organism was more commonly isolated from the peri-anal region (58.50%), vaginal mucosa (41.30%), followed by the oral mucosa (34.00%) and prepuce (22.30%).

Mircean *et. al*. (2010) studied *Malassezia* infection in 194 dogs and recorded a prevalence of 37.60% (73/194).

Conkova *et. al*. (2011) isolated *Malassezia pachydermatis* from 44.80% and 38.90% of ear and dermal swabs respectively in dogs suspected for yeast dermatitis.

Eidi *et. al*. (2011) isolated *M. pachydermatis* species and recorded the prevalence of 41.20% from dogs with skin lesions.

Kumar *et. al*. (2011a) reported an incidence of 30.00 per cent of *Malassezia* dermatoses among various dermatological disorders.

George *et. al*. (2012) recorded the prevalence of skin ailments as 10.69% of which *Malassezia* accounted for 7.83%.

2.1.1.1 Age

Bond *et. al*. (1996) in their study on incidence of cutaneous *Malassezia pachydermatis* in dogs with pruritic skin disease reported that three to seven years age group were mostly affected.

Kiss *et. al*. (1997) studied otitis externa and reported that *Malassezia* occurred in dogs aged between two to five years.

Scott *et. al*. (2001) reported that *Malassezia* dermatitis can occur in any age group.

The highest percentage of dogs having *Malassezia* otitis externa were recorded in age group of the two to four years (Kumar *et. al*., 2002a).

Cafarchia *et. al*. (2005a) reported that *Malassezia* was frequently isolated from dogs under 5 years of age.

Girao *et. al*. (2006) reported that majority of dogs affected with *M. pachydermatis* were aged between 1 to 3 years.

Nardoni *et. al*. (2007) observed that there was no age or sex predilection, but *Malassezia* dermatitis or otitis was more often diagnosed in dogs aged between 1 to 3 years.

Khosravi *et. al*. (2008) recorded that the animals affected with *Malassezia* species were aged between 1-5 years.

Mircean *et. al.* (2010) opined that the average age of dogs infected with *Malassezia pachydermatis* varied with breed.

Eidi *et. al*. (2011) observed that the dogs with *Malassezia* infection was found to be highest in the age group of 1-5 years followed by less than 1 year.

2.1.1.2 Sex

Bond *et. al*. (1996) studied the factors associated with cutaneous *Malassezia pachydermatis* and concluded that there was no sex predilection.

Kiss *et. al*. (1997) studied the incidence of *Malassezia* otitis extema in canines and reported that, 55.90% were males.

Kumar *et. al*. (2002a) reported that dogs of either sex were found equally susceptible to *Malassezia* otitis externa infection.

Girao *et. al*. (2006) reported that both male and female dogs are equally susceptible to *Malassezia* yeast.

Machado *et. al*. (2011) observed no significant difference in the prevalence of *Malassezia* dermatitis among male and female dogs.

2.1.1.3 Breed

Bond *et. al*. (1996) studied the factors associated with cutaneous *Malassezia pachydermatis* in various breeds of dogs and concluded that Basset Hound, Dachshund, Cocker Spaniel, and West Highland White Terriers were more affected.

Bond *et. al*. (1998) reported that Basset Hound had higher susceptibility.

Bruner and Blakemore (1999) recorded the breed predisposition in dogs to *Malassezia* dermatitis and concluded that Poodle, West Highland White Terrier, American Cocker Spaniel, Australian Terrier, Silky Terrier, German Shepherd, Cavalier Charles Spaniel, Shill Tzu, Dachshund, Basset Hound, Springer Spaniel and Boxer were frequently affected.

Marsella *et. al*. (2000) in their study on *Malassezia* dermatitis in dogs, found that German Shepherd and Cocker Spaniel were highly susceptible.

Genetic predisposition appears to be important because breeds like West Highland White Terrier, Basset Hound, American Cocker Spaniel, Shih Tzu, English Setter, and Dachshund had a significantly higher risk for *Malassezia* dermatitis (Muller *et. al*., 2001).

Nobre *et. al*. (2001) in their study on incidence of *Malassezia pachydermatis* as a cause of external otitis in dogs reported that Cocker Spaniel, German Shepherd and Brazilian Fila were highly susceptible.

Scott *et. al*. (2001) recorded that the West Highland White Terrier, Basset Hound, Springer Spaniel, German Shepherd, Cocker Spaniel, Silky Terrier, Australian Terrier, Maltese, Chihuahua, Poodles, Shetland Sheepdog, Lhasa Apso, and Dachshund were predisposed to *Malassezia* dermatitis.

Kumar *et. al*. (2002a) reported that German Shepherd was the most frequently affected breed for *Malassezia* otitis followed by Labrador Retriever.

Malassezia dermatitis has been reported in many breeds but certain breeds such as American Cocker Spaniel, Basset Hound, Cocker Spaniel, Dachshund, English Setter, Poodle, West Highland White Terrier and Shih Tzu were at high risk.

Basset Hound had greater skin and mucosal populations which were colonized at all mucosal sites whereas Beagle had greater yeast populations in the external ear canal compared to other breeds (Saijonmaa-Koulumies, 2002).

Eluk *et. al*. (2003) in a comparative study on *Malassezia pachydermatis* in dogs with chronic dermatitis screened different breeds and concluded that there was no breed predilection.

Cafarchia *et. al*. (2005a) reported that the frequency and population size of *M. pachydermatis* varied markedly between breeds.

Jeong *et. al*. (2005) reported that the Maltese (22.00%), Cocker Spaniel (17.00%), Pekingese (11.00%), Vizsla (11.00%) breeds of dogs were highly susceptible to *Malassezia* dermatitis.

Brito *et. al*. (2009) reported that *M. pachydermatis* was most frequently isolated from oral and vaginal mucosa of Poodle and English Cocker Spaniel.

2.1.1.4 Month

Kumar *et. al*. (2002a) recorded the highest occurrence of *Malassezia* otitis externa in dogs during June suggesting that weather might influence the onset of condition.

Patterson and Frank (2002) reported that the occurrence of *Malassezia* dermatitis may increase during the summer months, which may correlate with allergy and higher humidity during the season.

Cafarchia *et. al*. (2005a) recorded highest occurrence of *Malassezia* dermatitis during autumn in dogs.

Chen and Hill (2005) reported that the prevalence of *Malassezia* dermatitis in dogs was more common in warm and humid climate.

Conkava *et. al*. (2011) opined that difference in temperature and humidity affect the development of *Malassezia* infection.

2.1.1.5 Concurrent infections / conditions

Plant *et. al*. (1992) reported that dogs under chronic therapy with antibiotics, particularly enrofloxacin or antibiotics in combination with glucocorticoids were predisposed to *Malassezia* dermatitis.

Mason (1993) reported that defect in keratinisation of canine cutis favoured the growth of *M. pachydermatis*.

Bond *et. al*. (1996) reported hyperadrenocorticism as a concurrent disease in dogs with *Malassezia* dermatitis.

Charach *et. al*. (1997) stated that most of the dogs with *Malassezia* dermatitis have concurrent dermatoses like pyoderma, seborrhoea, atopy, food and flea allergies and endocrine disorders.

Watson (1998) reported that zinc deficiency can predispose to *Malassezia* dermatitis in dogs.

Scott *et. al*. (2001) indicated that prolonged use of antibiotic can cause overgrowth of *Malassezia* species and result in dermatitis.

Nambi *et. al*. (2002) reported that *Malassezia* was a facultative pathogen noticed in altered host defense mechanisms and skin surface microclimate, excessive

sebum production, accumulation of moisture and disruption of normal barrier function.

Naresh *et. al*. (2002) reported refractory canine pododermatitis due to mixed infection of *Demodex canis* and *Malassezia pachydermatis* in a dog.

Outerbridge (2006) opined that the integrity of skin barrier function is an important predisposing cause for *Malassezia* dermatitis and otitis externa. Endocrinopathies and metabolic diseases alter surface lipids and immune responses, predisposing to secondary *Malassezia* infections.

Malassezia dermatitis is frequently a recurrent skin infection associated with an underlying disease process (Rosenkrantz, 2006).

Nardoni *et. al*. (2007) recorded the higher prevalence of *M. pachydermatis* yeasts in different body sites of atopic dogs.

Craig (2008) reported that the major predisposing factors in the pathogenicity of *Malassezia* dermatitis may include humidity, skin folds, immunological dysfunction, alterations in the micro-environment of the skin, allergies, keratinisation disorders, other skin conditions and genetic makeup. Elevated number of yeasts are found in association with high *Staphylococcal* populations since these organisms produce mutually beneficial growth factors and alterations in the skin micro-environment which favours their growth.

Negre *et. al*. (2008) opined that endocrine disorders like hypothyroidism and diabetes mellitus can predispose to *Malassezia* dermatitis in dogs.

Ganguly *et. al*. (2009) observed that stress due to whelping, allergic skin diseases and lactation could predispose to *Malassezia* yeast infection.

Mircean *et. al.* (2010) opined that allergic dermatitis was the major underlying disease for *Malassezia* dermatitis in dogs.

Reddy and Kumari (2011) reported recurrent pyoderma in a dog with *Malassezia* dermatitis and hyperadrenocorticism.

Yurayart *et. al*. (2011) reported that concurrent infection between *Candida parapsiloisis* and *Malassezia pachydermatis* resulted in canine seborrhoeic dermatitis.

Eidi *et. al*. (2012) recorded *Malassezia* dermatitis in association with demodicosis in an immunocompromised dog.

Srikala and Kumar (2014) reported *Malassezia* dermatitis in 36.60% of dogs suffering from hypothyroidism.

2.2 SYMPTOMATOLOGY

Plant *et. al*. (1992) observed that pruritus was the common sign mostly affecting the axillary and inguinal regions. Lesions were dry, oily or greasy, malodorous with erythema, alopecia, hyperpigmentation and keratosebaceous plaques with lichenification.

Mobley and Mayer (1994) studied dermatitis associated with *Malassezia* and stated that cutaneous lesions were characterized by areas of erythema, alopecia, and thickened skin on the ventrum and all the limbs.

Charach (1997) observed pruritus of moderate to severe nature in *Malassezia* dermatitis. Other lesions included erythema, hyperpigmentation, offensive odour, grey to white scales and waxy or oily seborrhoea.

Morgan *et. al*. (1997) stated that dogs with *Malassezia* dermatitis exhibited clinical signs of pruritus often intense, erythema with scaly and malodorous, greasy exudates and papulocrustous lesions that resemble bacterial folliculitis, while hyperpigmentation and lichenification were noticed in chronic cases.

Gorman (1998) stated that the skin affected with *Malassezia* dermatitis was usually erythematous with varying degrees of alopecia and scaling. Hyperpigmentation and lichenification were frequently observed in dogs with chronic disease. Abdominal lesions consisted of symmetrical, well demarcated circular or elliptical areas of erythema which developed into scaly plaques. Patches of erythema, alopecia and exudation might be found in the ventral neck. Inter digital lesions were common with erythema and alopecia extending to the accessory carpal areas and medial aspects of the limbs in severe cases. Severe limb lesions include marked skin thickening, resulting in the formation of erythematous, scaling, and alopecic ridges.

Greene (1998) described the symptoms of generalised malasseziosis as pruritus, alopecia, excoriation with erythema, seborrhoea, lichenification and hyperpigmentation. In severe cases, the dog might lick or rub the lesions against hard objects.

Griffin (1998) described the symptoms of cutaneous malasseziosis as erythematous lesions, light grey to yellow scales, crusts, pruritus with variable intensity, alopecia, hyperpigmentation, green to yellow or grey brown crusty surface, plaque or lichenified plaques. Dirty to greasy hair coat with foul rancid odour and papules with erythema might be present. These lesions are common on the ventrum and skin folds.

Bruner and Blakemore (1999) opined that *Malassezia* dermatitis could be suspected when regional or generalised dermatosis exhibited with moderate to severe pruritus, cutaneous erythema, hyperpigmentation and waxy or oily seborrhoea. Other characteristic features include scaling, crusting, alopecia and greasiness of the hair and skin on the ear pinnae, lips, muzzle, neck, axillae, ventrum, inguinal area, peri-anal area, forearms, caudal thighs and feet.

Lower *et. al*. (2000) recorded severe alopecia, hyperpigmentation, lichenification of the ventral neck and chest, abdomen and legs with thickened ears having brownish discharge in a West Highland White Terrier with *Malassezia* dermatitis.

The clinical signs in *Malassezia* dermatitis were variable consisting of erythema, mild to severe pruritus, greasy or waxy, scaly (yellow or slate grey) and crusty lesions associated with an offensive rancid or yeasty odour. Marked lichenification and hyperpigmentation are recorded in chronic cases (Muse, 2000 and Muller *et. al*., 2001).

Kumar *et. al*. (2002c) recorded otalgia, discomfort, ear scratching, head shaking, erythema with mild to moderate otic discharge from the dogs suffering from otitis externa due to *Malassezia pachydermatis*.

Patterson and Frank (2002) reviewed that *Malassezia* dermatitis was manifested either as localised or generalised lesions which were common in the areas rich with sebaceous glands. They also opined that the skin lesions are not specific for *Malassezia* dermatitis and reflect the existing seborrhoea and pruritus.

Saijonmaa-Koulumies (2002) recorded symmetrical distribution of lesions in *Malassezia* dermatitis cases in the regions of face, ears, ventral neck, axillae, groins, legs and paws.

Baksi *et. al*. (2004) conducted clinico therapeutic study of *Malassezia* dermatitis in dogs and recorded pruritus, erythema, exudation, scaling and poor coat condition in the affected dogs.

Jeong *et. al*. (2005) reported that commonly affected areas in *Malassezia* dermatitis dogs were ear canal (41.00%), axillae (18.00%), groin (15.00%), peri-anal (12.00%), ventral aspect of the neck (9.00%), interdigital spaces (1.00%) and muzzle (1%.00). The clinical signs recorded were crusts (31.00%), alopecia (25.00%), hyperpigmentation (25.00%), scales (19.00%), erythema (13.00%), lichenification (11.00%), pustule (11.00%), ear swelling (11.00%), papules (5.00%) and offensive odour (5.00%).

Kumar *et. al*. (2006) performed clinical and therapeutic study on canine malasseziosis and observed that the skin lesions varied from dry, scaly, erythematous to moist, exudatous nature.

Outerbridge (2006) noticed that skin lesions resulting from *Malassezia* dermatitis may be localised or generalised, while the localised lesions most commonly involve the external ear canal, interdigital skin, ventral neck, axillae, inguinal region, or intertriginous areas.

Craig (2008) reported that lesions in the *Malassezia* dermatitis are common on the feet (interdigital region and ventral paw), face, axillae, inguinal region, pinnae, and ventral neck. The major clinical signs include pruritus, erythema, scaling, crusting, lichenification, greasiness, a strong body odour and otitis externa.

Mircean *et. al*. (2010) conducted a retrospective study on *M. pachydermatis* in dogs and reported that thoracic limb phalangeal region (71.20%) was the most affected region followed by auricular (67.10%), face (50.70%), pelvic region (49.30%) and distal portions of pelvic limb (45.20%). Dominant symptoms included pruritus (95.50%), erythema (90.40%), alopecia (67.10%), hyperpigmentation (46.60%) and scales (45.20%).

Conkava *et. al*. (2011) recorded the clinical manifestation of *Malassezia* dermatitis in the back and sides of the body as seborrhoeic alopecia with severe pruritus and sweetish odour.

Machado *et. al*. (2011) who reported that primary lesions in *Malassezia* dermatitis were commonly associated with pruritus and secondary changes were erythema, alopecia, excoriations, seborrhoea, lichenifcation and hyperpigmentation.

2.3 DIAGNOSIS

2.3.1 Cytology

Plant *et. al*. (1992) prepared glass impression smears from suspected cases of *Malassezia* dermatitis in dogs, stained them with modified Wright's stain and observed bottle shaped yeast organisms under high magnification.

Kennis *et. al*. (1996) in their study of quantity and distribution of *Malassezia* organisms on the skin of clinically normal dogs used glass slide impression smears and swab method for the diagnosis.

Kumar *et. al*. (2002a) isolated *M. pachydermatis* from 82.18% of the otitic ears by using roll smear cytological examination.

Matousek and Campbell (2002) reported that tape impression smear could be used to diagnose the cases of *Malassezia* dermatitis in dogs as the method is inexpensive, less time consuming and does not require heat fixation.

Nambi (2002) in a review of *Malassezia* dermatitis in dogs opined that the use of adhesive tape was the best method of diagnosis in dry form.

Eluk *et. al*. (2003) reported that the tape impression smear proved to be superior diagnostic tool compared to other tests like glass slide impression smears and collection of surface skin debris and scales in the diagnosis of *Malassezia* dermatitis.

A clear cellophane tape applied over a glass slide can be used for taking impression smear from the affected area of the skin which can be further stained with Methylene blue to reveal the presence of foot print shaped unipolar budding yeast organisms (Ramprabhu *et. al*., 2003).

Baksi *et. al*. (2004) reported that cytological examination by Diff-quick method can be used for the identification of *Malassezia* from clinical cases of dermatitis by using cotton swab and adhesive tape from moist and dry lesions respectively.

Cafarchia *et. al*. (2005b) reported that cytological examination is useful for diagnosing *Malassezia* dermatitis or otitis (or both) when at least 10 *Malassezia* organisms for the ear or 5 for the skin were present in 5 fields at 40X magnification.

Jeong *et. al*. (2005) performed cytological examination by direct impression smears, tape impression smears, skin scraping and ear swab using modified Wright's stain and *Malassezia* dermatitis was diagnosed based on cytological evidence of at least one body site having a mean of one yeast organism / oil immersion field.

Rosales *et. al*. (2005) diagnosed *Malassezia* dermatitis in dogs by using the tape strip cytology and cytological evidence of yeast counts greater than 10 organisms visible in 15 random oil immersion fields.

Girao *et. al*. (2006) performed cytological examination and isolated *M. pachydermatis* in 57.53% of the samples from the dogs with otitis and suggested that more than ten *Malassezia* cells per microscopic field are indicative of *M. pachydermatis* pathogenicity.

Prasanna *et. al*. (2006) performed cytological examination of skin of lesions by using cotton swabs from moist exudatous lesions and by adhesive tape over dry scaly lesions. They revealed that the finding of 10 or more yeast organisms per 0.5 square inch of microscopic slide (roughly one organism per twenty seven oil immersion fields) is suggestive of *Malassezia* dermatitis.

Jain *et. al*. (2007) opined that cytological examination by tape strip method can be successfully used for routine diagnosis of *Malassezia* dermatitis, which reveals peanut shaped Gram positive unipolar budding yeast organisms on microscopic examination.

Bensignor (2008) reported that from cutaneous samples, a mean of greater than 5 yeasts per field under oil immersion indicates the pathogenicity of *Malassezia* organisms.

Eidi *et. al*. (2011) in a study performed roll smear cytology and recorded 41.20% of skin lesions and 61.00% of otitis externa in dogs were found positive for *M. pachydermatis.*

George *et. al*. (2012) opined that detection of *Malassezia* infection by culture independent cytological techniques using glass slides, cotton swabs, or skin scrapings are rapid and have more than 90% specificity.

2.3.2 Cultural Examination

Kiss *et. al*. (1997) reported that *M. pachydermatis* grow on Sabourauds's dextrose agar and modified malt extract agar by incubation at 37˚C for 48 hours. They observed sand coloured colonies with dry and friable consistency which could easily be lifted off their base.

Eichenberg *et. al.* (2003) recovered isolates of *M. pachydermatis* on Sabouraud's dextrose broth supplemented with 1 per cent Tween 80 and suggested it as the most appropriate medium for culture.

Girao *et. al*. (2006) reported that although direct microscopic examination gives immediate diagnosis, fungal culture is necessary for more accurate results and for the identification of *Malassezia* species.

Olive oil coated Sabouraud's dextrose cycloheximide chloramphenicol agar medium was used for culture of *Malassezia pachydermatis* with incubation at 37˚C (Jain *et. al*., 2007).

Kumar *et. al*. (2008a) isolated and identified *Malassezia pachydermatis* by inoculation in Sabouraud's dextrose agar and initially demonstrated raised, doomed or high convex and smooth colonies with cream colour, which later become dry and wrinkled.

Machado *et. al.* (2011) used modified Dixon's agar for culture of *Malassezia* organisms and isolated *Malassezia* yeasts from 52.9% of dogs with cutaneous lesions.

Hernandez-Escareno *et. al*. (2012) reported growth of *Malassezia pachydermatis* on Potato dextrose agar supplemented with cycloheximide and chloramphenicol (0.5g/L) upon incubation at 37˚C for 7 days.

2.3.3 Other methods

Muller *et. al*. (2001) explained that the skin biopsy findings in dogs with *Malassezia* infection as a superficial perivascular to interstitial dermatitis with irregular hyperplasia, diffuse spongiosis and diffuse lymphocytic exocytosis of the epidermis and follicular infundibulum.

Biopsy of the affected skin for histopathology can be useful for diagnosis of *Malassezia* dermatitis. Histopathological changes noted were orthokeratosis, focal parakeratosis, scale and crust formation. Budding oval yeast could be detected in the scale crusts (Nambi, 2002).

Kumar *et. al*. (2008b) concluded that atopy patch test with *M. pachydermatis* antigens was more sensitive diagnostic test than the routine tests used to diagnose canine malasseziosis.

2.4 *IN VITRO* ANTIFUNGAL SENSITIVITY TEST

2.4.1 Antifungal drugs

Staroniewicz *et. al*. (1995) studied the sensitivity of *Malassezia pachydermatis* isolated from otitis externa in dogs and reported that the strains were sensitive to ketoconazole (100.00%), miconazole (88.90%) and clotrimazole (66.70%).

Akerstedt and Vollset (1996) reported sensitivity of *M. pachydermatis* to ketoconazole, clotrimazole, econazole, itraconazole, nystatin and pimaricin.

Kiss *et. al*. (1997) isolated *M. pachydermatis* from dogs with otitis externa and tested against five antimycotic agents *in-vitro*, and found that majority of isolates were sensitive to ketoconazole, followed by econazole, clotrimazole, miconazole, and nystatin.

Schmidt (1997) studied the activity of climbazole, clotrimazole and silver sulphadiazine against isolates of *Malassezia pachydermatis* and concluded that the two azoles, climbazole and clotrimazole showed good *in-vitro* activity against *Malassezia* yeast.

Zdovc and Brglez (1997) subjected 98 isolates of *Malassezia pachydermatis* isolated from the skin and mucosa of dogs to antifungal sensitivity test and reported that all (100.00%) were sensitive to ketoconazole, 93.50% to clotrimazole, and 89.10% to econazole, 86.90% to miconazole, 84.80% to amphotericin-B and 63.00% to nystatin.

Kim and Choi (1999) tested the drug susceptibility of *Malassezia pachydermatis* isolates from canine external ear canals and reported that all isolates of *Malassezia pachydermatis* were highly sensitive to ketoconazole, miconazole, tolnaftate and all strains were resistant to pimaracin.

Kumar *et. al*. (2002c) in their study on treatment of otitis externa in dogs associated with *Malassezia pachydermatis* reported that the *in-vitro* chemotherapeutic studies revealed that all isolates of *Malassezia* yeasts were sensitive to clotrimazole whereas only 40% of the isolates were sensitive to fluconazole.

Brito *et. al*. (2009) studied the sensitivity of 20 *Malassezia pachydermatis* isolates against antifungal drugs and reported that all the isolates were sensitive to itraconazole, fluconazole, ketoconazole and amphotericin-B, but resistant to caspofungin.

Cafarchia *et. al*. (2012) reported that *Malassezia pachydermatis* strains were susceptible to itraconazole, ketoconazole and posaconazole while less susceptible to fluconazole and miconazole.

Yurayart *et. al*. (2013) tested the antifungal sensitivity of *M. pachydermatis* isolates obtained from seborrhoeic dermatitis in dogs and reported that all the isolates were susceptible to itraconazole, ketoconazole, nystatin and terbinafine but resistant to 5-flurocytocine.

2.4.2 Herbal agents

Abubacker *et. al*. (2008) investigated the *in-vitro* antifungal activity of *Cassia alata* Linn. flower extract against aflatoxin producing fungi, plant pathogenic fungi, and human pathogenic fungi and emphasized that it could be used as a potential antifungal agent.

Chungasamarnyart *et. al*. (2008) tested the *in-vitro* fungicidal activity of *Annona squamosa, Cymbopogon nardus* Rendle, *Rhinacanthus nasutus* Kurz, *Cymbopogon citrates* Staf, *Piper betel* extracts and opined that these lotions could be used in the treatment of *Malassezia* dermatitis.

Esimone *et. al*. (2008) evaluated the *in-vitro* antimicrobial potency of a herbal soap formulated with ethanolic extract of *Cassia alata*. They recorded that the soap

exhibited excellent antimicrobial effect against Gram positive bacteria and opportunistic yeasts.

Rusenova and Parvanov (2009) studied the *in-vitro* sensitivity of different oils against *M. pachydermatis* and recorded that *Cinnamomum aromaticum, Origanum vulgare, Thymus vulgaris* and *Cymbopogon citrates* inhibited the *Malassezia* growth in decreasing order of their efficacy.

Lee and Lee (2010) evaluated the *in-vitro* antifungal activity of plant essential oils and itraconazole and reported that the essential oils of *Ocimum basilicum* L., *Melaleuca alternifolia* (Maid. & Bet.) Cheel, and *Rosa damascene* Mill. were more effective against *M. pachydermatis*.

Kumar *et. al*. (2011b) compared the *in-vitro* efficacy of plant oils with azole derivatives and reported a high degree of sensitivity (100.00%) for essential oil of *Cymbopogon flexuosus* followed by fluconazole and ketoconazole (91.30% each) and itraconazole (78.20%) against *Malassezia* recovered from clinically infected dogs.

Naeini *et. al*. (2011) studied the *in-vitro* activity of *Zataria multiflora* essential oil against various *Malassezia* species and opined that the oil may be used as an alternative for treatment of *Malassezia* associated diseases in dogs.

Omobuwajo *et. al*. (2011) compared the *in-vitro* antimicrobial activity of *Cassia alata* crude herbal soap with the leaf extract and recorded equal efficacy of both the products and opined that soaps incorporated with 5% *Cassia alata* in crude form reduced the production costs.

Sule *et. al*. (2011) tested *in-vitro* antifungal activity of *Senna alata* crude bark extract against dermatophytes i.e *Microsporum canslaslomyces, Trichophyton verrucosum, Trichophyton mentagrophytes* and *Epidermophyton floccosum* and concluded that the MIC of *Senna alata* crude bark extract on all the above tested dermatophytes was 5 mg/ml.

Pistelli *et. al*. (2012) investigated the *in-vitro* antimycotic activity of some aromatic plant essential oils against canine isolates of *Malassezia pachydermatis* based on minimum fungicidal activity (MFC) and minimum inhibitory concentrations (MIC). They reported that *Thymus serpillum, Origanum vulgare, Ocimum basilicum, Citrus limon,* and *Mentha piperita* showed a good antifungal action against *M. pachydermatis* inhibiting mycotic development at high dilution.

2.5 SERUM BIOCHEMICAL FINDINGS

2.5.1 Alanine aminotransferase (ALT)

Bensignor (2001) recorded elevated hepatic alanine aminotransferase (ALT) after oral administration of ketoconazole @ 5-10 mg/kg b. wt. for 2-3 weeks or more in dogs affected with *Malassezia* dermatitis.

Tiwari and Varshney (2003) conducted a study on the effect of drugs on liver specific enzymes in dogs and reported that use of ketoconazole resulted in decline of serum ALT and GGT activities which might be due to enzyme inhibition.

Mayer *et. al*. (2008) reported that elevated serum ALT levels were rare in dogs treated with ketoconazole.

Kumar and Sahoo (2011) conducted therapeutic trials by using ketoconazole and fluconazole in *Malassezia* dermatitis dogs. They recorded mean ALT (IU/L) values as 9.42 and 11.14 before therapy while the same were elevated to 20.42 and 16.14 after treating with ketoconazole and fluconazole respectively. The elevated ALT values might be associated with sub-clinical hepatopathy.

2.5.2 Gamma-glutamyltransferase (GGT)

Normal Gamma-glutamyltransferase (GGT) levels in dogs were reported as 1.2 to 6.4 (3.5±1.8) IU/L (Kaneko *et. al*., 1997).

Tiwari and Varshney (2003) conducted a study on effect of drugs on liver specific enzymes in dogs and reported that use of ketoconazole resulted in a decline of serum GGT levels.

2.6 THERAPY

2.6.1 Antifungal drugs

Dufait (1985) conducted therapeutic trials on *Malassezia* dermatitis and opined that ketoconazole should be administered at an average dose of 10-20 mg/kg b. wt. daily orally for a minimum period of 8-12 weeks.

Mason and Evans (1991) in a study on canine dermatitis associated with *Malassezia pachydermatis* reported that oral administration of ketoconazole, selenium sulphide shampoo and rinsing with povidone iodine along with external application of miconazole cream were effective in resolving the dermatitis.

Mobley and Mayer (1994) successfully treated 3 dogs suffering with *Malassezia* dermatitis with oral ketoconazole @ 10 mg/kg b. wt along with external

application of 2% ketoconazole cream on the affected areas once daily with lime sulphur dip twice weekly for four weeks.

Groux and Heripret (1995) successfully treated a Basset suffering from *Malassezia* dermatitis by using ketoconazole, but recurrence was recorded after 7 months. They further advised ketoconazole bath in place of oral ketoconazole for a month to overcome hepatic problems and concluded that incomplete relief was observed after 5 months of usage.

Carlotti and Dassot (1996) found that the therapy with oral ketoconazole along with topical enlconazole was effective in *Malassezia* dermatitis with 75% clinical recovery while no *Malassezia* organisms were noticed on cytological examination after one month of administration.

Griffin (1996) reported that the most commonly used drug against *Malassezia* dermatitis was ketoconazole @ 10 mg/kg b. wt. per day through oral route.

Charach (1997) reported that the systemic therapy with ketoconazole @ 10 mg/kg b. wt. given orally twice daily for 20 to 30 days along with topical therapy in the form of creams, shampoos or rinses were more effective for treating *Malassezia* dermatitis.

Pal *et. al*. (1997) treated two dogs with *Malassezia* infection by oral administration of ketoconazole and lime sulphur bath and noticed regression of lesions by 10 to 16 days, which disappeared by 31-34 days after initiation of therapy.

Christine (1998) stated that chlorhexidine has antibacterial and antifungal properties.

Gorman (1998) stated that ketoconazole is very effective when given at 5-10 mg/kg body weight twice daily with food for 14-28 days. But, large doses have side effects and involve high cost in large breeds of dogs.

Fernandez *et. al*. (1998) concluded that azoles, such as ketoconazole and itraconazole, are antifungal agents available in clinical practice, but they are highly toxic and expensive for prolonged therapy.

Lower *et. al*. (2000) treated a West Highland White Terrier with elephant like skin by using benzyl peroxide shampoo initially twice weekly and then afterwards once a week application followed by leave on application of 2% chlorhexidine conditioner. Ketoconazole was also given orally at 10 mg/kg b. wt. daily. Cephalexin was given @ 22 mg/kg b. wt. orally TID for eight weeks and twice daily topical application of an ointment containing gentamicin sulphate-betamethasone valerate-clotrimazole were also administered with improvement noticed after 30 days of treatment.

Marsella *et. al*. (2000) in their study on *Malassezia* dermatitis in dogs concluded that ketoconazole, itraconazole, and fluconazole are effective against *Malassezia*. However, all these drugs are quite expensive and had the potential for adverse effects. Topical therapy has the advantage of being extremely safe and relatively inexpensive.

Bensignor (2001) successfully treated *Malassezia* dermatitis in dogs by using ketoconazole @ 5 mg/kg b. wt and 10 mg/kg b. wt once daily orally.

Scott *et. al*. (2001) reported that ketoconazole is a good choice for treatment of *Malassezia* dermatitis as it is excreted through sebum and exocrine glands. It is also indicated that efficiency of ketoconazole in treating *Malassezia* dermatitis might

be related to its immunomodulatory and anti-inflammatory effects, with effects on leucotriene synthesis and antiproliferative action on keratinisation by altered metabolism of alltrans-retinoic acid.

Kumar *et. al*. (2002b) evaluated the therapeutic efficacy of different drugs in the treatment of *Malassezia* dermatitis and recorded faster clinical recovery in dogs treated with a combination of oral and topical preparations of ketoconazole.

Saijonmaa-Koulumies (2002) in a review on pyoderma and *Malassezia* dermatitis in dogs stated that topical therapy with a shampoo containing ketoconazole in combination with chlorhexidine three times a week could effectively reduce cutaneous yeast counts. Systemic use of ketoconazole 10 mg/kg b. wt once daily was recommended and it should be continued at least one week beyond clinical cure.

Jasmin *et. al*. (2003) suggested that the 3 per cent chlorhexidine shampoo used twice a week for three weeks, was effective to control elevated *Malassezia* population associated skin lesions and pruritus.

Baksi *et. al*. (2004) evaluated the therapeutic efficacy of different drugs in clinical cases of *Malassezia* dermatitis and reported that the dogs treated with ketoconazole orally @ 5-10 mg/kg b. wt. along with topical application yielded faster clinical recovery.

Jeong *et. al*. (2005) obtained good clinical response in dogs with *Malassezia* dermatitis when treated with itraconazole @ 5 mg/kg b. wt. orally.

Rosales *et. al*. (2005) studied the comparative efficacy of cephalexin alone and cephalexin in combination with terbinafine and ketoconazole in the treatment of *Malassezia* dermatitis in dogs and revealed that cephalexin combined with

terbinafine or ketoconazole was more effective than cephalexin alone though all the treatments decreased the yeast counts.

Kumar *et. al*. (2006) conducted therapeutic studies on *Malassezia* dermatitis and concluded that the ketoconazole preparations along with immuno-stimulants were suggested in treatment of canine malasseziosis.

Rosenkrantz (2006) reported that the topical application of ketoconazole, miconazole or chlorhexidine along with oral administration of ketoconazole was proved to be more effective in dogs with generalised *Malassezia* dermatitis.

Bensignor (2008) performed therapeutic studies with ketoconazole daily @ 10 mg/kg b.wt and itraconazole @ 5 mg/kg b.wt twice a week against *Malassezia* dermatitis in dogs. They reported that the pulse therapy with itraconazole was as effective as daily administration of ketoconazole.

Mayer *et. al*. (2008) conducted a retrospective study in 632 dogs treated with ketoconazole @ 2.6–33.4 mg/kg b. wt. and recorded adverse effects in 14.60% (92) of the dogs which include vomiting (7.10%), anorexia (4.90%), lethargy (1.90%), diarrhoea (1.10%), pruritus (0.60%), erythema (0.30%) and other effects (2.50%).

Negre *et. al*. (2008) opined that topical application of 2% miconazole in combination with 2% chlorhexidine for twice a week for three weeks was found effective against *Malassezia* dermatitis in dogs.

Srivastava *et. al*. (2008) opined that systemic therapy with antifungal agents is necessary when clinical signs were severe and wide spread while in cases with localised lesions topical application of antifungal agents like chlorhexidine and lime sulphur are useful.

George *et. al*. (2010) stated that topical application of chlorhexidine (2.5%) was found more effective than ketoconazole (2%) and selenium sulphide (2.5%) in the management of localised *Malassezia* dermatitis in dogs.

Sickafoose *et. al*. (2010) compared the efficacy ketoconazole and fluconazole @ 5-10 mg/kg b. wt. in the treatment of dogs with *Malassezia* dermatitis and reported that both the drugs had similar efficacy. Adverse effects like anorexia, vomiting, and diarrhoea were seen in 50% and 46% of the dogs treated with ketoconazole and fluconazole respectively.

Kumar and Sahoo (2011) studied the efficacy of ketoconazole and fluconazole against *Malassezia* dermatitis in dogs and observed no adverse side effects like anorexia, vomiting and diarrhoea during the course of treatment or subsequent observation period. Further they opined that topical medications may be used in conjunction with systemic therapy especially in generalised form of infection to hasten the relief and resolution of the disease without recurrence.

Saranya *et. al*. (2012) successfully treated a dog with *Malassezia* infection by using ketoconazole (Tab. Nizral-200mg) at a dose rate of 10 mg/kg body weight along with ketoconazole chlorhexidine shampoo (Ketochlor). Supportive therapy was given with herbal immunomodulating syrup (Immunol) and hepato-protectant (Tefroli forte pet syrup). Clinical improvement was noticed after three weeks of treatment which was determined by clinical examination and repeated cytological examination.

2.6.2 Herbal therapy

Palanichamy and Nagarajan (1990) reported the use of aqueous extracts of the leaves of *Senna alata* to treat eczema, itching and skin infections in humans.

Murdirati and Marurung (1991) concluded that aqueous extract of leaves of *Senna alata* could be used in the treatment of *Psoroptes cuniculi* mite infestation in rabbits.

Damodaran and Venkataraman (1994) conducted trials on the therapeutic efficacy of *Cassia alata* in superficial mycosis and concluded that topical application of *Cassia alata* leaf extract was effective in the treatment of ring worm and pityriasis versicolor in humans.

Ting (2000) performed a comparative study on the efficacy of *Cassia alata* cream and topical ketoconazole cream in the treatment of cutaneous fungal infection and recorded no significant difference between the two treatments in terms of resolution of clinical signs.

Ali-Emmanuel *et. al*. (2003) reported that the topical application of ointments prepared with the ethanolic leaf extracts of *Senna alata*, *Lantana camara* and *Mitracarpus scaber* were effective in the treatment of bovine dermatophilosis.

Herbal essential oils are promising sources for new natural antifungal drugs. These are effective against pathogenic fungi when compared to commercial synthetic antifungal drugs (Faleiro *et. al*., 2003).

Awal *et. al*. (2004) reported that leaves of *Cassia alata* showed antibacterial activity on Gram positive and negative bacteria.

Phongpaichit *et. al*. (2004) reported that some of the components of *Cassia alata* viz. rhein, emodol, 4,5-dihydroxy-1-hydroxy methyl anthrone and 4,5-dihydroxy-2-hydroxy methyl anthraquinone had antifungal activity.

Chungasamarnyart *et. al*. (2008) studied the efficacy of *Cymbopogon citrates, Cymbopogon nardus, Rhinacanthus nasutus, Piper betel* and *Annona squamosa* lotions in canine *Malassezia* dermatitis and emphasized that once a day application

of the herbal lotions for 2-5 weeks were 100% effective in alleviation of the clinical signs.

Esimone *et. al*. (2008) evaluated the antiseptic potentials of *Cassia alata* based herbal soap. They reported a high potency against common pathogens and opined that crude preparations of soapy plants soften the skin epidermis, enhance greater penetration, cleansing and promote rapid healing.

Anandan *et. al*. (2009) evaluated the hepato-protective activity of alcoholic extract of dried leaves of *Cassia alata*, Linn. and attributed the hepato-protective property to the flavonoids present in the leaves.

Eja *et. al*. (2009) reported that the leaf extract of *Cassia alata* is more active against yeast than griseofulvin and clotrimazole.

Rahman *et. al*. (2009) recommended the use of ointments of *Cassia alata* and neem leaves for the treatment of skin lesions in goats and calves.

Gidwani *et. al*. (2010) recommended the use of *Cassia alata* leaves in the treatment of skin disorders as the leaves contain triterpenoid, which has a good anti-inflammatory activity.

Oladele *et. al*. (2010) reported that *Cassia alata* soap 1.5% w/w is effective in the management of superficial fungal skin infections in humans and also opined that a faster therapeutic action could be achieved by using higher concentrations of *C. alata*.

Neharkar and Gaikwad (2011) investigated the hepatoprotective activity of *Cassia alata* (Linn.) leaves against Paracetamol induced hepatic injury in rats and reported that treatment with alcoholic extract of *Cassia alata* leaves at 200 mg/kg

and 400 mg/kg significantly reduced the levels of various biomarkers indicative of hepatic damage.

Sahoo *et. al*. (2011) reported that the essential oil of *Cymbopogon fiexuosus* is efficacious against *Stapphylococcal* and/or *Malassezia* otitis externa.

Rathish *et. al*. (2012) successfully treated malasseziosis in a Labrador Retriever by using 2% ointment daily for 5 weeks prepared from alcoholic extract of *Cassia alata* leaves.

CHAPTER - III

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Location and source of animals

Dogs presented with different dermatological ailments at Teaching Veterinary Clinical Service Complex, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada from March 2012 to August 2012 were taken up for the present study.

3.1.2 Clinical material

Clinical samples collected for this study included serum sample, cellophane tape impression smears, glass slide impression smears, skin scrapings from cutaneous lesions and swab exudates from the affected ears. These samples were subjected to laboratory examination and further diagnostic studies.

During the period under study, a total of 362 dogs having dermatological involvement were screened for the presence of *Malassezia pachydermatis*.

3.1.3 Laboratory material

3.1.3.1 Chemicals

All the dehydrated media and antifungal discs used in the present study were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai, chemicals from S. D. Fine Chemicals Ltd., Mumbai and the glassware from M/S Borosil, Mumbai.

3.1.3.2 Biochemical kits

ALT (SGPT) kit- Beacon diagnostics Pvt. Ltd., Navsari.

GGT kit- Accurex Biomedicals Pvt. Ltd., Mumbai.

3.1.3.3 Culture media

Sabouraud's dextrose broth

Sabouraud's dextrose agar

Brain heart infusion broth

3.1.3.4 Staining reagents

New methylene blue

Gram’s staining kit

3.1.3.5 Antifungal discs

Ketoconazole 10 mcg

Clotrimazole 10mcg

Fluconazole 10mcg

Nystatin 100 units

Amphotericin-B 100 units

3.1.4 Therapeutic agents

a. Ketoconazole cream (Nizral M/S Johnson and Johnson Ltd., Bangalore) containing ketoconazole 2% (w/w)
b. Ketoconazole tablet (Keto M/S Cipla Ltd., Mumbai) containing 200mg of ketoconazole

c. Ketochlor® shampoo, M/S Virbac, Mumbai consisted of Chlorhexidine gluconate 2.3% w/w (2% in free form; 0.3% encapsulated), Ketoconazole 1% w/w

d. *Cassia alata* ointment

Materials:

Cassia alata leaf powder

Soft white paraffin

Preparation of ointment:

Cassia alata ointment was prepared as per the procedure described by Rahman *et. al*. (2009) with slight modification. *Cassia alata* leaves were collected in and around the areas of Gannavaram. The collected leaves were dried at room temperature for 7-10 days, while drying the leaves were turned over every day. The leaves were finally dried at 50°C for 48 hours and reduced to coarse powder (Plate 2) by using a grinder. Then an ointment (Plate 3) was prepared by mixing 30 parts of leaf powder with 70 parts of soft white paraffin.

e. *Cassia alata* soap

Materials:

Cassia alata leaf powder

Palm kernel oil (PKO)

Distilled water

Sodium hydroxide pellets

Plate 1: *Cassia alata* shrub with characteristic candle stick appearance of flowers

(It belongs to the family Caesalpinaceae and commonly known as Candlebra bush / Ringworm bush / Dadrughna)

Plate 2: *Cassia alata* leaf powder

Plate 3: *Cassia alata* ointment

Plate 4: *Cassia alata* soap with different moulds

Preparation:

Cassia alata soap was prepared by using the procedure as described by Omobuwajo *et*. *al*. (2011)

Soap formula:

2.3 liters of Palm kernel oil

0.36 kg of NaOH

0.9 liters of distilled water

The cold method of soap making was used to prepare the herbal soap. The NaOH was dissolved in water in a beaker and allowed to cool, poured gradually into the palm kernel oil with continuous stirring. The 7% *Cassia alata* leaf powder was added gradually by stirring with a glass rod. The herbal soap mixture was then poured into moulds of desired shapes and allowed to solidify (Plate 4).

3.2 METHODS

3.2.1 Analysis of prevalence

The prevalence of *Malassezia* dermatitis was calculated taking into account, the number of cases positive for *Malassezia pachydermatis* out of total number of dermatological cases screened. The data were further analysed in relation to age, sex, breed and month. The age groups among the dogs were classified as puppies (<7 months), young adult (7 months to 2 years), adult (>2 years to 7 years) and aged (>7 years), as per the classification explained by George *et*. *al*. (2012).

3.2.2 Diagnosis

3.2.2.1 History

Thorough history was collected from the owners / attendants and the information thus obtained along with the observations made on the patient were recorded in the proforma developed for this purpose (Appendix).

Dogs of all ages, breeds, of either sex were included. The criteria for including dogs were history of pruriginous dermatitis and lesions characterised by erythema, papules, alopecia, lichenification, hyperpigmentation and oily seborrhoeic keratosis. In all these cases the underlying disease or predisposing factors were determined.

3.2.2.2 Clinical examination

Dogs presented with dermatological disorders were thoroughly examined for the presence of symptoms such as pruritus, alopecia, erythema, hyperpigmentation, keratosebaceous plaques, lichenification, rancid odour from the body, excessive foot licking, excoriation, head shaking and for the presence of otic discharge.

Lesions on different body parts were recorded to know the region wise occurrence of *Malassezia* dermatitis. The dogs were categorised into generalised *Malassezia* dermatitis and localised *Malassezia* dermatitis based upon the extent of clinical lesions. After detailed history collection and thorough clinical examination of the dogs, they were evaluated clinically based on pruritus score and clinical index score.

3.2.2.2.1 Pruritus Score

Pruritus was graded and scored by the owners. Each dog was assigned a value from 1 to 5 for pruritus based on a five-point scale where higher numbers signifying more severe clinical signs. An average score for the week was calculated based on the daily scores given by the owners as per the method adopted by Marsella *et. al*., 2000. The pruritus score was recorded at weekly intervals prior to therapy and after initiation of therapy. The definition of each score was given below.

Score	Definition
1	Mild pruritus (scratching, rubbing, chewing, or licking for less than 10% of day)
2	Mild-moderate pruritus (scratching, rubbing, chewing, or licking for less than 10-30% of day)
3	Moderate pruritus (scratching, rubbing, chewing, or licking for 30-50% of day)
4	Moderate-severe pruritus (scratching, rubbing, chewing, or licking for less than 50-75% of day but still able to relax and sleep at night)
5	Severe pruritus (scratching, rubbing, chewing, or licking all the time – even at night and during a meal)

3.2.2.2.2 Clinical Index Score (CIS)

The dogs were also assessed for the degree of severity of clinical signs and a grading scale was used to score the clinical signs as per the procedure described by Sickafoose *et. al*., (2010) with slight modification. Each dog was assigned a value from 0 to 3 for each of five clinical features typical of *Malassezia* dermatitis viz. erythema, greasy exudates, scaling, hyperpigmentation and lichenification. The total

score of five clinical features was taken as CIS with maximum of 15 for each dog. CIS was recorded at weekly intervals prior to therapy and after initiation of therapy.

Score	Severity
0	Absent
1	Mild
2	Moderate
3	Severe

3.2.2.3 Cytology

3.2.2.3.1 Examination of tape impression smears

Tape impression smears were examined based on the technique used by Eluk *et. al*. (2003). These were used to collect the samples from dry lesions. Hair present around the skin lesion was trimmed with a curved scissor. A piece of clear one sided cellophane acetate tape of 5.5 cm length and 2.5 cm width was taken from the roll and applied to the affected area, pressed twice firmly for two seconds. After removal, the strip was placed onto a pre cleaned microscopic glass slide with the sticky surface facing upward and fixed properly at both the ends. The prepared smears were stained with new methylene blue for one minute, the stain was removed, rinsed with distilled water and allowed to air dry. The stained slides were then examined under oil immersion objective.

3.2.2.3.2 Glass slide impression smears

Glass slide impression smears were used to collect the samples from wet lesions (Muse, 2000). The hair around the lesions was clipped and cleaned with spirit. The adjacent skin was gently squeezed and the glass slide pressed over the

lesion. The slide was air dried and stained with new methylene blue for one minute and examined under oil immersion.

3 .2.2.3.3 Examination of roll smear cytology

Roll swab smears were collected from external ear canal based on the technique used by Gotthelf and Young (1997). Ear exudates were collected from infected ears with a sterile swab, rolled over a clean glass slide, heat fixed, stained with new methylene blue, rinsed with distilled water, air dried and examined under oil immersion.

The stained smears were examined under oil immersion to observe for the presence of *Malassezia*, cocci, rods and neutrophils. Number of *Malassezia* yeasts present in ten microscopic fields was counted and the average was taken. (Nobre *et. al*., 2001 and Bensignor, 2008). Presence of at least 10 from the ear and 5 yeasts from the skin samples were considered positive.

3.2.2.4 Examination of skin scrapings for mites

Skin scrapings were collected from cases of *Malassezia* dermatitis to confirm the possibility of presence of mites along with *Malassezia* yeasts. Skin scrapings were collected till blood oozed from active lesions of the dogs included in the study. The sample thus collected was transferred into a test tube containing about 5 ml of 10% KOH and heated gently for 3-5 minutes. The sample was centrifuged at 1500 rpm for 3-5 minutes and supernatant was discarded. A drop of sediment was taken on a clean glass slide covered with a cover slip and examined under microscope (10X and 40X) for presence of adult mites or their developmental stages which were identified as per their morphological characters as described by Soulsby (1986).

3.2.2.5 Cultural examination

3.2.2.5.1 Isolation and identification of *Malassezia pachydermatis*

The samples that were positive on cytological examination were subjected for culture. Samples were collected with sterile swab and immediately transferred to Sabouraud's dextrose broth with 0.05mg of chloramphenicol (Huang *et. al*., 1993) and incubated at 37°C for 24 hours and pure culture was made by streaking 24 hour culture onto Sabouraud's dextrose agar added with chloramphenicol at the same dose rate and incubated at 37°C and that was monitored daily for 5 days. Colony characters were studied by observing shape, size, colour and consistency of colonies. Individual colonies were picked up, smeared over clean glass slides stained with new methylene blue solution, washed with distilled water, air dried and examined under oil immersion microscope for the presence of characteristic foot print or peanut shape organisms. Identification of species *Malassezia pachydermatis* was based on macroscopic and microscopic appearance of colonies and its ability to grow on the medium with no lipid supplementation.

3.2.3 *In-vitro* sensitivity testing

3.2.3.1 *In-vitro* antifungal sensitivity testing

The samples collected from 15 dogs with *Malassezia* dermatitis were subjected to *in-vitro* antifungal sensitivity test by disc diffusion method as a preliminary study to select the appropriate antifungal agent based on their sensitivity (Kiss *et. al*., 1997).

A small amount of growth from the isolated colonies obtained on Sabouraud's dextrose agar (SDA) with chloramphenicol was transferred into a tube of Brain heart

infusion broth with help of platinum loop and incubated at 37°C for 48 hours. The broth culture was evenly smeared over the surface of SDA plates with the help of sterile cotton swab. Antifungal discs i.e. ketoconazole, clotrimazole, fluconazole, nystatin and amphotericin-B discs were placed on the surface of the agar with sterile forceps keeping uniform spacing between two discs and pressed gently to ensure full contact. The results were recorded after 48-72 hours of incubation at 37°C by measuring the sizes of zone of inhibition around each disc and compared with the chart.

3.2.3.2 *In-vitro* antifungal sensitivity of *Cassia alata* leaf powder

The antifungal sensitivity of *Cassia alata* was tested as per the method described by Abubacker *et. al*. (2008) with slight modifications. Dry leaf powder of *Cassia alata* @ 200 mg was mixed with 20 ml of Sabouraud's dextrose agar (SDA) medium (10 mg/ml). The broth culture of *Malassezia* was evenly smeared over the surface of the SDA plates with help of sterile cotton swab and incubated at 37°C for 48 hours and observed for the growth of *Malassezia.*

3.2.4 Serum biochemistry

About 5 ml of blood was collected aseptically from cephalic vein of the affected dogs before and after therapy and the serum was separated out. Similarly serum was collected from eight apparently healthy dogs for estimation of the serum alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT).

3.2.4.1 Alanine aminotransferase (ALT)

Serum alanine aminotransferase (IU/L) was estimated by IFCC method by using reagents supplied by M/S Beacon Diagnostics Pvt. Ltd., Navsari, in UV-Vis-spectrophotometer 10 S, Thermoscientific Genesys Pvt. Ltd., Mumbai.

3.2.4.2 Gamma-glutamyltransferase (GGT)

Serum gamma-glutamyltransferase (GGT) was estimated by IFCC method by using reagents supplied by Accurex Biomedicals Pvt. Ltd., Mumbai, in UV-Vis-spectrophotometer 10 S, Thermoscientific Genesys Pvt. Ltd., Mumbai.

3.2.5 Therapeutic trials

Therapeutic agents ketoconazole and *Cassia alata* were selected based upon *in-vitro* antimycotic sensitivity test results. Dogs treated with systemic antibiotics or antifungal medication within the previous 30 days were not included in the therapeutic trials.

Thirty two dogs were randomly assigned to four groups each consisting of eight upon confirmation of etiology as *Malassezia* dermatitis. Dogs showing generalized *Malassezia* dermatitis were allotted to Group I and II, while dogs showing localized *Malassezia* dermatitis were allotted to Group III and IV. Dogs with concurrent conditions were given specific therapy along with above therapeutic regimen. In dogs with *Malassezia* dermatitis and pyoderma as a concurrent condition, antibiotic cephalexin was administered orally @ 22 mg/kg b. wt. BID (Rosales *et. al*., 2005) till bacteriological recovery.

3.2.5.1 Generalised *Malassezia* dermatitis

3.2.5.1.1 Group I (Tab. Ketoconazole, Ketochlor® shampoo)

Dogs of Group I were subjected to treatment with oral administration of Tab. ketoconazole @ 10 mg/kg b. wt SID (Bensignor, 2008) and was continued for one week beyond clinical cure. Ketochlor® shampoo bath was advised twice a week initially for a week and then afterwards for once a week. The owners were advised to continue the shampoo bath for two weeks even after the regression of symptoms.

3.2.5.1.2 Group II (Tab. Ketoconazole, *Cassia alata* soap and *Cassia alata* ointment)

Dogs of Group II were subjected to treatment with oral administration of Tab. ketoconazole @ 10 mg/kg b. wt SID and was continued one week beyond clinical cure. *Cassia alata* soap bath was advised twice a week initially for one week and then afterwards for once a week. *Cassia alata* ointment was applied twice a day in the first week and then afterwards for once a day. The owners were advised to continue the soap bath for two weeks, even after the regression of the symptoms.

3.2.5.2 Localised *Malassezia* dermatitis

3.2.5.2.1 Group III (Ketochlor® shampoo and Ketoconazole cream)

Dogs of Group III were subjected to treatment with ketoconazole cream twice a day in the first week and then afterwards for once a day. Ketochlor® shampoo bath twice a week initially for a week and then afterwards for once a week. The owners were advised to continue the shampoo bath for two weeks even after the regression of symptoms.

3.2.5.2.2 Group IV (*Cassia alata* soap and *Cassia alata* ointment)

Dogs of Group IV were subjected to treatment with *Cassia alata* ointment twice a day in the first week and then afterwards for once a day. *Cassia alata* soap bath was advised twice a week initially for one week and then afterwards for once a week. The owners were advised to continue the soap bath for two weeks, even after the regression of the symptoms.

3.2.5.3 Evaluation of different therapeutic regimens

The therapeutic efficacy of the drugs was assessed based upon clinical cure (pruritus score and clinical index score) and mycological cure (cytology). Pruritus score, clinical index score (CIS) and cytology were recorded at weekly intervals after initiation of therapy. The owners were requested to inform about drug related adverse effects in generalised *Malassezia* dermatitis dogs.

3.2.6 Statistical analysis

The results obtained were subjected to statistical analysis as per the methods described by Snedecor and Cochran (1994) and by using SPSS 20.0.0 version.

CHAPTER - IV

4. RESULTS

The present study was conducted to investigate *Malassezia* dermatitis in dogs presented to the Teaching Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada, Krishna (Dt), Andhra Pradesh. The prevalence, symptomatology and therapeutic efficacy of different drugs were studied and recorded.

4.1 PREVALENCE

During period under study (March 2012 to August 2012), a total of 3849 canine cases were registered at Teaching Veterinary Clinical Complex, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada. Out of these, 362 dogs with dermatological affections were screened and *Malassezia* dermatitis accounted for 10.22 per cent with 37 affected dogs.

4.1.1 Age-wise prevalence

The age-wise prevalence of *Malassezia* dermatitis in dogs is depicted in Table 1 and Figure 1. The prevalence percentage of *Malassezia* dermatitis in adult and young adult age groups was 51.35 and 27.02 respectively, whereas in puppies and aged dogs the prevalence was 10.81%. Among the total number of dogs positive for *Malassezia* dermatitis, the prevalence was high in adult dogs (2-7 years) and less in puppies and dogs aged above 7 years. There was no significant difference ($P>0.05$) among different age groups studied.

4.1.2 Sex-wise prevalence

Out of 37 dogs with *Malassezia* dermatitis, 24 (64.86%) were male and 13 (35.14%) were female. In the present study no statistically significant difference (P>0.05) between male and female dogs was noticed in the prevalence. The findings were presented in Table 2 and Figure 2.

4.1.3 Breed-wise prevalence

Observations shown in Table 3 and Figure 3 revealed breed-wise prevalence of *Malassezia* dermatitis in the present study and it was found to be highest in Labrador Retriever accounting for 16 cases (43.24%), followed by Pomeranian 8 (21.62%), mixed breed 6 (16.21%), Daschund 3 (8.10%), German Shepherd 2 (5.40%) and Lhasa Apso 2 (5.40%). In the present study *Malassezia* dermatitis was not recorded in the breeds of Pug, Saint Bernard, Dalmatian and Doberman. The prevalence percentage did not vary significantly (P>0.05) among the breeds.

4.1.4 Month-wise prevalence

The month-wise prevalence of *Malassezia* dermatitis in dogs is depicted in Table 4 and Figure 4. The prevalence percentage during the months of May, June, July, April, March and August was 37.83, 27.02, 10.81, 10.81, 8.10 and 5.40 respectively. Higher number of dogs were affected during the months of May and June and there was no significant difference (P>0.05) between the month-wise prevalence.

Table 1: Age wise prevalence of *Malassezia* dermatitis in dogs

Sl. No	Age group	Total number screened	Number affected	Per cent prevalence out of affected	Chi-square value
1	Puppies (< 7 months)	91	4	10.81	8^{NS}
2	Young adult (>7months-2years)	71	10	27.02	
3	Adult (>2years-7years)	123	19	51.35	
4	Aged (7years)	77	4	10.81	
Total		**362**	**37**	**100.00**	

NS : Not significant (P>0.05)

Table 2: Sex wise prevalence of *Malassezia* dermatitis in dogs

Sl. No	Sex	Total number screened	Number affected	Per cent prevalence out of affected	Chi-square value
1	Male	241	24	64.86	2^{NS}
2	Female	121	13	35.14	
Total		**362**	**37**	**100.00**	

NS : Not significant (P>0.05)

Table 3: Breed wise prevalence of *Malassezia* dermatitis in dogs

Sl. No	Breed	Total number screened	Number affected	Per cent prevalence out of affected	Chi-square value
1	Labrador Retriever	57	16	43.24	
2	German Shepherd	10	2	5.40	
3	Pomeranian	152	8	21.62	
4	Mixed	77	6	16.21	
5	Dachshund	16	3	8.10	
6	Lhasa Apso	10	2	5.40	**50^{NS}**
7	Pug	15	0	0	
8	Saint Bernard	6	0	0	
9	Dalmatian	11	0	0	
10	Doberman	8	0	0	
Total		**362**	**37**	**100.00**	

NS : Not significant (P>0.05)

Table 4: Month wise prevalence of *Malassezia* dermatitis in dogs

Sl.No	Month	Total number screened	Number affected	Per cent prevalence out of affected	Chi-square value
1	March	48	3	8.10	
2	April	46	4	10.81	
3	May	102	14	37.83	
4	June	78	10	27.02	**24^{NS}**
5	July	46	4	10.81	
6	August	42	2	5.40	
Total		**362**	**37**	**100.00**	

NS : Not significant (P>0.05)

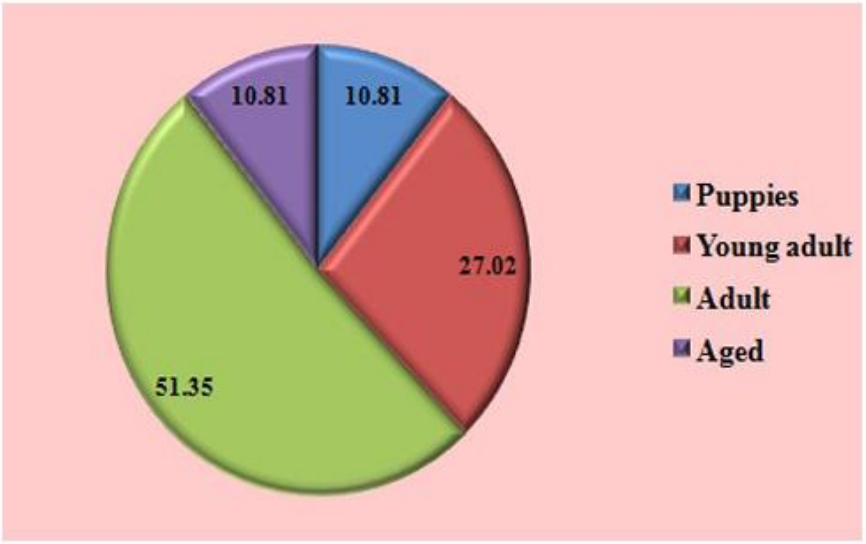

Figure 1: Age wise prevalence of *Malassezia* dermatitis in dogs

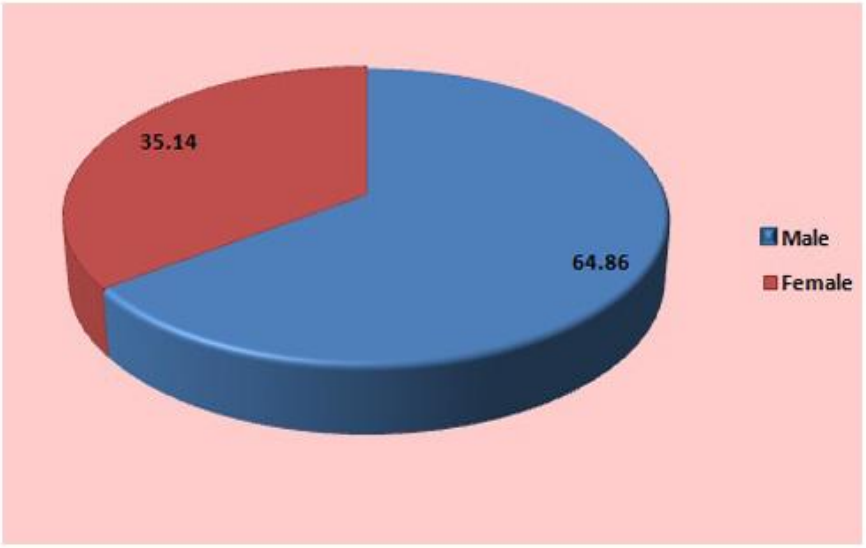

Figure 2: Sex wise prevalence of *Malassezia* dermatitis in dogs

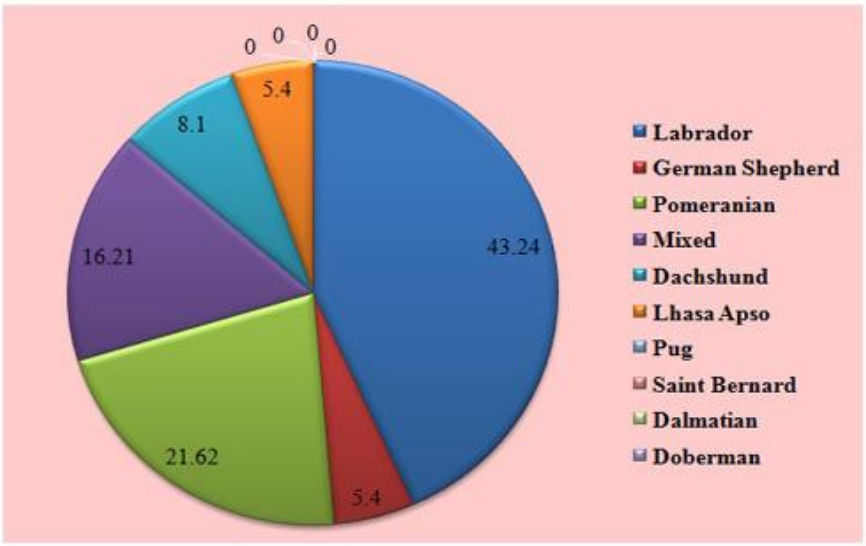

Figure 3: Breed wise prevalence of *Malassezia* dermatitis in dogs

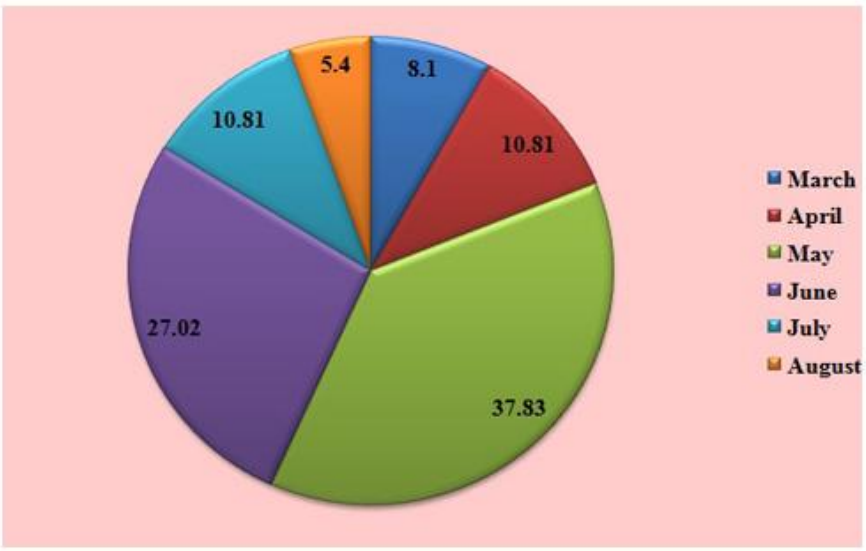

Figure 4: Month wise prevalence of *Malassezia* dermatitis in dogs

4.2 SYMPTOMATOLOGY

The dogs suffering from *Malassezia* dermatitis revealed a wide variety of clinical manifestations and were presented in Table 5 and Figure 5. Clinical examination of 37 dogs with *Malassezia* dermatitis revealed symptoms of pruritus in all the dogs (100%), erythema in 29 (78.37%), scaling in 22 (59.45%), exudation in 24 (64.86%), alopecia in 20 (54.05%), offensive odour in 33 (89.18%), hyperpigmentation in 18 (48.64%) and lichenification in 16 (43.24%) dogs (Plates 5 to 8). Among the clinical signs recorded in the present study pruritus, offensive odour and erythema were more common.

In dogs affected with *Malassezia* dermatitis the mean pruritus scores were 4.00±0.26, 4.12±0.29, 2.75±0.25 and 2.87±0.22 in Group I, Group II, Group III and Group IV respectively. The pruritus score ranged from 2-5 in the affected dogs. The mean clinical index score values in dogs affected with *Malassezia* dermatitis were 9.37±0.53, 9.62±0.53, 5.12±0.35 and 4.62±0.41 in Group I, Group II, Group III and Group IV respectively (Table 6). The clinical index score ranged from 4 to13 in dogs affected with either generalised *Malassezia* dermatitis or localised *Malassezia* dermatitis.

The topography of lesions in dogs with *Malassezia* dermatitis was presented in the Table 7 and Figure 6. The lesions were localised or multifocal. The assessment of clinical cases was done mainly on the basis of distribution pattern of lesions (either generalised or localised) which in turn helped in planning the therapeutic management of the same. It was noticed that the most affected region in dogs with *Malassezia* dermatitis in the present study was the auricular (81.08%). The other regions affected were thoracic limbs (distal regions) in 27 dogs (72.97%), pelvic

Table 5: Symptomatology of *Malassezia* dermatitis in dogs (n=37)

Clinical Sign	Number positive	Percentage (%)
Pruritus	37	100.00
Erythema	29	78.37
Scales	22	59.45
Exudation	24	64.86
Alopecia	20	54.05
Offensive odour	33	89.18
Hyperpigmentation	18	48.64
Lichenification	16	43.24

Table 6: Pruritus score, clinical index score, *Malassezia* count in dogs with *Malassezia* dermatitis (Mean±SE)

Group n=8	Pruritus score	Clinical index score	*Malassezia* / field
I	4.00±0.26	9.37±0.53	16.71±5.26
II	4.12±0.29	9.62±0.53	14.80±5.11
III	2.75±0.25	5.12±0.35	11.07±1.68
IV	2.87±0.22	4.62±0.41	10.38±0.82

Symptoms in dogs with *Malassezia* dermatitis

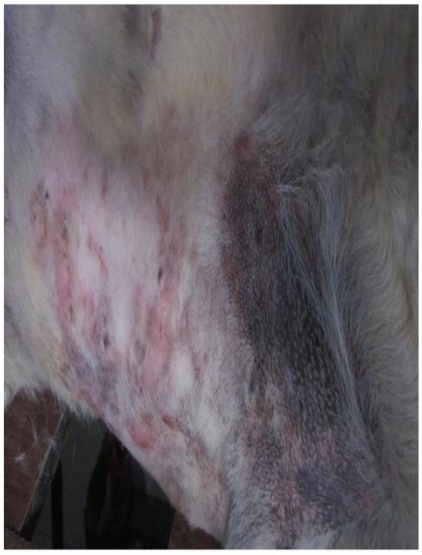

Plate 5: A dog showing patches of erythema, alopecia, exudation and hyperpigmentation on the ventral abdomen

Plate 6: Alopecia in a dog with generalised *Malassezia* dermatitis

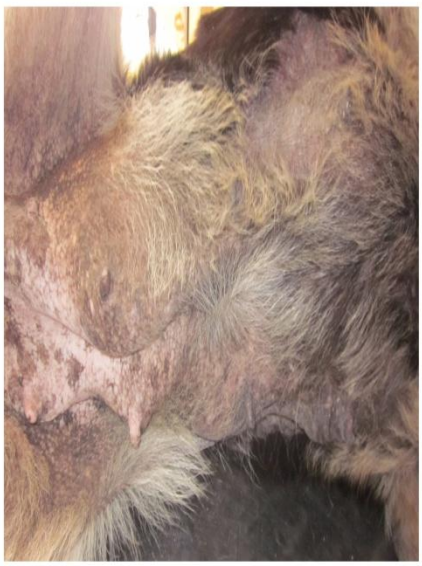

Plate 7: A bitch showing characteristic hyperpigmentation on the ventral abdomen

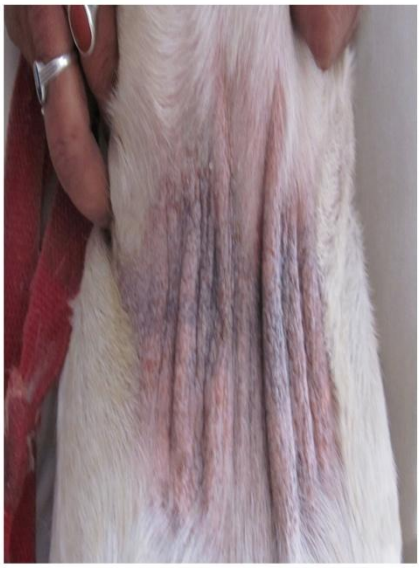

Plate 8: Marked lichenification on the ventral neck of a Labrador Retriever

Table 7: Topography of lesions in dogs with *Malassezia* dermatitis (n=37)

Region	Number affected	Percentage (%)
Thoracic limbs (distal regions)	27	72.97
Auricular	30	81.08
Face	10	27.02
Pelvic	18	48.64
Pelvic limbs (distal regions)	25	67.56
Neck	8	21.62
Abdominal	20	54.05
Pectoral	6	16.21
Dorsum	3	8.10

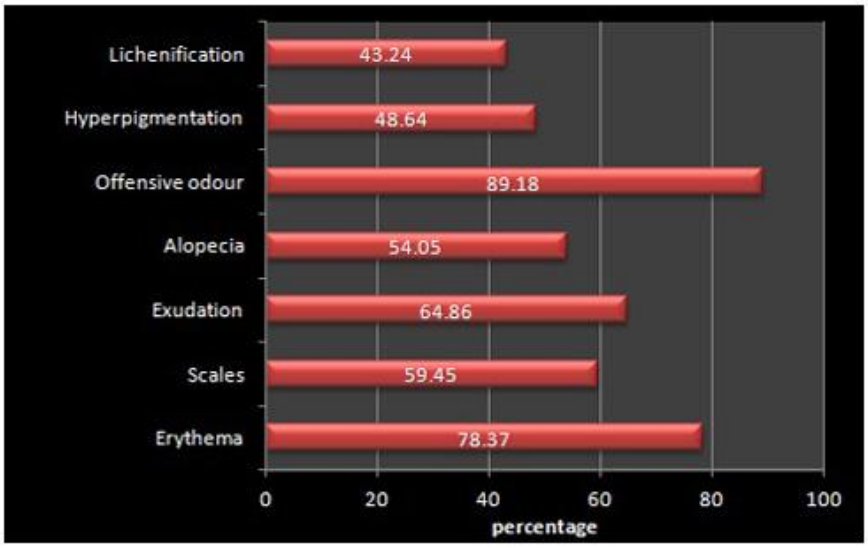

Figure 5: Symptomatology of *Malassezia* dermatitis in dogs (n=37)

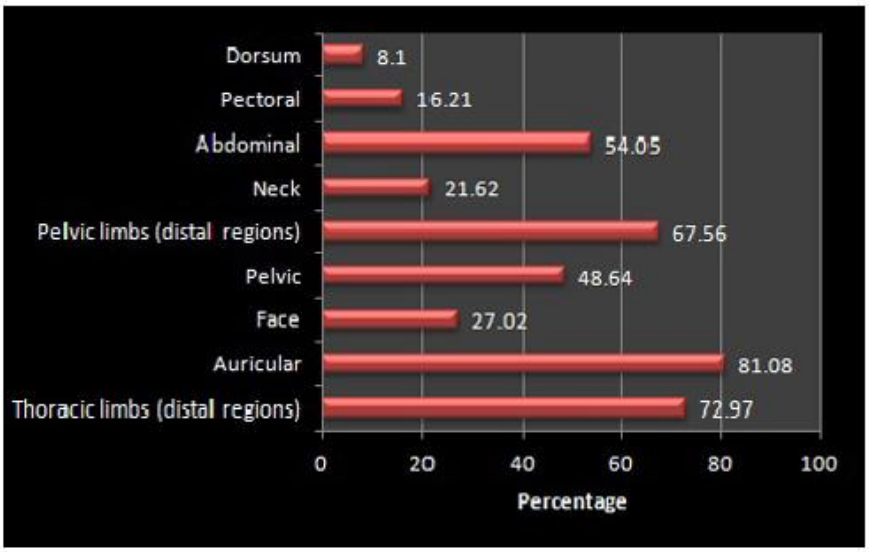

Figure 6: Topography of lesions in dogs with *Malassezia* dermatitis (n=37)

Topography of lesions and symptoms in dogs with *Malassezia* dermatitis

Plate 9: Chronic erythemato-ceruminous otitis associated with *Malassezia* in a German Shepherd

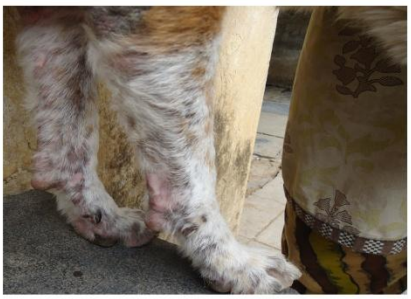

Plate 10: A dog showing erythema, lichenification and alopecia on the distal region of forelimbs

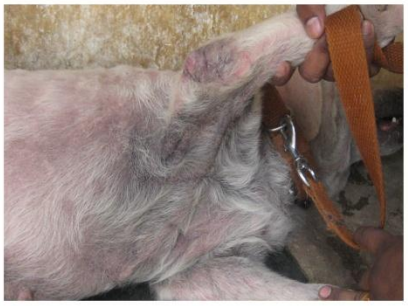

Plate 11: Hyperpigmentation and lichenification on the ventral thoracic and axillar region of a Labrador Retriever

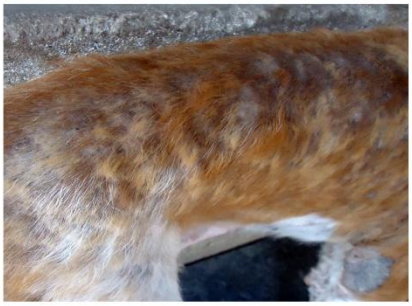

Plate 12: Presence of scales, crusts and diffuse alopecia on the dorsal region

Plate 13: A dog showing characteristic hyperpigmentation in the peri-anal region

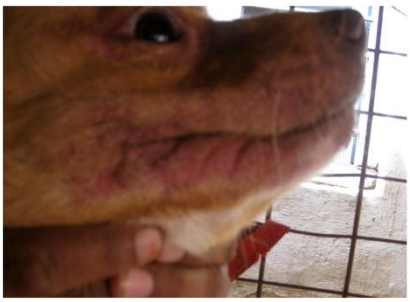

Plate 14: Facial erythema in a mixed breed dog

limbs (distal regions) in 25 dogs (67.56%), abdominal region in 20 dogs (54.05%) and pelvic region in 18 dogs (48.64%). Involvement of face, neck, pectoral, dorsum were seen in 10, 8, 6 and 3 dogs with a percentage prevalence of 27.02, 21.62, 16.21 and 8.10 respectively (Plates 9 to 14). Out of 37 affected dogs 18 were categorized under generalised *Malassezia* dermatitis and in 19 dogs it was localised.

4.3 DIAGNOSIS

4.3.1 Cytology

Out of 362 dogs screened, 37 dogs were diagnosed as *Malassezia* dermatitis by cytological examination. Cytology revealed characteristic foot print / peanut shaped *Malassezia pachydermatis* organisms. The number of organisms ranged from 6 to 50 per microscopic field (oil immersion) (Plate 15). The mean *Malassezia* count in Group I, Group II, Group III and Group IV were 16.71±5.26, 14.80±5.11, 11.07±1.68 and 10.38±0.82 respectively (Table 6).

In the present study *Malassezia* alone was noticed in 12 cases (32.44%), while it was as a mixed infection with plenty of bacterial cocci in 20 cases (54.05%) and with bacterial cocci, rods and neutrophils in 5 cases (13.51%). On the whole *Malassezia* in conjunction with bacteria was seen in 25 cases (67.56%) (Table 8 & Figure 7).

4.3.2 Isolation and identification of *Malassezia pachydermatis*

Thirty seven dogs which were positive for *Malassezia* organisms on cytological examination were subjected for culture on Sabouraud's dextrose agar. The species identification of *Malassezia pachydermatis* was confirmed by its ability

Table 8: Prevalence of *Malassezia* as monomicrobial / mixed infection in affected dogs

Sl. No	Type of micro-organism (Malassezia/Cocci/Rods)	Number affected	Per cent prevalence out of affected
1	*Malassezia* alone	12	32.44
2	*Malassezia* + bacteria	25	67.56
	a) *Malassezia* + cocci	20	54.05
	b) *Malassezia* + cocci +rods	5	13.51
Total		**37**	**100.00**

Table 9: Concurrent infections / conditions noticed along with *Malassezia* dermatitis in dogs (n=37)

Sl. No	Name of the infection /condition	Frequency (n)	Percentage (%)
1	Pyoderma	5	13.51
2	Hypothyroidism	1	2.70
3	Renal failure	1	2.70
4	Tick infestation	3	8.10
5	Demodicosis	4	10.81
6	Scabies	1	2.70
Total		**15**	**40.54**

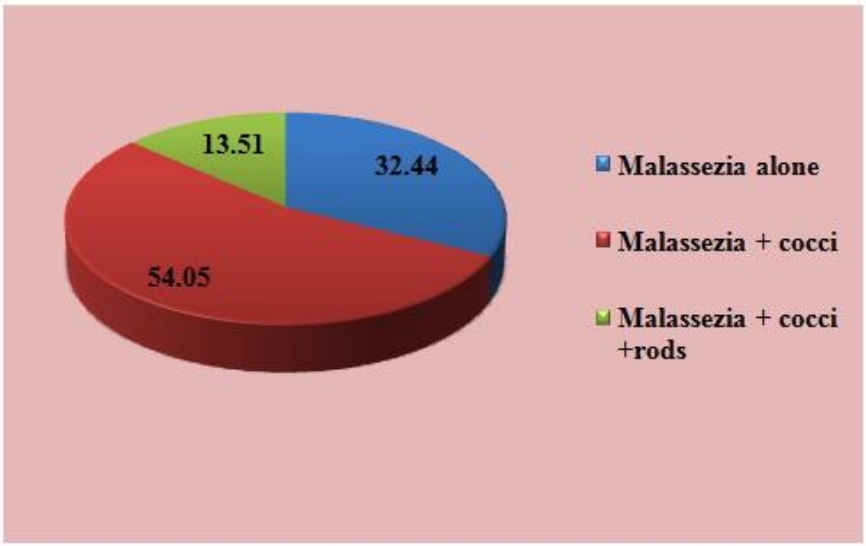

Figure 7: Prevalence of *Malassezia* as monobicrobial / mixed infection in affected dogs

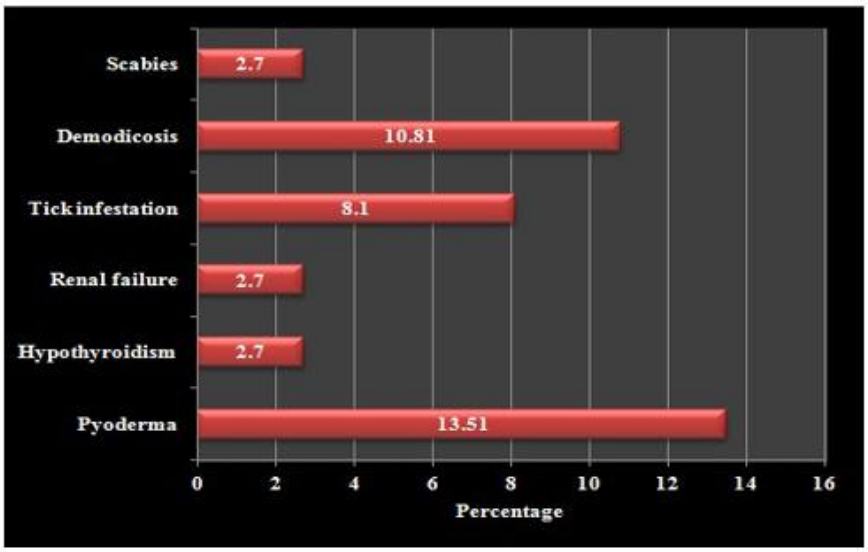

Figure 8: Concurrent infections / conditions noticed along with *Malassezia* dermatitis in dogs (n=37)

to grow on SDA without addition of any lipids. In the present investigation *Malassezia pachydermatis* colonies were observed as smooth, convex with pasty texture after 36 to 48 hours incubation at 37ºC. Initially the colonies were white to cream in colour and darkened to orange to light brown in colour with advancement of age (Plates 17 & 18). Individual colonies were picked up, stained with new methylene blue and examined under oil immersion for the presence of *Malassezia* organisms. *Malassezia* yeasts were pear shaped by 48 hours and peanut shaped by 72 hours of incubation.

4.3.3 Concurrent infections / conditions

The concurrent infections / conditions associated with *Malassezia* dermatitis were listed in Table 9 and Figure 8. Out of 37 dogs with *Malassezia* dermatitis, 15 dogs (40.54%) were in association with concurrent infection / condition. Out of these, pyoderma was recorded in 5 dogs (13.51%), hypothyroidism in 1 (2.70%), renal failure in 1 (2.70%), tick infestation in 3 (8.10%), demodicosis in 4 (10.81%) and scabies in 1 (2.70%) case (Plates 19 to 22).

4.4 *IN-VITRO* ANTIFUNGAL SENSITIVITY TEST

4.4.1 Antifungal drugs

The results of antifungal sensitivity test carried out on 15 samples positive for *Malassezia pachydermatis* were presented in the Table 10 and Figure 9. The pattern of antifungal sensitivity in descending order were ketoconazole (93.33%) followed by clotrimazole (53.33%), amphotericin-B (33.33%), fluconazole and nystatin

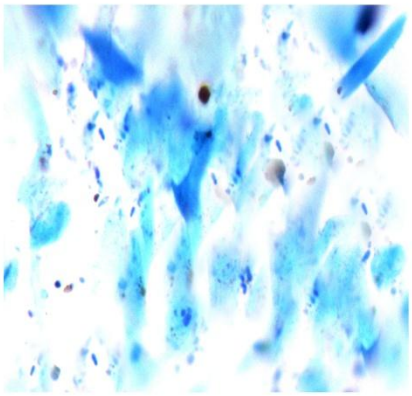

Plate 15: Photomicrograph of cytology smear revealing number of *Malassezia* organisms

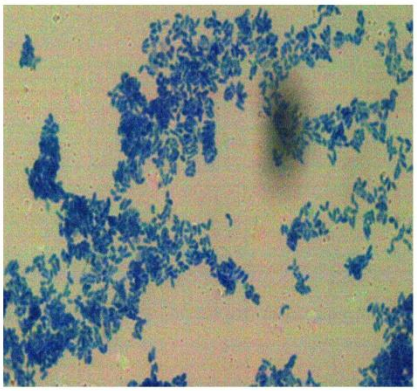

Plate 16: Photomicrograph of *Malassezia pachydermatis* obtained from pure culture

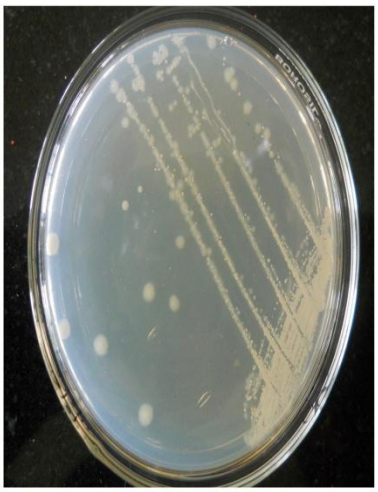

Plate 17: Colonies of *Malassezia pachydermatis* after 48 hours on SDA

Plate 18: Smooth, convex and round cream colour colonies of *Malassezia pachydermatis* after 72 hours on SDA

Concurrent conditions noticed along with *Malassezia* dermatitis in dogs

Plate 19: *Malassezia* dermatitis associated with pyoderma in a mixed breed dog.

Plate 20: A dog with generalised *Malassezia* dermatitis associated with demodicosis showing lichenification, alopecia and intense erythema on all the limbs and the ventral abdomen.

Plate 21: *Malassezia* dermatitis in association with hypothyroidism in a Labrador Retriever showing erythema, alopecia and typical rat tail appearance

Plate 22: A dog with generalised *Malassezia* dermatitis associated with scabies showing lichenification, hyperpigmentation and alopecia

Table 10: *In-vitro* antifungal sensitivity pattern of *Malassezia pachydermatis* (n=15)

Sl.No	Name of the antimycotic disc	No. of isolates sensitive	Percentage	No. of isolates resistant	Percentage
1	Ketoconazole	14	93.33	1	6.66
2	Fluconazole	4	26.66	11	73.33
3	Clotrimazole	8	53.33	7	46.66
4	Amphoterin-B	5	33.33	10	66.66
5	Nystatin	4	26.66	11	73.33

Table 11: Serum biochemical findings (Mean±SE) in healthy and dogs with *Malassezia* dermatitis (each group n=8)

Parameter	Healthy control	Group I	Group II	Group III	Group IV
ALT (IU/L)	18.76 ± 1.30^{a}	18.98 ± 2.58^{a}	18.33 ± 2.35^{a}	19.20 ± 1.61^{a}	18.98 ± 1.16^{a}
GGT (IU/L)	4.77 ± 0.74^{b}	4.92 ± 0.36^{b}	4.63 ± 0.43^{b}	5.06 ± 0.53^{b}	4.63 ± 0.75^{b}

Means bearing different superscripts within a row differ significantly ($P<0.05$)

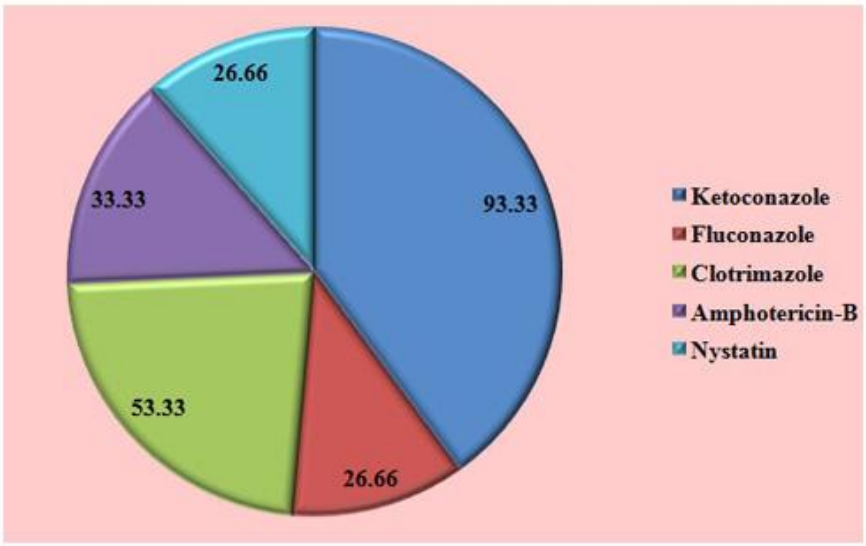

Figure 9: *In-vitro* antifungal sensitivity pattern of *Malassezia pachydermatis* (n=15)

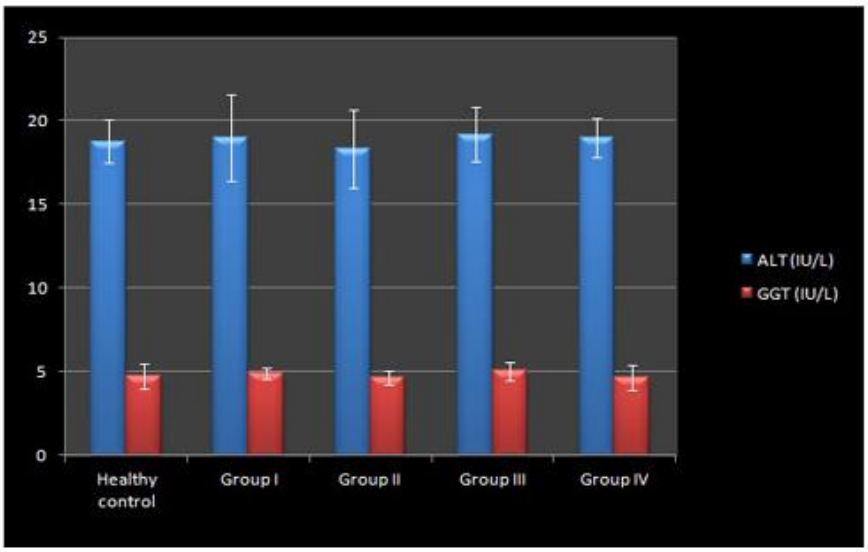

Figure 10: Serum biochemical findings (Mean±SE) in healthy and dogs with *Malassezia* dermatitis (each group n=8)

(26.66%). Higher number of isolates (93.33%) were sensitive to ketoconazole and 73.33% of isolates were resistant to fluconazole and nystatin.

4.4.2 *Cassia alata* leaf powder

Sabouraud's dextrose agar plates supplemented with 200 mg of *Cassia alata* leaf powder (10 mg/ml) were daily observed for the growth of *Malassezia pachydermatis*. No growth was observed after 72 hours of incubation.

4.5 SERUM BIOCHEMICAL FINDINGS

4.5.1 Alanine aminotransferase (ALT)

The mean serum ALT (IU/L) value recorded in the healthy control was 18.76±1.30 with the values ranged from 15.71 to 26.19. In dogs affected with *Malassezia* dermatitis the values were 18.98±2.58, 18.33±2.35, 19.20±1.61 and 18.98±1.16 in Group I, Group II, Group III and Group IV respectively (Table 11 & Figure10). The values did not differ significantly (P>0.05) from those of healthy control.

4.5.2 Gamma-glutamyltransferase (GGT)

In the present study the mean serum GGT (IU/L) value in the healthy dogs was 4.77±0.74 which ranged from 2.31 to 6.94. In affected dogs the same were 4.92±0.36, 4.63±0.43, 5.06±0.53 and 4.63±0.75 in Group I, Group II, Group III and Group IV respectively (Table 11 & Figure 10). No significant difference (P>0.05) was observed between healthy and affected dogs.

4.6 THERAPEUTIC TRIALS

4.6.1 Generalised *Malassezia* dermatitis

Sixteen dogs with generalised *Malassezia* dermatitis were randomly selected to form two groups i.e. Group I and Group II. Group I was treated with Tab. ketoconazole @ 10 mg/kg. b. wt. per orally and shampoo containing ketoconazole and chlorhexidine. Group II was treated with Tab. ketoconazole @ 10 mg/kg. b. wt. per orally and topical application of *Cassia alata* soap and *Cassia alata* ointment. Therapeutic efficacy was assessed by studying the regression of symptoms periodically by comparing pruritus score and clinical index score in combination with cytological examination. Dogs with concurrent conditions were also given specific therapy. Dogs with hypothyroidism and renal failure were not included in the therapeutic trial.

4.6.1.1 Group I

In Group I dogs with generalised *Malassezia* dermatitis the mean pruritus score, mean clinical index score and mean *Malassezia* per field were 4.00±0.26, 9.37±0.53 and 16.71±5.26 respectively on the day of presentation to the hospital.

It is evident from Table 12 that upon initiation of therapy the pruritus score decreased by day 3 (3.12±0.39) and further progressive reduction in the pruritus scores on day 7, 14 and 21 with the mean values of 1.62±0.49, 0.75±0.25 and 0.12±0.12.respectively. Significant reduction in pruritus score ($P<0.001$) was noticed by day 7 with complete regression in all the dogs by day 28.

Upon initiation of therapy improvement of clinical signs were recorded with a non-significant decline in clinical index score by 3rd day (7.50±0.62) and significant

($P<0.001$) decline in clinical index score from day 7 onwards. The mean clinical index score on day 7, 14, 21, 28, 35 and 42 were 6.50±0.50, 3.12±0.69, 1.62±0.49, 0.87±0.39, 0.50±0.26 and 0.37±0.18 respectively (Table 13) (Plates 23 to 26).

Generalised *Malassezia* dermatitis in dogs - before and after therapy

(Group I: Tab. Ketoconazole + Ketochlor® Shampoo)

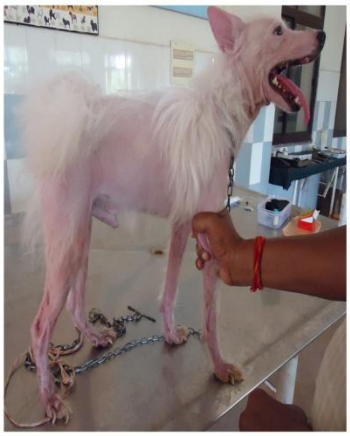

Plate 23: A Pomeranian dog with generalised *Malassezia* dermatitis with clinical index score of 9 before therapy

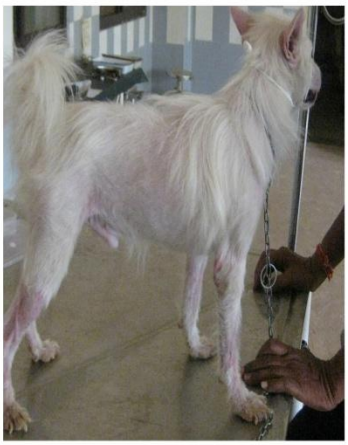

Plate 24: Same dog with clinical index score of 4 on day14 after initiation of therapy

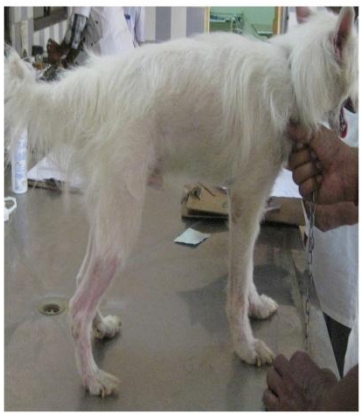

Plate 25: Improvement in clinical index score (2) on day 28

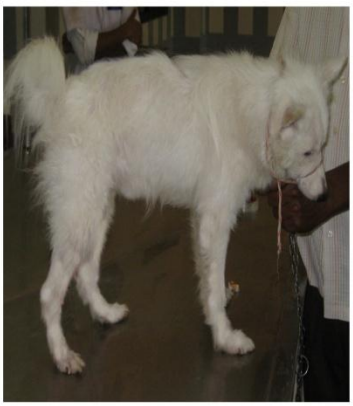

Plate 26: Same dog showing complete recovery on day 42 after therapy

Table 12: Day wise pruritus score (Mean±SE) in dogs with generalised *Malassezia* dermatitis (each group n=8)

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
I	4.00±0.26	3.12±0.39	1.62±0.49***	0.75±0.25***	0.12±0.12***	0	0	0
II	4.12±0.29	2.25±0.31***	1.50±0.32***	0.87±0.29***	0.25±0.16***	0.12±0.12***	0	0

*** Significant at (P<0.001) with day 0 / before therapy

Table 13: Day wise clinical index score (Mean±SE) in dogs with generalised *Malassezia* dermatitis (each group n=8)

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
I	9.37±0.53	7.50±0.62	6.50±0.50***	3.12±0.69***	1.62±0.49***	0.87±0.39***	0.50±0.26***	0.37±0.18***
II	9.62±0.53	7.50±0.77	5.12±0.78***	3.62±0.70***	2.25±0.45***	1.50±0.42***	0.75±0.31***	0.50±0.18***

*** Significant at (P<0.001) with day 0 / before therapy

Table 14: Day wise cytological enumeration (Mean±SE) of *Malassezia* in dogs with generalised *Malassezia* dermatitis (each group n=8)

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
I	16.71±5.26	14.06±4.98	8.76±3.27	3.06±1.07*	1.37±0.59**	0.58±0.11**	0.38±0.10**	0.21±0.10*
II	14.80±5.11	12.46±4.70	7.40±1.90	3.85±1.02	2.23±0.69*	1.30±0.44**	0.88±0.10**	0.45±0.13*

* Significant at (P<0.05) with day 0 / before therapy

** Significant at (P<0.01) with day 0 / before therapy

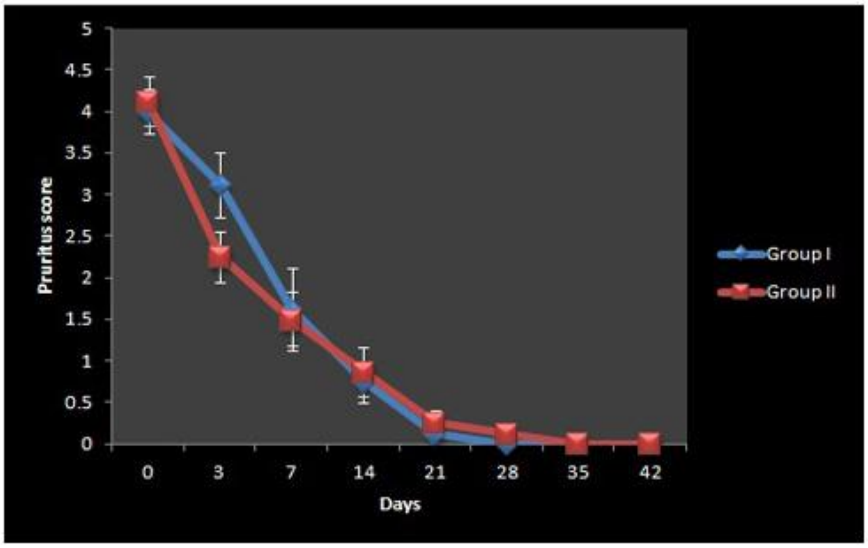

Figure 11: Day wise priritus score (Mean±SE) in dogs with generalised *Malassezia* dermatitis (each group n=8)

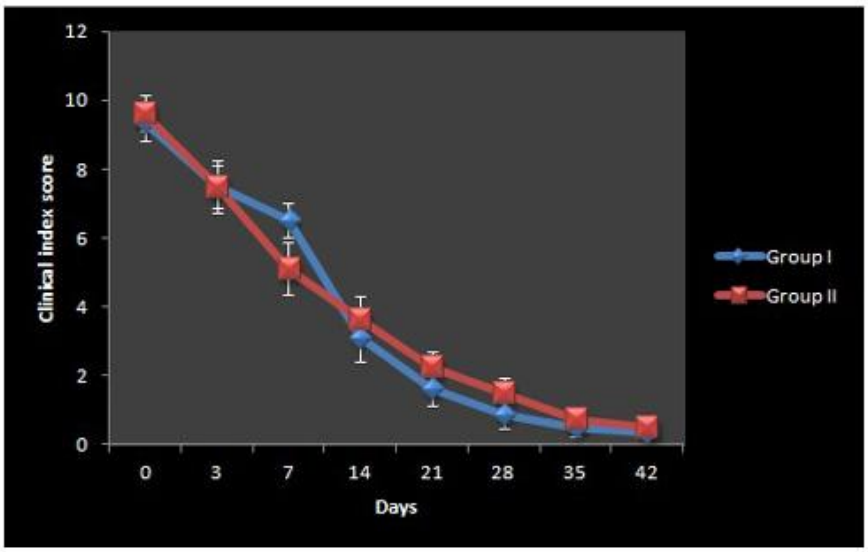

Figure 12: Day wise clinical index score (Mean±SE) in dogs with generalised *Malassezia* dermatitis (each group n=8)

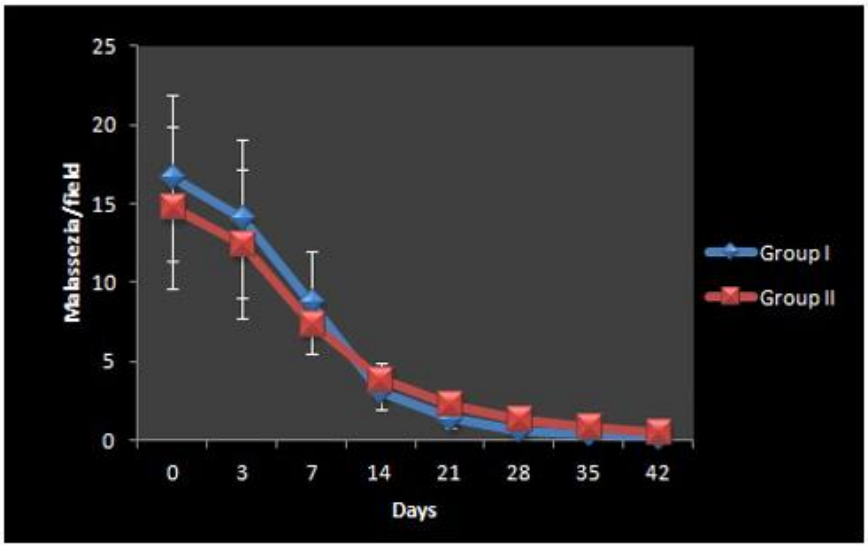

Figure 13: Day wise cytological enumeration (Mean±SE) of *Malassezia* in dogs with generalised *Malassezia* dermatitis (each group n=8)

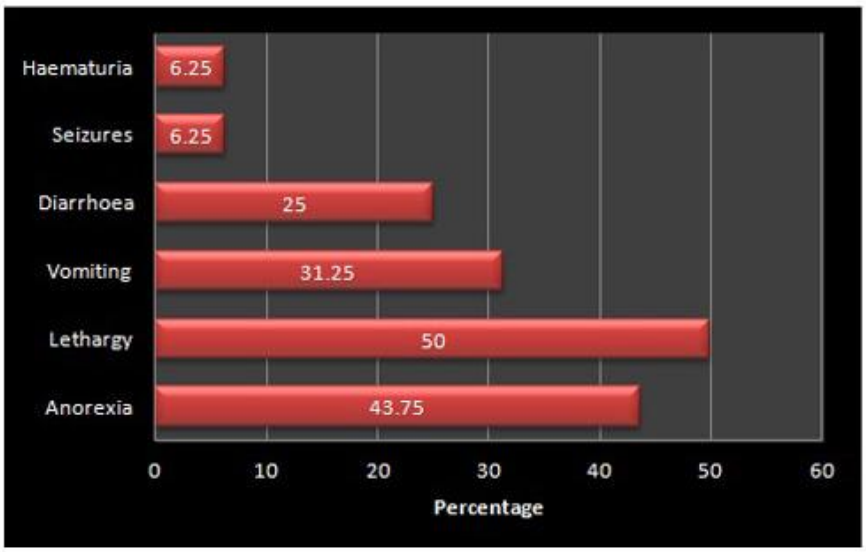

Figure 14: Adverse effects recorded in Group I and II dogs (n=16) treated with oral ketoconazole

The mean number of *Malassezia* per field before therapy was 16.71±5.26 and the same values after initiation of therapy were 14.06±4.98, 8.76±3.27, 3.06±1.07, 1.37±0.59, 0.58±0.11, 0.38±0.10 and 0.21±0.10 on days 3, 7, 14, 21, 28, 35 and 42 respectively (Table 14). Significant ($P<0.01$) reduction in mean *Malassezia* count was noticed by day 21. The density of bacteria and neutrophils reduced gradually and completely disappeared at the time of recovery.

Recovery path (Table 15) revealed complete recovery of 2 dogs (25.00%) by 14^{th} day, 3 dogs (62.50%) by 21^{st} day, 1 dog (75.00%) by 28^{th} day and 2 dogs (100.00%) by 35^{th} day. In this group all the 8 dogs recovered (100.00%) in an average period of 23.62±2.93 days (Table 16).

The post therapeutic mean ALT (IU/L) value (30.55±2.13) was found to be significantly ($P<0.05$) elevated when compared to pre therapeutic value (18.98±2.58). The pre and post therapeutic mean GGT (IU/L) values of dogs affected with *Malassezia* dermatitis were 4.92±0.36 and 5.21±0.78 respectively (Table 17). No significant difference ($P>0.05$) was noticed between pre and post therapeutic GGT values.

4.6.1.2 Group II

In Group II dogs with generalized *Malassezia* dermatitis the pre therapeutic mean values of pruritus score and clinical index score and *Malassezia* per field were 4.12±0.29, 9.62±0.53, 14.80±5.11 respectively.

It is evident from Table 12 that the post therapeutic mean pruritus scores were 2.25±0.31, 1.50±0.32, 0.87±0.29, 0.25±0.16 and 0.12±0.12 on days 3, 7, 14, 21 and

28 respectively. There was significant decline in pruritus score by day 3 ($P<0.001$) with complete disappearance of pruritus in all the dogs by day 35.

The clinical index scores showed improvement after initiation of therapy. The mean clinical index scores were 7.50±0.77, 5.12±0.78, 3.62±0.70, 2.25±0.45, 1.50±0.42, 0.75±0.31 and 0.50±0.18 on days 3, 7, 14, 21, 28, 35 and 42 respectively (Table 13). There was significant reduction ($P<0.001$) in mean CIS values by day 7 when compared to pre therapeutic value (Plates 27 to 30).

A reduction in the number of *Malassezia* was observed upon initiation of therapy with the mean values of 12.46±4.70, 7.40±1.90, 3.85±1.02, 2.23±0.69, 1.30±0.44, 0.88±0.10 and 0.45±0.13 on days 3, 7, 14, 21, 28, 35 and 42 respectively (Table 14). The mean *Malassezia* count was significantly ($P<0.01$) reduced by day 28.

Recovery path (Table 15) revealed complete recovery in 1 dog (12.50%) by 14^{th} day, 1 dog (25.00%) by 21^{st} day, 3 dogs (62.50%) by 28^{th} day, 2 dogs (87.50%) by 35^{th} day and 1dog (100.00%) by 42^{nd} day. In this group all the 8 dogs were recovered (100.00%) in an average period of 28.87±3.08 days (Table 16).

The post therapeutic mean ALT (IU/L) value (28.15±2.64) was found to be significantly ($P<0.05$) increased when compared to pre therapeutic value (18.33±2.35). The pre and post therapeutic mean GGT (IU/L) values of dogs affected with *Malassezia* dermatitis were 4.63±0.43 and 5.35±0.87 respectively (Table 17). No significant difference ($P>0.05$) was noticed between pre and post therapeutic GGT values.

Generalised *Malassezia* dermatitis in dogs - before and after therapy

(Group II: Tab. Ketoconazole + *Cassia alata* soap and *Cassia alata* ointment)

Plate 27: A Pomeranian dog with generalised *Malassezia* dermatitis showing erythema, alopecia on all the limbs and ears before therapy

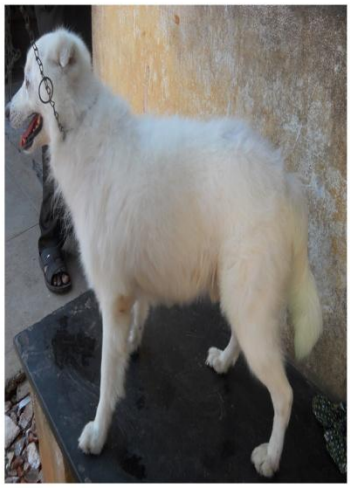

Plate 28: Same dog after complete recovery

Plate 29: Same dog from the plate 27 showing alopecia, lichenification and intense erythema of ear before therapy

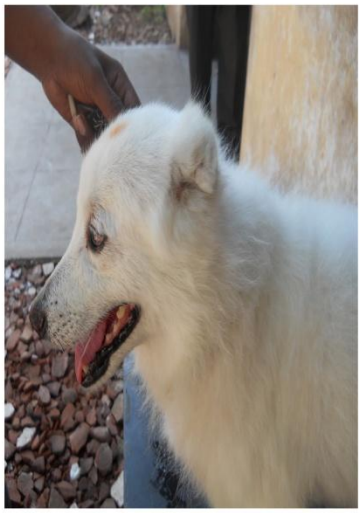

Plate 30: Same dog after therapy

Table 15: Recovery path exhibited by dogs with generalised *Malassezia* dermatitis

Group (n=8)	No. of dogs recovered (day-wise)								Average days taken for recovery
	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	
I	–	–	–	2 (25.00%)	3 (62.50%)	1 (75.00%)	2 (100.00%)	–	23.62±2.93
II	–	–	–	1 (12.50%)	1 (25.00%)	3 (62.50%)	2 (87.50%)	1 (100.00%)	28.87±3.08

Figures in the parenthesis indicate percentage of recovered dogs

Table 16: Therapeutic efficacy of treatment regimens in generalised *Malassezia* dermatitis dogs

Group	Name of the drug combination	No. of dogs treated	No. recovered	Per cent recovered	Average days taken for recovery
I	Tab. Ketoconazole + Ketoconazole and Chlorhexidine shampoo	8	8	100	23.62 ± 2.93^{NS}
II	Tab. Ketoconazole + *Cassia alata* soap and *Cassia alata* ointment	8	8	100	28.87 ± 3.08^{NS}

NS indicates no significant difference (P>0.05) between two groups

Table 17: Serum biochemical findings (Mean±SE) in dogs with generalised *Malassezia* dermatitis

before and after therapy (each group n=8)

Parameter	Healthy control n=8	Group I		Group II	
		Before treatment	After treatment	Before treatment	After treatment
ALT (IU/L)	18.76±1.30[a]	18.98±2.58[a]	30.55±2.13[b]	18.33±2.35[a]	28.15±2.64[b]
GGT (IU/L)	4.77±0.74[a]	4.92±0.36[a]	5.21±0.78[a]	4.63±0.43[a]	5.35±0.87[a]

Means bearing different superscripts within a row differ significantly ($P<0.05$)

4.6.1.3 Adverse effects

The adverse effects noticed in dogs (Group I and Group II) treated with ketoconazole orally and were presented in the Table 18 and Figure 14. In the present study the adverse effects that occurred were anorexia in 7 dogs (43.75%), lethargy in 8 dogs (50.00%), vomiting in 5 dogs (31.25%), diarrhoea in 4 dogs (25.00%), seizures and haematuria in 1 dog each (6.25%).

4.6.1.4 Comparative therapeutic efficacy

The efficacy of drugs used in Group I and Group II was assessed based upon clinical cure (pruritus score and clinical index score) and cytological examination. An improvement in the pruritus score was noticed in both the groups. But significant reduction in pruritus score ($P<0.001$) was noticed by day 7 in Group I while the same in Group II was on day 3 itself (Table 12 & Figure 11). The clinical index scores in both the groups were significantly reduced ($P<0.001$) by day 7 (Table 13 & Figure 12). A reduction in the number of *Malassezia* was observed after initiation of therapy. The mean *Malassezia* count showed significant decline ($P<0.01$) with pre therapeutic values by day 21 and 28 in Group I and Group II respectively (Table 14 & 13).

In Group I two dogs (25.00%) showed complete recovery by 14 days and by 35 days all the dogs (100.00%) were recovered. While in Group II only one dog (12.50%) recovered by day 14 with complete recovery of all the dogs (100.00%) by day 42 (Table 15).

On the whole 100.00% recovery was noticed in both the groups. The average time taken for recovery in Group I and Group II was 23.62±2.93 and 28.87±3.08

Table 18: Adverse effects recorded in Group I and II dogs (n=16) treated with oral ketoconazole

Sl. No	Adverse effect	No. of dogs affected	Percentage (%)
1	Anorexia	7	43.75
2	Lethargy	8	50.00
3	Vomiting	5	31.25
4	Diarrhoea	4	25.00
5	Seizures	1	6.25
6	Haematuria	1	6.25

respectively (Table 16). The average time taken for therapy did not vary significantly (P>0.05) between Group I and Group II.

4.6.2 Localised *Malassezia* dermatitis

Sixteen dogs with localised *Malassezia* dermatitis were randomly allocated to form two groups i.e. Group III and Group IV. Group III was treated with topical application of ketoconazole cream and shampoo containing ketoconazole and chlorhexidine. Group IV was treated with topical application of *Cassia alata* soap and *Cassia alata* ointment. Therapeutic efficacy was assessed by studying the regression of symptoms periodically by comparing pruritus score and clinical index score in combination with cytological examination.

4.6.2.1 Group III

In Group III dogs with localised *Malassezia* dermatitis the mean values of pruritus score, clinical index score and *Malassezia* count were 2.75±0.25, 5.12±0.35 and 11.07±1.68 respectively on the day of presentation to the hospital.

The mean pruritus scores upon initiation of therapy were 2.25±0.41, 1.25±0.49 and 0.50±0.32 on day 3, 7 and 14 respectively (Table19 & Figure 15). Significant (P<0.001) reduction in pruritus score was noticed by day 14 with complete regression in all the dogs by day 21.

It is evident from Table 20 and Figure 16 that following therapy, there was regression of symptoms with the clinical index scores of 3.87±0.39, 2.75±0.52, 1.62±0.49, 0.87±0.35, 0.50±0.26, 0.25±0.16 and 0.12±0.12 on days 3, 7, 14, 21, 28, 35 and 42 respectively. There was significant reduction (P<0.001) in mean clinical index score values by day 7 (Plates 31 & 32).

Localised *Malassezia* dermatitis in dogs - before and after therapy

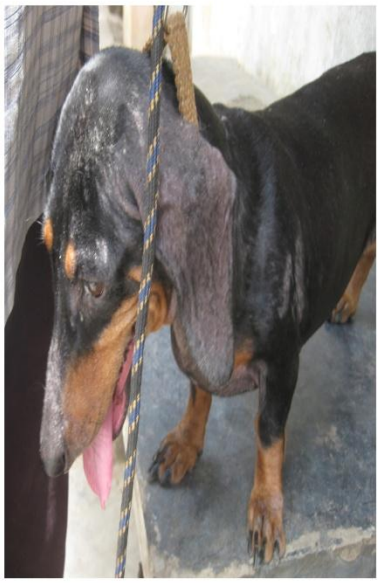

Plate 31: A Dachshund dog with localised *Malassezia* dermatitis (Group III) showing seborrhoea and alopecia in auricular and axillary region before therapy

Plate 32: Same dog showing complete recovery after treatment with-ketoconazole cream and Ketochlor shampoo on day 21

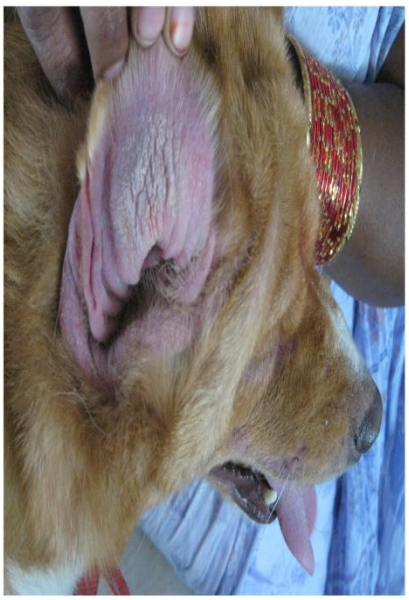

Plate 33: A dog with localised *Malassezia* dermatitis (Group IV) showing lichenification and scaling of ear before therapy

Plate 34: Same dog showing complete recovery after treatment with *Cassia alata* soap and *Cassia alata* ointment

Table 19: Day wise pruritus score (Mean±SE) in dogs with localised *Malassezia* dermatitis (each group n=8)

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
III	2.75±0.25	2.25±0.41	1.25±0.49**	0.50±0.32***	0	0	0	0
IV	2.87±0.22	1.85±0.22**	1.25±0.31***	0.87±0.22***	0.37±0.26***	0	0	0

** Significant at (P<0.01) with day 0 / before therapy
*** Significant at (P<0.001) with day 0 / before therapy

Table 20: Day wise clinical index score (Mean±SE) in dogs with localised *Malassezia* dermatitis (each group n=8)

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
III	5.12±0.35	3.87±0.39	2.75±0.52***	1.62±0.49***	0.87±0.35***	0.50±0.26***	0.25±0.16***	0.12±0.12***
IV	4.62±0.41	3.75±0.41	2.75±0.25**	1.75±0.36***	1.37±0.41***	0.75±0.31***	0.37±0.26***	0.25±0.16***

** Significant at (P<0.01) with day 0 / before therapy

*** Significant at (P<0.001) with day 0 / before therapy

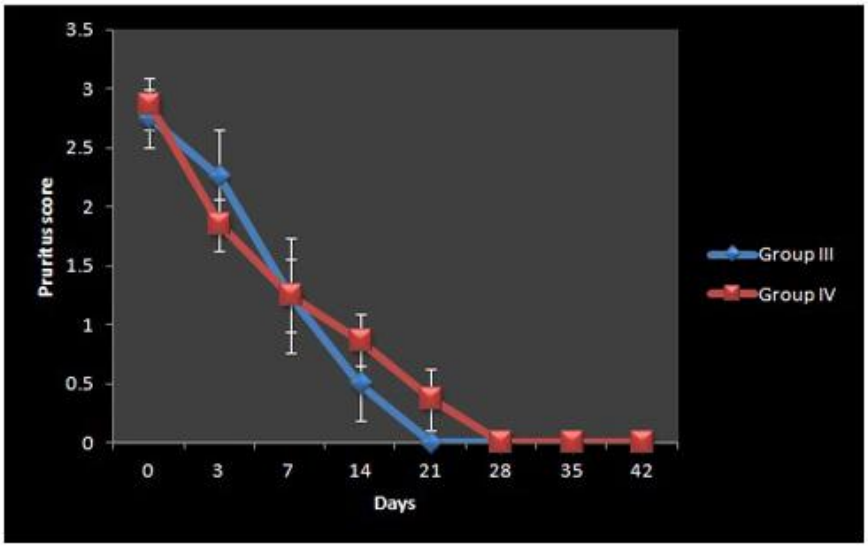

Figure 15: Day wise pruritus score (Mean±SE) in dogs with localised *Malassezia* dermatitis (each group n=8)

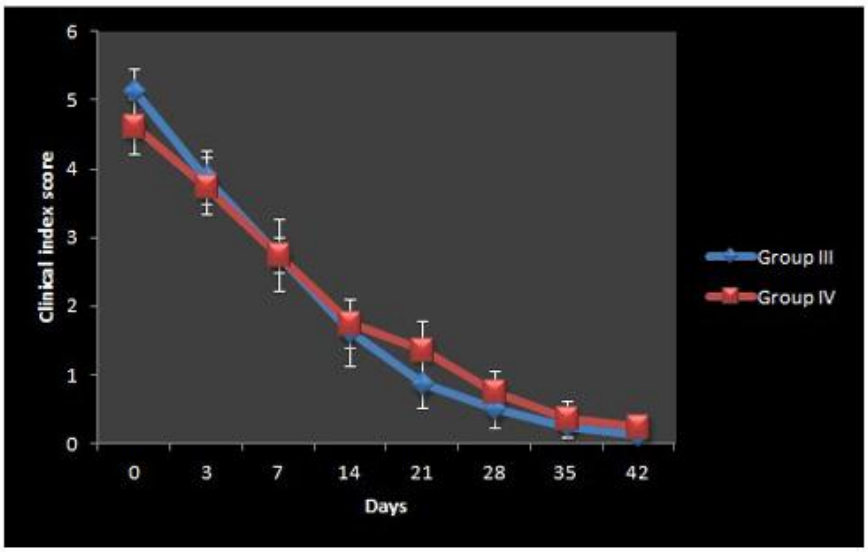

Figure 16: Day wise clinical index score (Mean±SE) in dogs with localised *Malassezia* dermatitis (each group n=8)

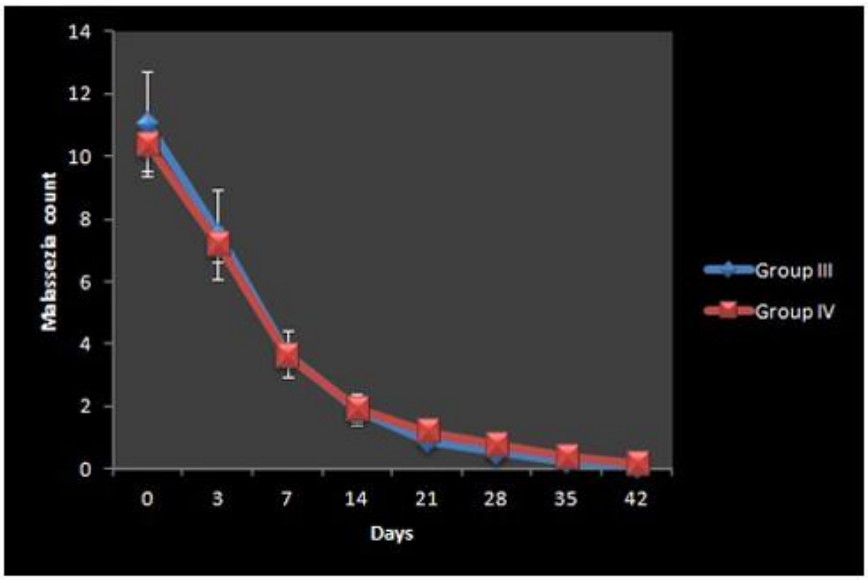

Figure 17: Day wise cytological enumeration (Mean±SE) of *Malassezia* in dogs with localised *Malassezia* dermatitis (each group n=8)

The mean of *Malassezia* count was 11.07±1.68 before therapy which gradually declined to 7.52±1.43, 3.68±0.73, 1.88±0.49, 0.90±0.22, 0.53±0.08, 0.25±0.04 and 0.08±0.02 on days 3, 7, 14, 21, 28, 35 and 42 respectively (Table 21 & 17). The mean *Malassezia* count significantly ($P<0.001$) reduced by day 7. The density of bacteria and neutrophils reduced gradually and they completely disappeared by the time of recovery.

Recovery path (Table 22) revealed complete recovery of 1 dog (12.50%) by 7^{th} day, 3 dogs (50.00%) by 14^{th} day, 3 dogs (87.50%) by 21^{st} day and 1 dog (100.00%) by 28^{th} day. All the 8 dogs in this group showed recovery (100.00%) in an average period of 17.50±2.29 days (Table 23).

The pre and post therapeutic mean ALT values of the affected dogs were 19.20±1.61 and 18.55±1.31 while the GGT values of the same were 5.06±0.53 and 4.48±0.70 respectively (Table 24). The values did not differ significantly ($P>0.05$) before and after therapy.

4.6.2.2 Group IV

The mean pruritus score, clinical index score and mean *Malassezia* count in Group IV dogs with localised *Malassezia* dermatitis were 2.87±0.22, 4.62±0.41 and 10.38±0.82 respectively.

It is evident from Table 19 and Figure 15 that upon initiation of therapy the reduction in the pruritus score was noticed by day 3 (1.85±0.22) and it significantly reduced ($P<0.001$) by day 7 (1.25±0.31). Further reduction in pruritus score was recorded on day 14 (0.87±0.22) and day 21 (0.37±0.26). In this group pruritus was completely resolved in all the dogs by day 28.

Table 22: Recovery path exhibited by dogs with localised *Malassezia* dermatitis

Group (n=8)	No. of dogs recovered (day-wise)								Average days taken for recovery
	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	
III	–	–	1 (12.50%)	3 (50.00%)	3 (87.50%)	1 (100.00%)	–	–	17.50±2.29
IV	–	–	–	2 (25.00%)	3 (62.50%)	2 (87.50%)	1 (100.00%)	–	22.75±2.56

Figures in the parenthesis indicate percentage of recovered dogs

Table 23: Therapeutic efficacy of treatment regimens in localised *Malassezia* dermatitis dogs

Group	Name of the drug combination	No. of dogs treated	No. recovered	Per cent recovered	Average days taken for recovery
III	Ketoconazole cream + Ketoconazole and Chlorhexidine shampoo	8	8	100	17.50 ± 2.29^{NS}
IV	*Cassa alata* soap and *Cassia alata* ointment	8	8	100	22.75 ± 2.56^{NS}

NS indicates no significant difference (P>0.05) between two groups

Table 24: Serum biochemical findings (Mean±SE) in dogs with localised *Malassezia* dermatitis

before and after therapy (each group n=8)

Parameter	Healthy control	Group III		Group IV	
		Before treatment	After treatment	Before treatment	After treatment
ALT (IU/L)	18.76±1.30[a]	19.20±1.61[a]	18.55±1.31[a]	18.98±1.16[a]	19.42±2.34[a]
GGT (IU/L)	4.77±0.74[a]	5.06±0.53[a]	4.48±0.70[a]	4.63±0.75[a]	4.92±0.56[a]

Means bearing different superscripts within a row differ significantly ($P<0.05$)

Upon initiation of therapy regression of symptoms was noticed by 3rd day with the clinical index scores of 3.75±0.41, 2.75±0.25, 1.75±0.36, 1.37±0.41, 0.75±0.31, 0.37±0.26 and 0.25±0.16 on days 3, 7, 14, 21, 28, 35 and 42 respectively (Table 20 & Figure 16). Significant reduction (P<0.001) in mean clinical index score was noticed by day 14 (Plates 33 & 34).

The mean *Malassezia* count was 10.38±0.82, 7.20±0.55, 3.62±0.37, 1.92±0.38, 1.18±0.34, 0.75±0.26, 0.37±0.10 and 0.17±0.05 on days 0, 3, 7, 14, 21, 28, 35 and 42 respectively (Table 21 & 17). Significant (P<0.001) reduction in mean *Malassezia* count was noticed by day 3. The density of bacteria and neutrophils reduced gradually and they completely disappeared by the time of recovery.

Recovery path (Table 22) revealed complete recovery of 2 dogs (25.00%) by 14th day, 3 dogs (62.50%) by 21st day, 2 dogs (87.50%) by 28th day and 1 dog (100.00%) by 35th day. In this group all the 8 dogs were recovered (100.00%) in an average period of 22.75±2.56 days (Table 23).

The pre and post therapeutic mean ALT (IU/L) values of affected dogs were 18.98±1.16 and 19.42±2.34 while the same of GGT (IU/L) values were 4.63±0.75 and 4.92±0.56 respectively (Table 24). No significant difference (P>0.05) was noticed in ALT and GGT values in *Malassezia* dermatitis dogs before and after treatment.

4.6.2.3 Comparative therapeutic efficacy

The clinical cure was assessed by means of pruritus score and clinical index score. There was no observable difference in the pruritus score and clinical index score in the two groups. Significant reduction (P<0.001) in pruritus score was

observed by day 14 in Group III, while the same in Group IV it was observed by day 7 (Table 19). But the clinical index score showed significant decline ($P<0.001$) by day 7 in Group III where as it was observed by day 14 in Group IV (Table 20). The mycological cure was assessed by cytological examination which revealed significant reduction ($P<0.001$) in mean *Malassezia* count by day 7 and day 3 in Group III and Group IV respectively (Table 21). From Table 22, it was evident that one dog (12.50%) in Group III was recovered by day 7 itself while in Group IV none of the dogs showed complete recovery by 7^{th} day. But all the dogs were recovered by 28 days in Group III while in Group IV all the dogs exhibited complete recovery by day 35.

On the whole 100.00% recovery was noticed in both the groups. The average time taken for recovery in Group III and Group IV was 17.50±2.29 and 22.75±2.29 days respectively with no significant difference ($P>0.05$) between the two groups (Table 23).

CHAPTER - V

5. DISCUSSION

5.1 PREVALENCE

In the present investigation dogs with dermatological ailments presented to the Teaching Veterinary Clinical Complex, N. T. R College of Veterinary Science, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada were screened to study the prevalence of *Malassezia* dermatitis. The overall prevalence of *Malassezia* dermatitis was observed to be 10.22 per cent. This finding was in close agreement with the earlier reports of Feijo *et. al*. (1998) and George *et. al*. (2012) who reported the prevalence of *Malassezia* dermatitis in dogs as 11.40 and 7.83 per cent respectively. On the contrary, most of the workers have reported higher prevalence ranging from 22 to 83 per cent (Girao *et. al*., 2006; Eidi *et. al*., 2011 and Kumar *et. al*., 2011a). These differences could be attributed to the varied geographical conditions.

5.1.1 Age wise prevalence

On perusal of Table 1 it is evident that the *Malassezia* dermatitis was more prevalent in adult dogs (51.35%) and less in puppies and dogs aged above 7 years (10.81%). There was no statistically significant difference (P>0.05) among different age groups studied. *Malassezia* dermatitis studies were conducted previously by various workers in different age groups of dogs and reported. The findings of present study are almost in concurrence with those of Bond *et. al*. (1996); Kiss *et. al*. (1997) and Kumar *et. al*. (2002a) who recorded similar observations. On the contrary,

Cafarchia *et. al*. (2005a) and Eidi *et. al*. (2011) recorded higher number of *Malassezia* dermatitis dogs aged between 1-5 years, while Girao *et. al*. (2006) reported majority of dogs affected with *M. pachydermatis* were aged between 1 to 3 years while Scott *et. al*. (2001) stated that *Malassezia* dermatitis could occur in any age group. Mircean *et. al*. (2010) opined that the average age of dogs infected with *M. pachydermatis* varied with breed.

5.1.2 Sex wise prevalence

Higher prevalence of *Malassezia* dermatitis was noticed in males (64.86%) than females (35.14%). Similarly Kiss *et. al*. (1997) reported higher predisposition of male dogs to *Malassezia* associated infection than the female dogs which could be attributed to the presence of androgens that were responsible for increased sebum production predisposing for infection. From the present study it was evident that the prevalence of *Malassezia* dermatitis did not differ significantly (P>0.05) in males and females. This finding was in close agreement with earlier reports of Girao *et. al*. (2006) and Machado *et. al*. (2011) who opined that gender has got no influence on the prevalence of *Malassezia* dermatitis.

5.1.3 Breed wise prevalence

It was observed from the present study that majority of the dogs affected were Labrador Retriever (43.24%), followed by Pomeranian (21.62%), mixed breed (16.21%), Daschund (8.10%), German Shepherd (5.40%) and Lhasa Apso (5.40%). These findings were in close agreement with Kumar *et. al*. (2002a) who reported Labrador Retriever as one of the most oftenly affected breed for *Malassezia* dermatitis. Cafarchia *et. al*. (2005a) reported that the frequency and population size of *M. pachydermatis* vary markedly between different breeds. However, in the present study there was no significant difference (P>0.05) in the prevalence among

the breeds which was similar to the earlier reports of Saijonmaa-Koulumies (2002) and Eluk *et. al*. (2003) who stated that there was no breed predilection. As the number of *Malassezia* dermatitis dogs studied were limited in the present study, generalised conclusions could not be drawn with regards to breed predilection.

5.1.4 Month wise prevalence

Higher number of dogs were affected with *Malassezia* dermatitis during the months of May and June. These findings were in accordance with Kumar *et. al*. (2002a); Patterson and Frank (2002) and Chen and Hill (2005) who recorded higher prevalence in summer months. High ambient temperature and humidity are favourable for increased *Malassezia* infections (Cafarchia *et. al*., 2005a).

5.2 SYMPTOMATOLOGY

The clinical signs observed in 37 dogs with *Malassezia* dermatitis revealed symptoms of pruritus in all the dogs (100%), erythema in 29 (78.37%), scaling in 22 (59.45%), exudation in 24 (64.86%), alopecia in 20 (54.05%), offensive odour in 33 (89.18), hyperpigmentation in 18 (48.64%) and lichenification in 16 (43.24%) dogs. Many earlier workers have also reported similar clinical findings in *Malassezia* dermatitis (Baksi *et. al*., 2004; Kumar *et. al*., 2006 and Machado *et. al*., 2011).

In the present study pruritus was noticed in all the dogs. This observation gained support by the findings of Charach (1997) and Mircean *et. al*. (2010) who opined that pruritus could be due to release of certain enzymes like lipase and protease which contribute to cutaneous inflammation through proteolysis and lipolysis. Other symptoms like erythema, exudation, scaling, alopecia and offensive odour might be due to the release of chemical mediators such as serotonin, prostaglandins, peptides and leukotrienes at the site of inflammation. The chronic inflammatory skin disease associated with sebaceous gland hyperplasia allows the

lipophilic yeast to proliferate resulting in odoriferous byproducts. In this study lichenification and hyperpigmentation were recorded in dogs with chronic infections and was in accordance with the findings of Muller *et. al.* (2001); Mircean *et. al.* (2010) and Machado *et. al.* (2011).

It was noticed that the auricular region (81.08%) was mostly affected in the present study. This finding was in agreement with the reports of earlier workers (Jeong *et. al.*, 2005; Outerbridge, 2006 and Mircean *et. al.*, 2010). The higher incidence might be due to greater relative humidity, stable temperature, stagnation of ceruminous discharge within the ear canal (rich in lipid) which in turn creates an ideal environment for *Malassezia pachydermatis* to grow and multiply to produce the lesions (Prado *et. al.*, 2008 and George *et. al.*, 2012).

The other regions affected were thoracic limbs (distal regions) in 27 dogs (72.97%), pelvic limbs (distal regions) in 25 dogs (67.56%), abdominal region in 20 dogs (54.05%) and pelvic region in 18 dogs (48.64%). Similar observations were reported by Nardoni *et. al.* (2007) and Brito *et. al.* (2009). Frequent occurrence of *Malassezia pachydermatis* in the above regions could be attributed to physical (humidity, warmth) and anatomical (skin folds, skin rich in sebaceous / ceruminous glands) skin characteristics (Patterson and Frank, 2002 and Mircean *et. al.*, 2010).

It was revealed from the study that localised *Malassezia* dermatitis was noticed in 19 dogs and generalised in 18 dogs. This was in agreement with findings of Charach (1997) who reported that localised *Malassezia* dermatitis was common than generalised form.

5.3 DIAGNOSIS

5.3.1 Cytology

In the present study cytological examination was performed as per the recommendations of the previous workers (Nambi, 2002; Ramprabhu *et. al*., 2003; Jain *et. al*., 2007 and Eidi *et. al*., 2011). Tape impression smears were used to collect sample from dry lesions, glass slide impression smears from wet greasy lesions and roll swab method was used to collect samples from auricular lesions. These sampling techniques could be done with ease and yielded consistent results. Eluk *et. al*. (2003) also reported that the method is inexpensive, less time consuming, easy to access the infected areas. Another advantage is that the tape preparation does not require any heat fixation (Matousek and Campbell, 2002). Detection of *Malassezia* infection by culture independent cytological techniques using glass slides, cotton swabs, or skin scrapings are rapid and have more specificity (Cafarchia *et. al*., 2005b and George *et. al*., 2012).

In the present investigation the number of organisms ranged from 6 to 50 per microscopic field (oil immersion). The population of *Malassezia* organisms considered to be pathogenic is controversial and hence, difficult to define as the number varied from 1 / oil immersion field (Bruner and Blakemore, 1999; Nobre *et. al*., 2001 and Khosravi *et. al*., 2008) to more than 40 / oil immersion field (Marsella *et. al.*, 2000 and Rosales *et. al.*, 2005). This variation in the yeast population could be attributed to the site of collection, method of collection and breeds of the dogs included in the study. However, more number of yeasts per field may be of better diagnostic value for *Malassezia* infection (Scott *et. al*., 1995).

In the present study presence of at least 10 yeasts from the ear and 5 yeasts from the skin samples were considered positive and the average number of *Malassezia* yeasts present in ten microscopic fields was taken as opined by earlier investigators (Nobre *et. al*., 2001 and Bensignor, 2008). In this study *Malassezia*

alone was seen in 32.44% dogs and mixed infection with plenty of bacterial cocci, rods and a combination of cocci and rods along with neutrophils were seen in 67.56% dogs. Rosales *et. al*. (2005) and Sickafoose *et. al*. (2010) also reported that *Malassezia* and bacterial dermatitis often occur concurrently in dogs. Craig (2008) reported that presence of *Staphylococci* favours the growth of *Malassezia* and probably these two organisms are mutually beneficial through utilization of products formed by bacterial and yeast lipases.

5.3.2 Isolation and identification of *Malassezia pachydermatis*

Malassezia pachydermatis was confirmed by its ability to grow on SDA without addition of any lipids (Eidi *et. al*., 2011; Naeini *et. al*., 2011 and Crosaz *et. al*., 2013) and reported that *Malassezia pachydermatis* is the only lipid independent species available. In the present investigation mycological cultural examination of 37 samples that were positive for cytology revealed regular round colonies which were observed after 48 hours of incubation, they were sand coloured, friable in consistency and could be easily lifted of their base. Similar colony characteristics were also reported by Kiss *et. al*. (1997) and Kumar *et. al*. (2008a). In this study the samples that were positive on cytology were also positive for cultural examination revealing that cytological examination was as good as cultural examination. This finding was in agreement with Nambi (2002), Jain *et. al*. (2007) and George *et. al*. (2012) who reported that culture independent cytological examination could be performed to diagnose *Malassezia* dermatitis in dogs. On the contrary, Girao *et. al*. (2006) and Prado *et. al*. (2008) opined that though direct microscopic examination gives immediate diagnosis, fungal culture is necessary for more accurate results and for the identification of *Malassezia* species. Cafarchia *et. al*. (2005b) however found that cytological examination was more sensitive than fungal culture.

5.3.3 Concurrent infections / conditions

In the present study *Malassezia* dermatitis was associated with concurrent conditions like pyoderma (13.51%), hypothyroidism and renal failure (2.70%), demodicosis (10.81%), tick infestation (8.10%) and scabies (2.70%) in 40.54% of dogs. These findings were similar to earlier observations made by Naresh *et. al*. (2002) and Eidi *et. al*. (2012) who reported *Malassezia* dermatitis was in association with demodicosis in dogs, while Charach *et. al*. (1997); Outerbridge (2006) and Negre *et. al*. (2008) stated that most of the dogs with *Malassezia* dermatitis have concurrent dermatoses like pyoderma, flea allergies and endocrine disorders. Nambi (2002) opined that any disease which compromises the skin barrier and suppresses the immune response of the host act as predisposing factors in canine malasseziosis.

5.4 *IN-VITRO* ANTIFUNGAL SENSITIVITY TEST

5.4.1 Antifungal drugs

The antifungal sensitivity test conducted in the present study revealed that 93.33% of the *Malassezia pachydermatis* isolates were sensitive to ketoconazole. Higher sensitivity of *Malassezia* isolates to ketoconazole was reported by Kiss *et. al*. (1997) and Kim and Choi (1999) who reported that azoles such as ketoconazole inhibits the synthesis of ergosterol of the fungal cell membranes which alters the cell permeability resulting in its death.

5.4.2 *Cassia alata* leaf powder

No growth was observed on Sabouraud's dextrose agar plates supplemented with *Cassia alata* leaf powder (10 mg/ml) and these findings were in accordance with Abubacker *et. al*. (2008) and Esimone *et. al*. (2008) who reported the antifungal properties of *Cassia alata* leaves.

5.5 SERUM BIOCHEMICAL FINDINGS

Serum biochemical studies revealed no significant difference (P>0.05) in the alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) values in dogs affected with *Malassezia* dermatitis when compared to healthy dogs. The ALT levels in *Malassezia* dermatitis dogs were similar to the earlier findings made by Kumar and Sahoo (2011). The GGT levels were within the reference range (Kaneko *et. al*. (1997). Perusal of literature showed scanty reports with regards to these biochemical parameters in dogs affected with *Malassezia* dermatitis.

5.6 THERAPEUTIC TRIALS

5.6.1 Generalised *Malassezia* dermatitis

Sixteen dogs with generalised *Malassezia* dermatitis were randomly allocated to form two groups i.e. Group I and Group II. Group I was treated with Tab. ketoconazole @ 10 mg/kg. b. wt. per orally and shampoo containing ketoconazole and chlorhexidine (Ketochlor®). Group II was treated with Tab. ketoconazole @ 10 mg/kg. b. wt. per orally and topical application of *Cassia alata* soap and *Cassia alata* ointment.

5.6.1.1 Group I

Antifungal agent ketoconazole was selected based upon *in-vitro* results and efficacy reports of earlier workers (Bensingnor, 2008 and Sickafoose *et. al.*, 2010). In this study topical therapy was also used along with systemic therapy to hasten the relief and resolution of the disease without recurrence (Kumar and Sahoo, 2011). Use of systemic ketoconazole and topical application of shampoo containing ketoconazole and chlorhexidine in dogs with generalised *Malassezia* dermatitis has gained support from the findings of Rosenkrantz (2006) and Saranya *et. al*. (2012). In the present study ketoconazole was given orally @ 10 mg/kg. b. wt. as suggested

by Bensignor (2008), while Baksi *et. al*. (2004) reported that ketoconazole was effective at the dose rate of 5-10 mg/kg. b. wt.

Significant reduction (P<0.001) in the mean value of pruritus score and clinical index score by day 7 in Group I dogs, indicates the efficiency of ketoconazole in the resolution of clinical signs which might be due to its immunomodulatory, anti-inflammatory effects with effect on leukotriene synthesis and antiproliferative action on keratinization by altered metabolism of alltrans-retinoic acid (Scott *et. al*., 2001 and Rosales *et. al*., 2005).

The mean *Malassezia* count significantly (P<0.01) reduced by day 21. The density of bacteria and neutrophils reduced gradually and they completely disappeared by the time of recovery. The improvement in clinical signs could be due to the combined action of ketoconazole and chlorhexidine. Rosenkrantz (2006) and George *et. al*. (2010) also recommended the use of chlorhexidine along with ketoconazole in generalised *Malassezia* dermatitis owing to its anti-seborrhoeic and antibacterial properties.

In this group all the 8 dogs recovered (100%) in an average period of 23.62±2.93 days (Table 16). The duration of treatment reported by different authors is variable. Baksi *et. al*. (2004) successfully treated clinical cases of *Malassezia* dermatitis by using above treatment in 28 days, while Prasanna *et. al*. (2006) reported that the average duration of recovery was 20-25 days.

5.6.1.2 Group II

In this group *Cassia alata* soap and *Cassia alata* ointment were used along with oral ketoconazole. *Cassia alata* was selected, based on the reports of previous authors who reported the antifungal and antimicrobial properties (Damodaran and Venkataraman, 1994 and Esimone *et. al*., 2008).

It was evident from the Table 12 and 13 that, the mean pruritus score and clinical index score reduced significantly by day 3 and day 7 respectively. Significant reduction ($P<0.01$) in the mean *Malassezia* count was noticed by day 28 (Table 14). Phongpaichit *et. al*. (2004) reported that some of the components of *Cassia alata* viz. rhein, emodol, 4,5-dihydroxy-1-hydroxy methyl anthrone and 4,5-dihydroxy-2-hydroxy methyl anthraquinone had antifungal activity while ketoconazole achieves its antifungal effect by blocking ergosterol synthesis, an essential component of the fungal cell wall.

Recovery path (Table 15) revealed complete recovery in 1 dog by 14^{th} day, while all the dogs recovered in an average period of 28.87±3.08 days (Table 16). The complete cure achieved in this group was similar to the findings of Rathish *et al.* (2012) who successfully treated malasseziosis in a Labrador Retriever pup.

5.6.1.3 Adverse effects

In the present study the adverse effects noticed in dogs (Group I and Group II treated with oral ketoconazole) include anorexia (43.75%), lethargy (50.00%), vomiting (31.25%), diarrhoea (25.00%) and seizures and haematuria (6.25%). These recordings were similar to the earlier observations made by Marsella *et. al*. (2000) and Sickafoose *et. al*. (2010). The reversible adverse effects might be due to hepatotoxic effect of ketoconazole (Fernandez *et. al*., 1998 and. Tilley *et. al*., 2000). On the contrary Kumar and Sahoo (2011) observed no adverse side effects like anorexia, vomiting and diarrhoea during the course of treatment or subsequent observation period.

5.6.1.4 Comparative therapeutic efficacy

In the present investigation, the efficacy of drugs used in Group I and Group II was assessed based upon clinical cure (pruritus score and clinical index score) and

cytological examination. It was observed that the dogs treated with oral and topical ketoconazole (Group I) showed quick clinical recovery. Significant reduction in pruritus score ($P<0.001$) was noticed by day 7 in Group I, while the same in Group II was on day 3 itself (Table 12). The clinical index scores in both the groups were significantly reduced ($P<0.001$) by day 7 (Table 13). A reduction in the number of *Malassezia* was observed after initiation of therapy. The mean *Malassezia* count showed significant decline ($P<0.01$) with pre therapeutic values by day 21 and 28 in Group I and Group II respectively (Table 14). The improvement seen in Group I treated dogs could be due to potent antifungal or anti-inflammatory properties of ketoconazole and antiseborrhoeic action of chlorhexidine (Ramaprabhu *et. al*., 2003). The improvement seen in Group II could be attributed to the combined action of ketoconazole and *Cassia alata*. Further this combination also helped in significant reduction of pruritus score by day 3 itself in Group II, while it reduced on day 7 in Group I which could be due to triterpenoid a major constituent of *Cassia alata* with a good anti-inflammatory action (Gidwani *et. al*., 2010).

The time taken for recovery was 23.62±2.93 and 28.87±3.08 days respectively in Group I and Group II. In both the groups adverse effects and significant ($P<0.05$) elevation of ALT levels was noticed after therapy with oral ketoconazole might be due to the hepato toxic property of ketoconazole which was in accordance with the findings of Bensignor (2001); Mayer *et. al*. (2008) and Kumar and Sahoo (2011). But no significant ($P>0.05$) difference was noticed between pre and post therapeutic GGT levels in both the groups. On the contrary, Tiwari and Varshney (2003) observed a decline in GGT levels after ketoconazole therapy and opined that it might be due to enzyme inhibition.

From the Table 16 it was evident that all the dogs in both the groups responded to the therapeutic regimen but with a difference in the onset of resolution of clinical signs and time taken for recovery. When cost factor was considered Ketochlor® (ketoconazole and chlorhexidine) shampoo was expensive when compared to *Cassia alata* ointment and soap.

5.6.2 Localised *Malassezia* dermatitis

Sixteen dogs with localised *Malassezia* dermatitis were randomly allocated to form two groups i.e Group III and Group IV. Group III was treated with topical application of ketoconazole cream and shampoo containing ketoconazole and chlorhexidine (Ketochlor®). Group IV was treated with topical application of *Cassia alata* soap and *Cassia alata* ointment. Therapeutic efficacy was assessed by studying the regression of symptoms periodically by comparing pruritus score and clinical index score in combination with cytological examination. Localised *Malassezia* dermatitis dogs could be treated with the daily spot on application of an antifungal cream, ointment, lotion etc. (Mason and Stewart, 1993 and Charach, 1997). *Cassia alata* ointment and soap were selected based on the reports of previous investigators (Damodaran and Venkataraman, 1994 and Ali-Emmanuel *et. al*., 2003) and *in-vitro* study results. Use of *Cassia alata* soap in dogs with localised *Malassezia* dermatitis was also reported by Esimone *et. al*. (2008); Oladele *et. al*. (2010) and Omobuwajo *et. al*. (2011).

5.6.2.1 Group III

After topical therapy by using ketoconazole and chlorhexidine in Group III dogs, initial response started by 3 days. Jasmin *et. al*. (2003) also used 3 per cent chlorhexidine shampoo twice a week for three weeks in the treatment *Malassezia* dermatitis.

There was significant (P<0.001) reduction in pruritus score by day 14 with complete regression in all the dogs by day 21. In this study significant reduction (P<0.001) in mean clinical index score was observed by day 7. This finding was supported by Srivastava *et. al*. (2008) who reported topical application of antifungal agent and chlorhexidine in treatment of localised *Malassezia* dermatitis.

The significant (P<0.001) reduction in mean *Malassezia* count was noticed by day 7. The density of bacteria and neutrophils reduced gradually and they completely disappeared by the time of recovery. Saijonmaa-Koulumies (2002) in a review of pyoderma and *Malassezia* dermatitis in dogs stated that topical therapy with shampoo containing ketoconazole combined with chlorhexidine at three times a week application could effectively reduce cutaneous yeast counts which was attributed to anti-yeast and anti- bacterial properties of chlorhexidine (Christine, 1998).

On the whole all the 8 dogs in this group recovered in an average period of 17.50±2.29 days (Table 23). George *et. al*. (2010) stated that chlorhexidine (2.5%) topical application was found more effective than ketoconazole (2%) and selenium sulphide (2.5%) in the management of localised form of *Malassezia* dermatitis in dogs.

5.6.2.2 Group IV

It is evident from Table 19 that upon initiation of therapy the reduction in the pruritus score was noticed by day 3 and it significantly reduced (P<0.001) by day 7 which completely disappeared by day 28.

Significant reduction (P<0.001) in mean clinical index score noticed by day 14 was in accordance with Rathish *et. al*. (2012) who successfully treated the *Malassezia* dermatitis in a dog with 2% ointment prepared from alcoholic extract of

leaves of *Cassia alata*. Eja *et. al*. (2009) reported that the leaf extract of *Cassia alata* is more active against yeast than griseofulvin and clotrimazole.

In the present study a significant (P<0.001) reduction in the mean *Malassezia* count was noticed by day 3. Esimone *et. al.* (2008) evaluated the antimicrobial activity of *Cassia alata* and reported that the soap is predominantly effective against Gram-positive bacteria and opportunistic yeast. However Awal *et. al*. (2004) reported that the leaves of *Cassia alata* was effective both Gram-positive and negative bacteria.

In this group all the 8 dogs were recovered (100.00%) in an average period of 22.75±2.56 days (Table 23). This finding gained support by the observations of Ting (2000) and Oladele *et. al*. (2010) who had successfully treated skin lesions by using *Cassia alata*. They opined that incorporation of these leaves having antifungal property in the soap will ensure effective washing of the lesions as well as application of herb on to the skin. The treatment could however be extended beyond 2 weeks after clinical cure due to recalcitrative nature of fungal infections in order to ensure total clearance and to prevent recurrence of the lesions.

5.6.2.3 Comparative therapeutic efficacy

Therapeutic efficacy of two treatments adopted in Group III and Group IV were compared based on resolution of clinical signs and number of animals recovered and time taken for recovery.

It was evident from Table 23 that in both the groups 100% recovery was noticed but with mild variation in the time of disappearance of clinical signs. Significance improvement was noticed in Group III and Group IV in pruritus score by (day 14 and 7), clinical index score (day 7 and 14) and mean *Malassezia* count (day 7 and 3) respectively.

It was evident that one dog (12.50%) in Group III was recovered by day 7 itself, while in Group IV none of the dogs showed complete recovery by day 7. But all the dogs were recovered by 28 days in Group III while in Group IV all the dogs exhibited complete recovery by day 35. Though the overall therapeutic response was slightly delayed in Group IV dogs, pruritus resolved earlier when compared to Group III. Chlorhexidine is a broad spectrum biocide effective against Gram-positive bacteria, Gram-negative bacteria and fungi and has the property of quicker kill rate than other antimicrobials (Jasmin *et. al*., 2003).

Abubacker *et. al*. (2008) and Esimone *et. al*. (2008) reported the antifungal and antimicrobial activity of the leaves. While Gidwani *et. al*. (2010) reported that triterpenoid was the major constituent of *Cassia alata* with good anti-inflammatory activity.

The average time taken for recovery in Group III and Group IV was 17.50±2.29 and 22.75±2.29 days respectively with no significant difference (P>0.05) between the two groups (Table 23). This finding was in accordance with Ting (2000) who reported no significant difference was recorded between ketoconazole cream and *Cassia alata* cream in terms of resolution of clinical signs.

In Group III and Group IV pre and post therapeutic ALT and GGT values did not differ significantly (P>0.05) with no adverse reactions in both the groups but considering the cost of ketoconazole cream and shampoo for topical application *Cassia alata* ointment and soap could be used as an effective alternative. When cost economics of the therapy was compared topical application of ketoconazole cream and shampoo were expensive and could be affordable by people with high socio-economic status, where as *Cassia alata* ointment and soap were cheap alternatives to

the expensive standard drugs with acceptable potency in the management of skin infections.

5.7 CONCLUSIONS

The present study indicated that the prevalence of *Malassezia* dermatitis was 10.22 per cent with a higher rate in dogs aged between 2 to 7 years and in males. Higher prevalence was observed in Labrador Retriever and during the months of May and June. Ears were mostly affected followed by limbs, abdominal and pelvic region. Cytology proved to be a simple, quick and reliable test in the diagnosis of the condition. Generalised *Malassezia* dermatitis was effectively treated with oral administration of ketoconazole and topical Ketochlor® shampoo; however the condition could also be treated with oral administration of ketoconazole and topical application of *Cassia alata* ointment and soap with similar efficacy comparable with earlier treatment. External application of ketoconazole ointment and twice weekly use of Ketochlor® shampoo could successfully resolve the signs of localised *Malassezia* dermatitis. Use of *Cassia alata* ointment and soap could treat localised *Malassezia* dermatitis with similar efficacy as that of antifungal agents.

Further the study suggests that *Cassia alata* could be used as an alternative to synthetic antifungal agents as it is locally available and treatment is cost efficient with no adverse effects even after prolonged application. Hence, it is recommended that *Cassia alata* could be incorporated into soap and ointment and made commercially available for the treatment of canine *Malassezia* dermatitis as the plant has antifungal and anti-inflammatory properties.

CHAPTER - VI

6. SUMMARY

The present study was undertaken to record the prevalence and symptomatology of *Malassezia* dermatitis in dogs with an objective to determine the therapeutic efficacy of *Cassia alata* in comparison with conventional antifungal drugs. The study was conducted at Teaching Veterinary Clinical Complex, N. T. R College of Veterinary Science, Gannavaram and Super Speciality Veterinary Hospital, Vijayawada. The details of the study were summarized as follows.

In order to find out the prevalence, a total of 362 dogs with dermatological affections were screened from March 2012 to August 2012 of which 37 dogs were diagnosed with *Malassezia* dermatitis accounting for 10.22%.

The dogs belonging to the age group of 2-7 years showed greater susceptibility (51.35%) followed by young adults (7months to 2 years) and susceptibility was least in puppies and dogs aged above 7 years.

Higher prevalence was observed in the male dogs (64.86%). Results also revealed that Labrador Retriever (43.24%) were more susceptible followed by Pomeranian (21.62%). Higher prevalence was recorded during the months of May (37.83%) and June (27.02%).

Pruritus, offensive odour and erythema were the predominant signs in dogs with *Malassezia* dermatitis which were noticed in 100%, 89.18% and 78.37% of the dogs respectively. Ears (81.08%) were mostly affected followed by distal regions of thoracic limbs (72.97%) and pelvic limbs (67.56%).

Out of the 37 dogs, 18 were affected with generalised *Malassezia* dermatitis and 19 dogs with localised *Malassezia* dermatitis. The mean pruritus score was 4.00±0.26 and 4.12±0.29 in Group I and Group II dogs affected with generalised *Malassezia* dermatitis while the mean clinical index score in these groups was 9.37±0.53 and 9.62±0.53 respectively. In dogs with localised *Malassezia* dermatitis the mean pruritus score and clinical index score were 2.75±0.25 and 5.12±0.35 in Group III while the same in Group IV were 2.87±0.22 and 4.62±0.41 respectively.

Cytology revealed the presence of *Malassezia* organisms alone in 32.44% of cases and in combination with bacteria in 67.56% of the dogs. The number of *Malassezia* organisms ranged from 6 to 50 per microscopic field. Cultural examination of the same samples yielded positive results indicating that cytological examination was as good as cultural examination in the diagnosis of *Malassezia* dermatitis. In the present study, cultural examination revealed the growth of *Malassezia pachydermatis* on Sabouraud's dextrose agar without addition of any lipids.

In this study 40.54% of the dogs with *Malassezia* dermatitis were associated with other concurrent conditions like pyoderma (13.51%), demodicosis (10.81%), tick infestation (8.10%), hypothyroidism (2.70%), scabies and renal failure (each 2.70%).

Antifungal sensitivity test performed in 15 samples revealed that higher number of isolates were sensitive to ketoconazole (93.33%), followed by

clotrimazole (53.33%), amphotericin-B (33.33%), fluconazole and nystatin (26.66%).

In the present study, inhibition of growth of *Malassezia pachydermatis* on Sabouraud's dextrose agar plates supplemented with *Cassia alata* leaf powder revealed the efficacy of antifungal activity of *Cassia alata.*

The mean serum ALT (IU/L) in dogs with *Malassezia* dermatitis were 18.98±2.58, 18.33 ±2.35, 19.20±1.61 and 18.98±1.16 in Group I, Group II, Group III and Group IV respectively while GGT (IU/L) values were 4.92±0.36, 4.63±, 5.06±0.53 and 4.63±0.75 respectively in the above groups. Serum biochemical studies revealed no significant difference (P>0.05) in the alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) values in dogs affected with *Malassezia* dermatitis when compared to healthy dogs.

Sixteen dogs affected with generalized *Malassezia* dermatitis were treated by oral administration of ketoconazole @10 mg/kg. b. wt in combination with topical application of Ketochlor® shampoo (Group I) and *Cassia alata* ointment and *Cassia alata* soap (Group II) along with oral ketoconazole administration. Therapeutic efficacy was assessed periodically by observing the regression of symptoms, comparing the pruritus and clinical index scores along with cytological examination. Upon therapy significant improvement was noticed in the pruritus score, clinical index score and mean *Malassezia* count in both the groups. All the dogs in both the groups responded to therapy but with a difference in the onset of resolution of clinical signs. The average time taken for recovery was 23.62±2.93 and 28.87±3.08 days respectively in Group I and Group II respectively.

Localised *Malassezia* dermatitis was treated by using ketoconazole cream and Ketochlor® shampoo in Group III and with *Cassia alata* ointment and *Cassia alata*

soap in Group IV. Efficacy was 100% in both the groups and the average time taken for recovery was 17.50±2.29 days and 22.75±2.29 days in Group III and Group IV respectively.

In the present study, adverse effects were observed in dogs treated with oral ketoconazole which included anorexia, lethargy, vomiting, diarrhoea, seizures and hematuria. The post therapeutic ALT values in Group I dogs treated with oral ketoconazole were significantly ($P<0.05$) elevated when compared to pre therapeutic values.

It is concluded from the present study that the prevalence of *Malassezia* dermatitis was 10.22 per cent and higher in males and dogs aged between 2 to 7 years. Higher prevalence was observed in Labrador Retriever and was higher in May and June. Ears were mostly affected followed by limbs, abdominal and pelvic regions. Cytology proved to be a simple, quick and reliable test in the diagnosis of the condition. Generalised *Malassezia* dermatitis was effectively treated with oral administration of ketoconazole and topical Ketochlor® shampoo; however the condition could also be treated with oral administration of ketoconazole and topical application of *Cassia alata* ointment and soap with similar efficacy comparable with earlier treatment. External application of ketoconazole cream and twice weekly use of Ketochlor® shampoo could successfully resolve the signs of localised *Malassezia* dermatitis. However, *Cassia alata* ointment and soap could also treat localised *Malassezia* dermatitis with similar efficacy as that of antifungal agents.

Further the study suggests that *Cassia alata* could be used as an alternative to synthetic antifungal agents as it is locally available and treatment is cost efficient with no adverse effects even after prolonged application. Hence, it is recommended that *Cassia alata* could be incorporated into soap and ointment and made

commercially available for the treatment of canine *Malassezia* dermatitis as the plant has antifungal and anti-inflammatory properties.

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APPENDIX

CLINICAL CASE RECORD

Case no: Date:

Age: Breed:

Sex: Month:

I. History:

II. Clinical examination:

a). Pruritus score:

Day	0	3	7	14	21	28	35	42
Score								

b). Clinical index score (CIS):

Clinical sign	Day wise score for each clinical sign							
	0	3	7	14	21	28	35	42
Erythema								
Exudation								
Scaling								
Hyperpigmentation								
Lichenification								
Clinical index score								

Other lesions: Pustules / Crusts / Papules / Nodules

c). Topography of lesions:

Region affected	✓
Thoracic limbs (distal regions)	

Auricular	
Face	
Pelvic	
Pelvic limbs (distal regions)	
Neck	
Abdominal	
Pectoral	
Dorsum	

III. Laboratory examination:

a). Cytology:

Day	0	3	7	14	21	28	35	42
Average *Malassezia* / field								

b). Gram's staining: Cocci / Rods / Neutrophils

c). Cultural examination:

d). Serum biochemical findings:

Parameter	Before treatment	After treatment
ALT (IU/L)		
GGT (IU/L)		

IV Associated Conditions:

V. Treatment:

Generalised / Localised **Group I / II / III / IV**

Date	Therapy given

VI. Adverse effects observed: