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CAMEL MASTITIS

A TECHNICAL BULLETIN

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PREFACE

The camel generally known as beast of burden has now been considered as an animal of production function utility due to various functional qualities and therapeutic value of camel milk. The camel milk dairies have come up as commercial activity in most camel inhabiting countries. For all the milk producing animals the disease mastitis is most studied and researched area all over the world and similar care is to be taken in camels too if milk production function of camels is to be exploited safely. Therefore utmost importance is to be given to mastitis due to its wide occurrence and wide variety of the organisms affecting the udder. The preventive and therapeutic measures for this disease have to be regularly followed, with special care in using the antibiotics owing to emergence of drug resistance microbial strains. Mastitis, besides causing the loss in milk production has hazardous effects on human beings and suckling calves since most pathogenic organisms affecting udder health are secreted in the milk.

Due to therapeutic utility of camel milk and consumption of raw camel milk has been in practice and also advocated due to destruction of therapeutic value of camel milk either by pasteurization or heating, the control and proper management of mastitis is further warranted towards production of safe pathogen free milk. Regular check up of camel herd for mastitis is necessary to avoid secretion of pathogenic microbes in the milk and timely check of spread of the infection. This technical bulletin on camel mastitis covers various aspects of aetiology, diagnosis, treatment and prevention of mastitis in the Camel species. I am sure that the researchers, extension workers specially the field veterinarians will find this manuscript to be of use as reference material in dealing with camel mastitis.

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Director

INTRODUCTION

Mastitis is internationally recognized as one of the most important diseases of economic importance to the dairy industry, affecting almost all domesticated species of the animals and reported from all over the world. The disease causes colossal losses in terms of reduced milk production, cost of treatment, veterinarian's fee, discarding of milk *etc*. In India, the estimated loss due to mastitis is around Rs. 16,702 millions per annum (Yathiraj, 2006). In the U.S. an overall annual loss of $ 2 billions was reported by Cullor (1993). Beck *et al* (1992) estimated an annual loss of ₤ 110 millions in the U. K.

Mastitis in camels has been reported from almost all camel rearing countries. Mastitis, besides causing the loss in milk production has hazardous effects on human beings and suckling calves. Since some of the most pathogenic organisms like mycobacterium, brucellas *etc*. are excreted in the milk. The milk of camel, like that of other dairy animals, contains all the essential nutrients and provides a nutritious food. Camel milk has higher mineral contents including Ca, Na, Mg, Fe and Cu, low sugar and 40 % lower cholesterol than that of cow milk. Camel milk contains about 2-3 times more vitamin-C than cow milk. This vitamin-C is of paramount importance for human diet in arid and semi-arid areas where fruits and vegetables are scarce or not available. It also increases the shelf life of milk by increasing the acidity. Camel is proving to be a good source of milk in India. Camel continues lactating even under stress conditions like drought, when the production of other milch animals ceases. In camels the lactation length is longer and they may yield 5-6 liter of milk per day even during drought.

Mastitis is caused mainly by bacterial pathogens. Relative importance of different infections is likely to vary in different areas and countries. Therefore successful prevention and control of mastitis depends upon knowing the incidence and etiology of mastitis in a particular area. A total of 137 species, subspecies and serovarieties of organisms have been isolated from bovine mammary gland (Watts, 1988). In case of cattle it is estimated that about 50 % of the animals are infected at any time and 95 to 98 % of these infections are subclinical (Guidry, 1985).

Detection of subclinical mastitis is difficult and depends upon various test procedures aimed at detecting the cause or the products of inflammation in milk. Cultural examination is considered to be the golden test in order to establish any opinion about infection status of the udder. Various indirect tests such as somatic cell count (SCC), California mastitis test (CMT), electrical conductivity and pH estimation are based upon detection of products of inflammation or changes in milk and have a well established role as screening test for predicting disease status of mammary glands in cattle but their relevance for application to the camel is less known. These inflammatory markers can be reliable and easy source for detection of subclinical mastitis in camels also.

There are three main principles of mastitis control i.e. elimination of existing infection, prevention of new infection and monitoring udder health status. Elimination of existing infections can be achieved by appropriate therapy of the infected cases. Prevention of new infections and monitoring of udder health status can be achieved by proper supplementation of the required nutrients, hygienic measures and essential diagnostic and maintenance procedures. Numerous antimicrobial formulations are available for the treatment of mastitis, but their efficacy alone or in combination with minerals and immunomodulators is not fully established particularly in camels.

Elimination through antibiotics has simultaneously increased the resistance problem in diverse bacterial pathogens. Though plant derived products have been used for medicinal purposes for centuries and they possess almost limitless ability to synthesize aromatic substances such as flavanoids (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. *In vitro* many laboratories have found thousands of phytochemicals, which have inhibitory effects on all types of microorganisms. More of these compounds should be subjected to animal and human studies to determine their effectiveness in the whole organisms system. Although numerous antimicrobial formulations are available for the treatment of mastitis, but none of them is claimed to be of herbal origin, which could be considered safe, when milk is used after treatment, which leads to drug resistant bacterial strains not only for animals but also for human beings. Moreover success rate for treatment with the available antimicrobials is not always encouraging. World Health Organization also encourages herbal remedies in global health care programme such drugs are easily available at low cost, locally grown and comparatively safe. So this study will discuss about some aspects of prevalence,

etiology, diagnosis, treatment and control of mastitis in camels under the heads:

(1) Prevalence of mastitis by cultural examination and SCC.
(2) Identification and characterization of bacterial mastitis pathogens.
(3) Effect of stage of lactation on incidence of intra mammary infections.
(4) Odematous mastitis in camels.
(5) Physio chemical alterations in milk in different types of mastitis.
(6) Effectiveness of aqueous, crude and methanol extract of various medicinal herbs against dromedary mastitis pathogens.
(7) Efficacy of antibiotic, antioxidant and immuno modulation therapy alone or in combination for the treatment of different types of mastitis.
(8) Relationship between different types of mastitis with serum zinc and copper status and effect of mineral feeding on incidence of intra mammary infections in camels.

MATERIALS AND METHODS

(1) Prevalence of mastitis by cultural examination and SCC:

Collection of milk samples - The udders of camels were cleaned thoroughly with water and mopped to dry with clean cloth. The milkers' hands were thoroughly washed with soap and water and rinsed with 70% alcohol. After discarding first few streams of milk, the teat apices were cleaned with cotton swab soaked in 70% alcohol. Nearly 30 ml. of milk sample from each quarter was collected separately in sterile test tubes. The tubes were marked as right-fore (RF), right-hind (RH), left-fore (LF) and left-hind (LH). Collection was first done from the near side and then from the offside to avoid contamination of the disinfected teat apices. History and relevant information such as animal number, age, stage of lactation, milk yield and physical condition of the udders were recorded.

Cultural examination - Aseptically collected 282 quarter milk samples from 71 lactating camels were subjected to bacteriological examination as per standard procedures (Brown *et al*, 1981) using 5 % sheep blood agar and MacConkey's lactose agar. These plates were incubated at 37^0 C for 24 to 48 hours. The resulting growth from the respective plate of media was purified and identified on the basis of colony morphology including fermentation of lactose and haemolysin production, Gram staining reaction and morphology of the organisms. Staphylococci and streptococci were differentiated on the basis of catalase test. Gram +ve and catalase +ve cocci were differentiated into micrococci and staphylococci on the basis of oxidase teat. Staphylococci were further differentiated into coagulase +ve and coagulase -ve staphylococci on the basis of tube coagulase test (Gibbs and Skinner, 1966).

Somatic cell count - SCC of all the milk samples was performed as per the method of Schalm *et al* (1971) by using Giemsa stain.

Based on the results of cultural examination and SCC the non clinical cases were categorized into four types.
Type. 1: Negative (culturally -ve and SCC < 500000 per ml)

Type. 2: Subclinical mastitic (culturally +ve and SCC > 500000 per ml)

Type. 3: Latent infections (culturally +ve and SCC < 500000 per ml)

Type. 4: Non-specific cases (culturally -ve and SCC > 500000 per ml).

pH reaction of milk - H^+ ion concentration of milk was determined immediately after milking using digital pH meter. Normal camel milk pH is approx. 6.2, if this increases to 6.4 or more is indicative of sub clinical mastitis.

California Mastitis Test - Grading of the test is based on the number of SCC in milk, since breakage of cell leads to gel formation which is easy tool to interpret. Plastic paddle having four shallow cups is used. Equal amount (3 ml) of milk and the reagent were put into each cup of the paddle and the contents were mixed by a gentle circular motion of the paddle in a horizontal plane.

Chart 1: Grading and interpretation of CMT score.

Score	Suggested meaning	DESCRIPTION OF VISIBLE REACTION
-	No reaction	Mixture remains liquid
T	Trace	A slight slime with no tendency towards gel formation.
+	Weak	A distinct slime with no tendency towards gel formation.
++	Distinct +ve	Mixture thickens immediately with gel formation. On continued swirling, mass moves around the periphery leaving the bottom of the cup exposed.
+++	Strong +ve	A distinct gel forms, which tends to adhere to the bottom of the paddle and during swirling a distinct central peak is formed.

(2). Identification and characterization of bacterial mastitis pathogens:

Streptococci were further identified on the basis of CAMP test; in this test 5% sheep blood agar with 0.1% esculin was used. A culture of *Staph aureus* capable of producing a large zone of β-haemolysis was streaked across the center of a plate containing the blood agar esculin medium. A colony of streptococcus culture picked up from a blood agar plate was streaked perpendicular to and within 2 or 3 mm of the line of staphylococcus inoculation. Upto ten cultures can be tested on a plate. A known culture of *Str agalactiae* was included with each test run to provide a CAMP +ve control. The inoculated plates were incubated at 37^0C for 18-24 hours and the cultures

were examined for CAMP reaction, esculin splitting and haemolysis. A +ve CAMP reaction was indicated by a semicircular or arrowhead-shaped zone of complete lysis in the β-zone of haemolysis produced by the staphylococci. A browning of the medium around the streptococcal culture indicates a splitting of the esculin. The CAMP-esculin reactions of the three-streptococcal species, which most frequently cause mastitis, are *Str agalactiae, Str dysgalactiae* and other *streptococci* are shown in chart 2.

Chart 2: Distinguishing features of streptococci

Organism	CAMP	Esculin	Haemolysis
Str agalactiae	+	-	α β or γ
Str dysgalactiae	-	-	α or γ
Other streptococci	+	+	α or γ

The staphylococci were characterized to species level as per the identification scheme of Watts and Owens (1988). A total of 55 isolates of Gram +ve and catalase +ve cocci, which on preliminary examination were identified as staphylococci were differentiated into coagulase +ve and coagulase -ve staphylococci on the basis of tube coagulase test (Gibbs and Skinner, 1966). Coagulase -ve staphylococci were further grouped into novobiocin sensitive and novobiocin resistant strains on the basis of novobiocin sensitivity (30 µg discs - Hi Media). Oxidase +ve cultures were also included in the novobiocin resistant group as a few species are oxidase +ve. The isolates in each group were further characterized to species level by means of different physiological and biochemical tests as mentioned below:

(a) Coagulase +ve staphylococci: pigment production, haemolysin production, tube coagulase test, presence of clumping factor and utilization of mannitol and maltose.

(b) Coagulase -ve novobiocin sensitive staphylococci: urease production, DNase production, phosphatase production and utilization of trehalose, maltose, mannitol, sucrose, fructose, lactose and ribose.

(c) Coagulase -ve and novobiocin resistant staphylococci: oxidase production, urease production and utilization of sucrose, cellobiose, xylose, arabinose, melibiose and raffinose.

Pigment Production - pigment produced by different strains was studied on milk agar medium as mentioned by Cruickshank *et al* (1965). The pigmentation was recorded as white, golden and golden yellow after 24 hours of incubation at 37 ^{0}C.

Haemolysin Production - it was recorded by growing the culture on 5 % sheep blood agar plates. In case of α toxin there was a clear narrow zone of hemolysis, in case of

β in -toxin the heamolysis of erythrocytes was indicated by a wide zone of darkened red blood cells and in case of strain showing both α β toxin, there was narrow clear zone of haemolysis surrounding the colony surrounded by a wide and hazy zone of darkened red blood cells.

Coagulase Test - Coagulase test for different strains was performed as per the method of Gibbs and Skinner (1966).

Clumping Factor - For the demonstration of clumping factor the method described by Stokes and Ridgway (1980) was followed. In case of positive test clear agglutination of organisms was visible.

Urease Production - urease test was performed by the method of Christensen (1946). After inoculation of isolated colonies from overnight cultures. The tubes were incubated at 37^0C for five days and observed daily for the appearance of red, purple or crease colour, which indicated a positive test.

DNase Production - the method of Jeffries *et al* (1957) was followed for the detection of DNase production using Bacto DNase test agar (Hi- Media). The spot inoculated plates were incubated at 37 ^{0}C for 18-24 hours and then flooded with 1 N HCI. In case of positive test hollowing around the growth was visible.

Phosphatase Production - phosphatase test was carried out according to Barber and Kuper (1951). The plates of the medium inoculated with the test cultures were incubated at 37 ^{0}C for 24 hours. The plates were then exposed to ammonia vapours. The development of pink colour indicated a positive phosphatase test.

Aerobic Utilization of Sugars - for the utilization of mannitol, maltose, trehalose, sucrose, fructose, lactose, ribose, cellobiose, xylose, arabinose, melibiose and raffinose phenol red agar base medium was used. Carbohydrate fermentation discs (Hi Media) for the above sugars were used. After smearing of broth cultures these sugars discs were applied like antibiotic sensitivity discs. These plates were incubated at 37 ^{0}C for three days and observed daily for the appearance of yellow colour, which indicated a positive test.

Chart 3: Distinguishing features of coagulase +ve staphylococci

Organism	Pigment	β-toxin	Clumping factor	Tube coagulase	Utilization of	
					Maltose	Mannitol
Staph aureus	20-70%	+	+	+	+	+
Staph intermedius	-	+		+	-	+
Staph hyicus	-	-	-		-	-

Chart 4: Differentiation of coagulase -ve novobiocin sensitive staphylococci

Organism	Aerobic utilization									
	Urease	DNA	Phos	Trehalose	Maltose	Mannitol	Sucrose	Fructose	Lactose	Ribose
Staph hyicus	+	+	+	+	-	-	+	+		+
Staph chromogenes	+	-	+	+			+	+		+
Staph simulans	+	-	-	+	-		+	+	+	+
Staph epidermidis	+	-	+	-	+	-	+	+	+	+
Staph haemolyticus		-	+	+	+			+	+	
Staph hominis	+	-	-		+		+	+	+	-
Staph warneri	+	-	-	+			+	+		
Staph capitis	-	-	-	-	-	+		+	-	-
Staph auricularis	-	-	-	+		-		+	-	-
Staph caprae	+	-	+	+		-	-	-	+	-

Chart 5: Differentiation of coagulase -ve novobiocin resistance staphylococci

Organism	Aerobic utilization							
	Oxidase	Urease	Sucrose	Cellobiose	Xylose	Arabinose	Melibiose	Raffinose
Staph saprophyticus	-	+	+	-	-	-	-	-
Staph cohnii	-		-	-	-	-	-	-
Staph xylosus	-	+	+	-	+		-	-
Staph sciuri	+	-	+	+			-	-
Staph lentus	+		+	+	+		-	-
Staph gallinarum	-	+	+		+	+	-	-
Staph arlettae	-	-	+	-	+	+	+	+
Staph kloosii	-		-	-		-		+
Staph equorum	+	+	+	-	+	+	-	-

(3). Efficacy of antibiotic, antioxidant and immunomodulation therapy alone or in combination for the treatment of different types of mastitis.

(3a). *In vitro* antibiotic sensitivity of the bacterial mastitis pathogens: A total of 114 isolates of various species of organisms, comprising of *Staph aureus* (23), *Staph epidermidis* (32), *Str agalactiae* (12), *Str dysgalactiae* (12), other streptococci (24) and *Corynebacterium* spp. (11) recovered from intramammary infections, were subjected to *in vitro* chemotherapeutic sensitivity to 18 antimicrobials by the disc- diffusion method (Bauer *et al,* 1966). The concentration of different chemotherapeutic discs was chloramphenicol (30 µg), cephalexin (30 µg), amoxycillin (30 µg), amoxyclav (30 µg), tetracycline (30 µg), oxytetracycline (30 µg), cloxacillin (30 µg), gentamycin (10 µg), ciprofloxacin (5 µg), lincomycin (2 µg), penicillin (10 iu), kanamycin (30 µg), polymyxin-b (300 iu), nitrofurantoin (300 µg), neomycin (30 µg), ampicillin (10 µg), spiramycin (30 µg), erythromycin (15 µg) and furazolidone (50 µg). The susceptibility was interpreted as sensitive, intermediate and resistant according to the zone size interpretation chart supplied by the manufacturer.

(3b). Efficacy of treatment regimens against culturally +ve but apparently healthy quarters: Forty-five infected quarters of 19 camels were divided into four gps. First, three gps were given the treatment and the fourth gp was kept as untreated control.

Gp 1: For 13 quarter infections six animals were treated with amoxycillin and cloxacillin.

Gp ll: For 13 quarter infections five animals were treated with amoxycillin and cloxacillin + levamisole.

Gp lll: For 13 quarter infections five animals were treated with amoxycillin and cloxacillin + levamisole + vitamin-E and selenium.

Gp lV: For six quarter infections three animals were kept as untreated control.

On fourth day of last treatment the quarter milk samples from these animals were collected and examined bacteriologically.

Drugs used: Amoxycillin and cloxacillin were administered intramuscularly at the dose rate of 5 mg and 5 mg per kg body weight daily for three days. Levamisole was administered subcutaneous at the dose rate of 2.5 mg per kg body weight as a single dose. Vitamin-E and selenium were administered intramuscularly at the dose rate of 55 l.U. and 1.5 mg per 25 kg body weight respectively as a single dose.

From these cases, 45 isolates of various species of organisms, recovered were subjected to *in vitro* chemotherapeutic sensitivity to 10 antimicrobials by the disc-diffusion method (Bauer *et al*, 1966).

(3c). Efficacy of treatment regimens against culturally +ve apparently clinical cases: sixteen infected quarters out of 17 apparently clinical quarters of seven animals as detected by bacteriological examination were divided randomly into 2 gps and were subjected to systemic therapy.

Gp 1: for nine quarters four animals were given enrofloxacin intramuscularly @ 5 mg per kg b.wt. once daily for three days.

Gp ll: Seven quarters of three animals were kept as untreated control.

On the fourth day of the last treatment the quarter milk samples from these animals were collected and subjected to bacteriological examination as per standard procedures.

(3d). Therapeutic efficacy of ascorbic acid against intramammary infections: Aseptically collected 60- quarter milk samples from 15 lactating camels were examined bacteriologically using standard procedures.

The infected quarters as detected by the cultural examination, were divided randomly into two gps and were subjected to ascorbic acid therapy.

Gp I : for 13 infected quarters, nine animals were given ascorbic acid @50 gm once orally.

Gp II : for seven quarters infections six animals were kept as untreated control. After 72 hours of treatment the quarter milk samples from these animals were examined bacteriologically using standard procedures.

(4). Relationship between different types of mastitis with serum zinc and copper status and effect of mineral feeding on incidence of intramammary infections.

(4a). Relationship between different types of mastitis (intramammary infections/ SCC) with serum zinc and copper status: Aseptically collected 128 quarter milk samples from 32 apparently healthy camels were evaluated for cultural examination and SCC. Based on the results of cultural examination and SCC the animals were divided into four types as negative; subclinical mastitic; latent and non-specific cases as described earlier.

The blood samples were collected from these lactating female camels, taking due sterile precautions. Blood sample were harvested by jugular venepuncture and serum was separated. Serum (2.5 ml) was mixed with equal volume of nitric acid in Kjeldhal digestion tube. The samples were kept overnight at room temperature and then heated over digestion bench using low heat below 90^0 C till the volume of the samples was reduced to 0.5 ml. It was later cooled and 5.0 ml. of double acid mixture containing three parts of nitric acid and one part of 70% perchloric acid was added to it. The samples were again transferred to digestion bench for slow digestion. This procedure was repeated till white fumes emanated and the volume was reduced to 0.5 ml. The digested samples were cooled and diluted to 25 ml with distilled water. The digested samples were subjected to estimation of the minerals by atomic absorption spectrophotometer (Model AAS 4141 of ECIL, Hyderabad, India). Copper, Zn, Fe and Co ware estimated at wavelength 324.4 nm, 212.9 nm, 248.3 nm and 240.4 nm and sensitivity was checked at 1.50 ppm, 1.00 ppm, 2.00 ppm and 2.00 ppm, respectively.

(4b). Effect of mineral feeding on incidence of intramammary infections: Thirty-six infected quarters of 14 lactating camels as detected by bacteriological examination were divided into two gps.

Gp.1: Eight camels with 18 infected quarters including four quarters with mixed infections were fed with $CuSo_4$ (2 gm), $ZnSo_4$ (2 gm) and SeS_2 (Se-2mg) daily for 30 days.

Gp.2: Six camels with 18 infected quarters including two quarters with mixed infections were kept as untreated control.

Quarter milk samples from these animals were examined bacteriologically on 0, 15 and 30 days of mineral feeding.

(5). Physiochemical alterations in milk in different types of mastitis: Quarter milk samples from normal lactating camels (n=56), drying off camels (n=28) and clinical mastitic cases (n=17) were examined for change in colour and consistency. Ten samples from each category were subjected to Na+ and K+ estimation by atomic absorption spectrophotometer (Model EC4141). Na+ and K+ were estimated at wavelength 581.1 nm and 766.6 nm and sensitivity was checked at 300 ppm and 100 ppm, respectively.

(6). Effect of stage of lactation on incidence of intramammary infections: A total of 101 quarter milk samples were examined bacteriologically from 28 lactating camels, which comprised of 17 clinically apparent quarters of seven animals, 56 apparently healthy quarters of 14 lactating animals and 28 apparently healthy quarters of seven animals during the last week of drying.

(7). Odematous mastitis in camels: Odematous mastitis in camels is a managemental problem, due to inadequate exercise after calving. This inadequate exercise results in persistent post parturient udder odema for longer periods and interferes with the normal let down and milking process. Post parturient udder odema is more severe in case of camels and persists normally for 3-4 days. Thereafter a sort of udder involution starts and udder becomes normal in 15-20 days. In some cases, involution of udder is very slow; it leads to a chronic odematous condition of udder with cessation of milk. In very advanced cases, it leads to flabby udder with loosening and wrinkling of udder skin and there is complete stoppage of milk. Odematous condition of udder occurs more severe in dairy camels compared to camels with only suckling calves. Occurrence of this condition is observed both in the field conditions as well as at organized dairy farm. In district Udaipur of Rajasthan state where camels are reared mainly for the milk and this particular area is known as camel milk belt, this condition was more severely observed. At a single camel dairy out of the 10 lactating camels, six developed odematous mastitis with varying degree of severity. In this area, movement of the animals is comparatively restricted due to more land use under cultivation.

(7a). Gross examination of odematous mastitis cases: Observations of 17 such clinical field cases were recorded as per the history provided by the farmers and gross examination of the udder was carried out.

(7b). Bacteriological examination: Aseptically collected quarter milk samples from 66 lactating camels from field cases as well as from an organized herd were examined bacteriologically by using 5 % sheep blood agar and MacConkey's lactose agar following standard procedures (Brown *et al,* 1981). These animals belonged to three groups:

Gp 1: 40 quarter milk samples of 17 cases with clinical symptoms of odematous mastitis.

Gp 2: 80 quarter milk samples of 20 fresh calvers within one month of calving along with some animals having normal post parturient udder odema, and

Gp 3: 115 quarter milk samples of 29 apparently healthy lactating animals in the mid lactation.

(8). Effectiveness of aqueous, crude and methanol extract of medicinal herbs against dromedary mastitis pathogens.

(8a). ***Invitro*** **antibacterial activity of medicinal herbs.**

Test cultures: A total of 90 bacterial isolates recovered from intramammary infections, which comprised of *Staph epidermidis* (30), *Staph aureus* (19), *Corynebacterium* spp. (10), *Micrococcus* spp. (10), *Bacillus* spp. (9), *Pseudomonas* spp. (4), *Proteus* spp., *Streptococcus* spp., *Klebisella* spp., and *E. coli* (2 each) were taken for examination of antibacterial activity to aqueous extract of pardesi kiker (*Prosopis juliflora*), jal (*Salvadora oleoides*), mitha neem (*Murraya koenigii*), arlu (*Ailanthus excelsa*), shisham (*Dalbergia sissoo*), siris (*Albizia lebbeck*).

A total of 76 bacterial isolates comprising *Staph epidermidis* (34), *Staph aureus* (16), *Corynebacterium* spp. (9), *Micrococcus* spp. (4), *Bacillus* spp. (5) and *E. coli* (8) were selected to investigate antibacterial activity to crude juice and methanol extract of tulsi (*Ocimum sanctum*), ashwagandha (*Withania somnifera*), datura (*Datura metel*), peepal (*Ficus religiosa*), pardesi kiker (*Prosopis juliflora*), anar (*Punica granatum*) leaves, garlic (*Allium sativum*) bulb, karela (*Momordica charantia*) fruit and ginger (*Zingiber officinale*) root.

Source of herbs: Traditional medicinal herbs viz *Ocimum sanctum*, *Withania somnifera*, *Datura metel*, *Ficus religiosa*, *Punica granatum* and *Prosopis juliflora* leaves and bark of pardesi kiker (*Prosopis juliflora*), jal (*Salvadora oleoides*), mitha neem (*Murraya koenigii*), arlu (*Ailanthus excelsa*), shisham (*Dalbergia sissoo*), siris (*Albizia lebbeck*) were collected locally. Vegetable herbs viz. *Allium sativum, Momordica charantia*, *Zingiber officinale* were procured from the local market. All these herbs were identified by a botanist.

Extraction process:

Extraction of aqueous extract: Bark of the experimental herbs viz. *Prosopis juliflora*, *Salvadora oleoides*, *Murraya koenigii*, *Ailanthus excelsa*, *Dalbergia sissoo*, *Albizia lebbeck* were dried under shade. Small coarse particles of bark were made with the help of mixer grinder. These coarse bark particles were boiled in distilled water in ratio (1:16). When the water remained approximately one third the original volumes the heating

was stopped and aqueous extract so prepared was sieved and filtered using syringe driven filter (0.22 µm, HiMedia). Aqueous extract was stored in refrigerator till further use.

***Extraction of crude juice*:** Juices of fresh herbal plants viz. *Ocimum sanctum*, *Withania somnifera*, *Datura metel*, *Ficus religiosa*, *Punica granatum* and *Prosopis juliflora* leaves, *Allium sativum* bulb, *Momordica charantia* fruit and *Zingiber officinale* rhizome were extracted with a juicer-mixer (Phillips India home use type) in the laboratory. These juices were filtered through a Whatman filter paper no. 1 (Qualigens) and then scitz filtered. These juices were stored at 4^0C till further use.

***Extraction of methanol extract*:** All herbs as mentioned for extraction of crude juice were cut into small pieces, dried under shade and were coarse grounded. Five grams of the coarse powder were mixed with 100 ml of methanol in glass stoppered bottles which were kept overnight at room temperature. The following day, these mixtures were vortexed for 10 minutes and then centrifuged at 3000 rpm for 10 minutes. The resulting supernatant of each herb was collected separately in glass beakers and chilled in a freezer for two hours. Thereafter the liquid portion was poured into a clean separate beaker and the methanol evaporated at 37-40 ^{0}C. The final volume was reconstituted to five milliliter with normal saline and was stored in refrigerator till further use.

Antibacterial sensitivity: For conducting antibacterial sensitivity tests 2-3 pure isolated colonies of fresh cultures were suspended in three ml of sterilized nutrient broth and were incubated at 37^0C for appearance of turbidity. These cultures were then spread over nutrient agar plates with cotton swabs under sterilized conditions. For each culture for aqueous extract nutrient agar plates were divided into six parts and were marked as (J= Jal, M= Mitha neem, A= Arlu, S= Siris, P= Pardesi kiker, S= Shisham). Into the centre of each part, a well was punched and 10 µl of aqueous extract was pipetted into the wells as per markings, respectively. Plates were kept at room temperature for one hour to facilitate diffusion and were then incubated at 37^0C for 24 hours. The diameter of zone of inhibition was measured. Results were interpreted as positive when the diameter of zone of inhibition was more than 10 mm. The procedure for antibacterial sensitivity was carried out according to Novarro *et al* (1996).

For determination of antibacterial activity of crude juices as well as for methanol extract: 2-3 pure isolated colonies of fresh cultures were suspended in three ml of sterilized nutrient broth and were incubated at 37^0C for appearance of turbidity. These cultures were then spread over nutrient agar plates with cotton swabs under

sterilized conditions. For each culture, three plates were used and each plate was divided into six parts (Fig.1). The parts were marked as follows:

Plate 1: CT = crude tulsi, MT = methanol tulsi, CAg = crude ashwagandha, MAg = methanol ashwagandha, CD = crude datura, MD = methanol datura.

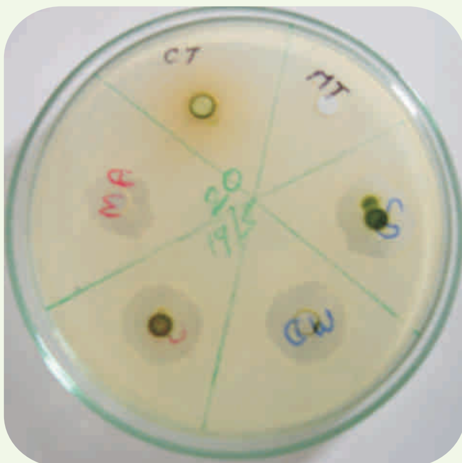
Plate 2: CP = crude peepal, MP = methanol peepal, CA = crude anar, MA = methanol anar, CK = crude kiker, MK = methanol kiker.

Plate 3: Cgr = crude garlic, Mgr = methanol garlic, Ckr = crude karela, MKr = methanol karela, CGn = crude ginger, Mgn = methanol ginger.

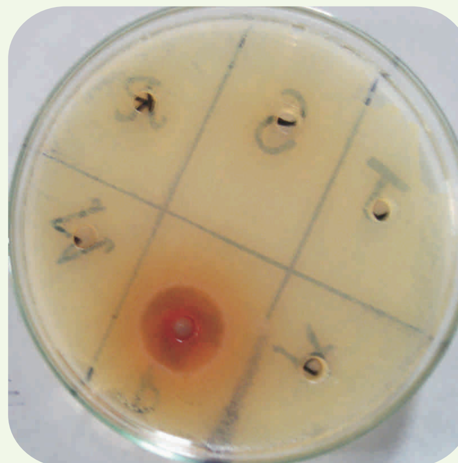
Into the centre of each part, a well was punched and 10 µl of either crude juice or methanol extract was pipetted into the wells accordingly. Plates were kept at room temperature for one hour to facilitate diffusion and were then incubated at 37^{0}C for 24 hours. The diameter of zone of inhibition was measured. Results were interpreted as positive when the diameter of the zone of inhibition was more than 10 mm.

Punching of Agar for Well

Loading of Well



Zone of Inhibition



Zone of Inhibition

(8b). Ultraviolet rays treatment of methanol extract: The methanol extracts of each plant were exposed to UV rays in a laminar flow (15Wx 40minutes) as one milliliter thick layer and were tested for antibacterial activity against eight isolates comprising *Staph aureus (5)*, *E. coli* (2) and *Pseudomonas* spp. (1) to screen for the presence of photosensitive phytochemicals.

(8c). Synergistic effect: To investigate the synergistic effect, the methanol extracts of two plants were mixed. All possible combinations were tested for all plants in 1:1 ratio (5µl+5µl) on six isolates comprising *Staph aureus* (3), *E. coli* (2) and *Pseudomonas* spp. (1).

(8d). *In vitro* minimum inhibitory concentrations of plant extracts: Methanol extracts of anar (*Punica granatum*) and pardesi kiker (*Prosopis juliflora*) were evaluated *in vitro* for MIC against eight isolates comprising *Staph aureus* (5), *E. coli* (2) and *Pseudomonas* spp. (1) in nutrient broth by dilution method.

(8e). *In vivo* effectiveness of garlic *(Allium sativum)* juice: Garlic clove were peeled and juice was extracted, this juice was diluted with distilled water (1:1), pH of this solution was 6.90.

Fourteen infected quarters of nine animals as detected by bacteriological examination were divided randomly into two gps.

Gp I: Seven infected quarters of four animals were infused with garlic solution 3.0-ml per quarter through teat canal route with the help of blind syringe to avoid tearing of teat canal septum.

Gp II. Seven infected quarters of five animals were kept as untreated controls.

After 72 hours of single dose treatment, the quarter milk samples from these animals were collected and subjected to bacteriological examination as per standard procedures.

RESULTS

(1). Prevalence of mastitis by cultural examination and SCC.

On the basis of infected quarters 39.72 % (112/282) were culturally +ve, whereas, 65.60 % (185/282) had SCC more than 500000 per ml of milk. Of these, 34.40 % (97/282) and 5.32 % (15/282) were having, subclinical (culturally +ve and SCC > 500000 ml); and Latent (culturally + ve and SCC < 500000/ ml) mastitis, respectively according to the International Dairy Federation criteria adopted for cattle. While, 31.20 % (88/282) of the quarters were having "non-specific (culturally -ve and SCC > 500000/ ml), mastitis (Table 1).

Table I: Cultural examination and SCC on 282 quarters from 71 apparently healthy camels. (Tuteja *et al*. 2003)

Quarters culturally +ve	Quarters showing SCC > 500000/ ml	Quarters showing SCC > 500000/ ml and culturally +ve	Quarters showing SCC< 500000/ml and culturally +ve	Quarters showing SCC > 500000/ml and culturally – ve
112/282	185/282	97/282	15/282	88/282
39.72%	65.60%	34.40%	5.32%	31.20%

Table 2: Mean SCC of quarters with CMT score (Tuteja *et al*. 2003)

CMT score	No of quarters	Mean SCC ($x10^{5}$)
-ve	137	4.8
Trace	103	9.9
+1	27	17.2
+2	12	65.6
+3	3	122.3

Table 3: Quarter wise incidence of subclinical infections and SCC >500000/ml. (Tuteja *et al*. 2003)

Quarter	No of quarter	No infected	Per cent	SCC >500000/ml	Per cent
RF	71	31	43.66	48	67.60
RH	70	25	35.71	47	67.14
LF	71	25	35.21	45	63.38
LH	70	31	44.29	45	64.29

Table 4. Mean pH of the quarter milk samples in different type of mastitis (Tuteja *et al*. 2003)

Type of mastitis	No of quarters	Mean pH
Negative	78	6.45
Sub clinical	89	6.39
Latent	14	6.32
Non specific	86	6.43
Clinical	5	7.19

As reported in other species of the animals the mean SCC was found to increase proportionately to CMT score (Table 2). No apparent difference was observed between fore and hind quarters as regards to both infection level as well as elevation of SCC (Table 3). As evident from Table (4) mean pH of quarter milk samples was within the normal range in all the non clinical quarters however in case of clinically infected quarters there was a significant rise in the mean pH (7.19).

2. Identification and characterization of bacterial mastitis pathogens.

Amongst 115 isolates from 282 apparently healthy quarters examined including three quarters with mixed infections (Table 5). *Staph epidermidis* was the most predominant (27.83%) organism followed by unclassified streptococci (20.87%), *Staph aureus* (20.0%), *Str agalactiae* (10.43%), *Str dysgalactiae* (10.43%), *Corynebacterium* spp.

(9.57%) and *Bacillus* spp. (0.87%). Mixed infections were present in the following combinations *Staph epidermidis* and unclassified streptococci (2), *Staph aureus* and *Str dysgalactiae* (1). Amongst *Staph aureus* strains 82.61 % were associated with a SCC > 5, 00,000 per ml. similarly, *Staph epidermidis* 90.00 %; *Str agalactiae* 58.33 %; *Str dysgalactiae*, 100 %; unclassified streptococci, 91.66 %; *Corynebacterium* spp. 81.81 % and *Bacillus* spp. 100.00 %, respectively, were associated with SCC > 5, 00,000 per ml. The mean SCC for the above pathogens was 11.1x10^5, 19.5 x10^5, 11.7 x10^5, 10.7x10^5, 12.4 x10^5, 8.2 x10^5 and 9.2 x10^5, respectively (Table 6).

Table 5: Relative frequency of 115 isolates from 112 culturally +ve quarters (Tuteja *et al*. 2003)

Organisms	No of isolates	Per cent
Staph epidermidis	32	27.83
Unclassified Streptococci	24	20.87
Staph aureus	23	20.0
Str agalactiae	12	10.43
Str dysgalactiae	12	10.43
Corynebacterium spp.	11	9.57
Bacillus spp.	1	0.87

Table 6: Quarters with different infections having SCC > 500000/ml (Tuteja *et al*. 2003)

Infection	Number of quarters	Quarters with SCC > 500000/ ml	Per cent	Mean SCC (x10^5)
Staph aureus	23	19	82.61	11.1
Staph epidermidis	30	27	90.00	19.5
Str agalactiae	12	7	58.33	11.7
Str dysgalactiae	12	12	100	10.7
Unclassified streptococci	24	22	91.66	12.4
Corynebacterium spp.	11	9	81.81	8.2
Bacillus spp.	1	1	100	9.2

A total of 55 isolates of staphylococci including 23 coagulase +ve isolates from camel intramammary infections were characterized to species level. The different species of staphylococci identified (Table.7) in order of their frequency were *Staph aureus* (30.91%), *Staph hyicus* (10.91%), *Staph intermedius* (7.27%), *Staph haemolyticus* (7.27%), *Staph auricularis* (7.27%), *Staph sciuri* (7.27%), *Staph hominis* (5.45%), *Staph epidermidis* (3.64%), *Staph capitis* (1.82%), and *Staph warneri* (1.82%). Out of the 55 isolates, nine isolates could not be identified with the present identification system used. Of these, *Staph aureus* strains 94.12 % (16/17), *Staph hyicus* 83.33 % (5/6), *Staph intermedius* 100 % (4/4), *Staph haemolyticus* 100 % (4/4), *Staph auricularis* 100 % (4/4),

Staph hominis 100 % (3/3), *Staph epidermidis* 100 % (2/2), *Staph capitis* 100 % (1/1) and *Staph warneri* 100 % (1/1) were associated with elevated SCC (> 5,00,000 per ml.) of milk.

Table 7: Characterization of 55 isolates of staphylococci (Tuteja *et al*. 2003)

Staphylococcal species	No of isolates	Per cent	Per cent of quarters with SCC > 500000/ ml
Staph aureus	17	30.91	16/17=94.12
Staph hyicus	6	10.91	5/6=83.33
Staph intermedius	4	7.27	4/4=100
Staph haemolyticus	4	7.27	4/4=100
Staph auricularis	4	7.27	4/4=100
Staph sciuri	4	7.27	4/4=100
Staph hominis	3	5.45	3/3=100
Staph epidermidis	2	3.64	2/2=100
Staph capitis	1	1.82	1/1=100
Staph warneri	1	1.82	1/1=100
Non- typable	9	16.37	-

3. Efficacy of antibiotic, antioxidant and immunomodulation therapy alone or in combination for the treatment of different types of mastitis.

(3a). *In vitro* antibiotic sensitivity of the bacterial mastitis pathogens: Results of *in vitro* chemotherapeutic sensitivity of the isolates from latent / subclinical intramammary infections are presented in table 8.

Amongst *Staph aureus* strains (23) tested, 100 % were sensitive to chloramphenicol, cephalexin, amoxycillin, amoxyclav, tetracycline, oxytetracycline, cloxacillin, gentamycim followed by ciprofloxacin, kanamycin, neomycin (95.6% each), polymyxin-b (91.3%), lincomycin, penicillin, ampicillin (86.9% each), furazolidone (78.3%), nitrofurantoin (73.9%) and spiramycin, erythromycin (56.5% each).

Of the 32 *Staph epidermidis* strains,100 % were sensitive to chloramphenicol, cephalexin, amoxycillin, amoxyclav, neomycin followed by tetracycline, oxytetracycline, cloxacillin, ciprofloxacin (96.9%), kanamycin (93.7%), gentamycin, polymxin-b (90.6% each), lincomycin (81.2%), penicillin (78.1%), nitrofurantoin, ampicillin, spiramycin (71.9% each), furazolidone (68.7%) and erythromycin (62.5%).

Amongst *Str agalactiae* strains (12) tested 100 % were sensitive to chloramphenicol, cephalexin, amoxycillin, amoxyclav, tetracycline, oxytetracyline, lincomycin, penicillin, nitrofurantoin, ampicillin, erythromycin followed by cloxacillin, ciprofloxacin, polymyxin-b (91.7% each), gentamycin, kanamycin, spiramycin (83.3% each) and neomycin and furazolidone (66.7% each).

Of the 12 *Str dysgalactiae* strains tested 100 % were found sensitive to chloramphenicol, cephalexin, amoxicillin, amoxyclav, tetracycline, oxytetracycline, cloxacillin, gentamycin, lincomycin, penicillin, nitrofurantoin, ampicillin followed by ciprofloxacin, spiramycin (91.7% each), erythromycin (83.3%), kanamycin, polymyxin-b (75.0% each) and neomycin, furazolidone (50.0% each).

24 unclassified streptococci strains tested were found 100 % sensitive to chloramphenicol, cephalexin, amoxicillin, amoxyclav, tetracycline, oxytetracycline, cloxacillin, lincomycin, penicillin followed by gentamycin, nitrofurantoin, spiramycin (95.8% each), polymyxin-b, ampicillin (91.7% each), erythromycin (87.5%), ciprofloxacin, kanamycin (83.3% each), furazolidone (75.0%) and neomycin (70.8%).

Of the, 11 strains of *Corynebacterium* spp. tested, 100 % were sensitive to chloramphenicol, cephalexin, amoxicillin, amoxyclav, tetracycline, oxytetracycline, cloxacillin, gentamycin, penicillin, neomycin, spiramycin, erythromycin, furazolidone followed by ciprofloxacin, lincomycin, nitrofurantoin (90.9% each), kanamycin, ampicillin (81.8% each), and polymyxin-b (72.7%).

Table 8: *In vitro* antibiotic sensitivity of the bacterial isolates (Tuteja *et al*. 2003)

Antibiotic	No. of isolates tested						
	Staph aureus (23)	*Staph epidermidis* (32)	*Str agalactiae* (12)	*Str dysgalactiae* (12)	Other Streptococci (24)	*Corynebacterium spp.* (11)	Total (114)
Chloramphenicol	22	32	12	12	24	11	114 (100%)
Cephalexin	23	32	12	12	24	11	114 (100%)
Amoxycillin	23	32	12	12	24	11	114 (100%)
Amoxyclav	23	32	12	12	24	11	114 (100%)
Tetracycline	23	31	12	12	24	11	113 (99.1%)
Oxytetracycline	23	31	12	12	24	11	113 (99.1%)
Cloxacillin	23	31	11	12	24	11	112 (98.2%)
Gentamycin	23	29	10	12	23	11	108 (94.7%)
Ciprofloxacin	22	31	11	11	20	10	105 (92.1%)
Lincomycin	20	26	12	12	24	10	104 (91.2%)
Penicillin	20	25	12	12	24	11	104 (91.2%)
Kanamycin	22	30	10	9	20	9	100 (87.7%)
Polymyxin-b	21	29	11	9	22	8	100 (87.7%)
Nitrofurantoin	17	23	12	12	23	10	97 (85.1%)
Neomycin	22	32	8	6	17	11	96 (84.2%)
Ampicillin	20	23	12	12	22	9	96 (84.2%)
Spiramycin	13	23	10	11	23	11	91 (79.8%)
Erythromycin	13	20	12	10	21	11	87 (76.3%)
Furazolidone	18	22	8	6	18	11	83 (72.8%)

In considering overall sensitivity irrespective of the species of the organisms, 100 % of the isolates were found sensitive to chloramphenicol, cephalexin, amoxicillin and amoxyclav. More then 90 % were sensitive to tetracycline, oxytetracycline, cloxacillin, gentamycin, ciprofloxacin, lincomycin and penicillin. Sensitivity to kanamycin, polymyxin-b, nitrofurantoin, neomycin and ampicillin was more than 80 %. Whereas, 79.8, 76.3 and 72.8 % of the isolates were sensitive to spiramycin, erythromycin and furazolidone, respectively.

(3b). Efficacy of treatment regimens against culturally positive but apparently healthy quarters.

Table 9: Efficacy of treatment regimens against intramammary infections in camels (Tuteja & Dixit, 2007)

Organism isolated	Gp. 1		Gp. 2		Gp. 3		Gp. 4	
	Quarters infected		Quarters infected		Quarters infected		Quarters infected	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Staphylococcus spp.	7	4	7	4	9	2	1	1
Streptococcus spp.	4	0	5	0	4	0	4	4
Corynebacterium spp.	2	0	1	0	0	0	1	1
Overall	13	4	13	4	13	2	6	6
Percent clearance	69.23		69.23		84.61		0	

Comparative efficacy of different treatment regimens is shown in table 9. Amoxycillin and cloxacillin could clear 100% of the infections due to streptococci and corynebacterium, but the cure rate against staphylococci was 42.85%. Similar results were obtained when levamisole was also given along with amoxycillin and cloxacillin. When amoxycillin and cloxacillin + levamisole + vitamin E and selenium were used, could clear 100% of the infections due to streptococci. But the cure rate against staphylococci increased to 77.78%. In control gp, infections persisted as such. No immunomodulatory effect of levamisole was observed in the treatment trial conducted. The overall efficacy of treatment was 69.23 % for gp.1 (amoxycillin and cloxacillin), 69.23 % for gp.2 (amoxycillin and cloxacillin + levamisole) and 84.61 % for gp.3 (amoxycillin and cloxacillin + levamisole + vitamin-E and selenium). In case of control (gp.4), neither spontaneous recovery nor new infections were observed. *In vitro* sensitivity testing of the isolates obtained from these cases showed staphylococci to be 100% sensitive against amoxycillin and cloxacillin.

(3c). Efficacy of treatment regimens against culturally positive apparently clinical cases.

The treatment efficacy of enrofloxacin against clinical mastitis cases in camels is shown in the table 10. The overall efficacy of treatment was 77.77 % with enrofloxacin. In case of control gp, neither spontaneous recovery now infections were observed. In all the treatments cure rate for staphylococci was lower than other microbes.

Table 10: Treatment efficacy of enrofloxacin against clinical mastitis in camels (Tuteja & Dixit, 2007)

Organisms isolated	Enrofloxacin treated		Untreated control	
	Quarters infected		Quarters infected	
	Before treatment	After treatment	Before treatment	After treatment
Staphylococcus spp.	7	2	6	6
Streptococcus spp.	2	0	1	1
Overall	9	2	7	7
Percent clearance	77.77		0	

(3d). Therapeutic efficacy of ascorbic acid against intramammary infections.

Table 11: Treatment efficacy of ascorbic acid against intramammary infections. (Tuteja & Dixit, 2007)

Organism	Ascorbic acid		Untreated control	
	Quarters infected		Quarters infected	
	Before treatment	After treatment	Before treatment	After treatment
Staphylococcus spp.	9	5	5	5
Micrococcus spp.	2	1	0	0
Streptococcus spp.	1	0	1	1
Corynebacterium spp.	1	1	1	1
Overall	13	7	7	7
Percent clearance of infections	46.1		0	

The overall efficacy of treatment was 46.1 % with ascorbic acid @ 50 gm per animal orally. In case of control gp, neither spontaneous recovery nor new infections were observed (Table 11).

(4). Relationship between different types of mastitis with serum zinc and copper status and effect of mineral feeding on incidence of intramammary infections in camels.

(4a). Relationship between different types of mastitis (intramammary infections/SCC) with serum zinc and copper status.

Table 12. Mean serum Zn, Cu, Co and Fe concentration of mastitic camels. (Tuteja *et al*. 2004)

Type of mastitis	Number of animals	Zn conc. (µg/ml)	Cu conc. (µg/ml)	Co conc. (µg/ml)	Fe conc. (µg/ml)	Mean SCC ($x10^5$)
Effect				*		
Negative	5	1.30±0.58	1.56±0.17	1.78±0.12	2.42±0.83	369025±209717
Sub clinical	16	2.62±0.33	1.19±0.10	1.34±0.18	3.94±0.48	1059593±117235
Latent	0	---	--	--	--	--
Non-specific	11	2.12±0.39	1.33±0.12	1.26±0.10	3.07±0.56	867606±141391
Clinical	1	2.20±1.30	1.80±0.39	0.70±0.00	6.90±1.86	Clumps of cells

A non- significant variation among negative, sub clinical, non-specific and clinical gps (P<0.05) was recorded in the mean serum Zn, Cu and Fe concentration. However Co concentrations in these gps recorded to be 1.78±0.12, 1.34±0.18, 1.26±0.10 and 0.70±0.41µg/ml, respectively, varied significantly among gps (*P<0.05) (Table 12).

Table 13. Relationship between mean SCC and mean Zn, Cu, Co and Fe concentration. (Tuteja *et al*. 2004)

SCC per ml of milk (lacs)	Number of animals	Mean± S.E. Zn conc. (µg/ml)	Mean ± S.E. Cu conc.(µg/ ml)	Mean ± S.E. Fe conc. (µg/ml)	Mean ±S.E. Co conc. (µg/ml)
Effect			*		
Upto 2.0	5	1.30±0.60	1.56±0.16	2.42±0.84	1.78±0.12
>2.0-5.0	18	2.37±0.32	1.35±0.08	3.93±0.46	1.26±0.10
>5.0-10.0	7	2.45±0.51	0.95±0.14	2.77±0.71	1.41±0.16
>10.0	2	2.60±0.95	1.25±0.25	3.35±1.33	1.35±0.29

*Significant (P<0.05).

Mean serum values of Zn, Co and Fe varied non-significantly among gps (*P<0.05). However Cu concentration varied significantly among gps (P<0.05), when relationship with variation in SCC was considered (Table 13).

(4b). Effect of mineral feeding on incidence of intramammary infections.

Feeding of specially designed mineral mixture for 30 days could clear an overall 54.4 % (12/22) of the infections, where as in control gp a self- cure for 15 % (3/20) of the infections was observed (Table 14).

Table 14: Efficacy of mineral feeding against intramammary infections in camels (Tuteja & Dixit, 2007)

Organism	Mineral feeding			Untreated control		
	Quarters infected			Quarters infected		
	0 day	15 day	30 day	0 day	15 day	30 day
Staph aureus	3	3	2	3	3	3
Staph epidermidis	12	10	8	12	11	10
Streptococcus spp.	6	-	-	5	4	4
Corynebacterium spp.	1	-	-	-	-	-
Overall	22	13	10	20	18	17

5. Physiochemical alterations in milk in different types of mastitis.

In lactating animals milk was normal whitish in colour and normal in consistency, which indicates the healthy status of the herd examined. Clots and flakes were found in the milk of the clinically infected quarters. Increase in sodium conc. in the milk of clinically infected compared to milk of healthy quarters shows increase permeability of sodium due to inflammation of the secretary tissue. Where as K+ conc. was found almost same between milk of normal and clinically infected quarters (Table 15).

Table 15: Physio chemical changes in milk.

Milk property	Lactating animals	Drying off animals	Clinical cases
Colour of milk	Normal whitish (n=56)	70% Normal whitish 30% slight yellowish (n=28)	90% Yellowish ; 10% creamy (n=17)
Consistency	Normal (n=56)	Normal (n=28)	90% clots, flakes; 10% pus like and watery (n=17)
Na+ conc. (ppm)	113.88±23.696 (n=10)	114.53±21.465 (n=10)	171.92±29.525 (n=10)
K+ conc. (ppm)	56.04±6.021(n=10)	66.89±6.507 (n=10)	65.56±3.221(n=10)

6. Effect of stage of lactation on incidence of intramammary infections:

The lower infection rate in milking animals as compared to drying off animals shows flushing action of milking. Since milking was reduced in the drying off period there might be less flushing of the infections entering through the teat canal. Recovery of bacteria from more than 90 % of the clinically infected quarters shows mastitis mainly due to bacterial pathogens (Table 16).

Table 16: Difference in infection level of lactating, drying off and clinical quarters (Tuteja & Dixit, 2007)

Type of Animal	Clinical Cases		Milking Animals		Drying off Animals	
	Quarters	Animals	Quarters	Animals	Quarters	Animals
Culturally examined	17	7	56	14	28	7
Culturally +ve	16	7	6	3	11	5
Per cent +ve	94.12	100	10.71	21.43	39.28	71.43

7. Odematous mastitis in camels.

(7a). Gross examination of odematous mastitis cases: Initial physiological udder odema shows moderate odematous fluid in the udder and teats. During different stages of development of mastitis, there is severe enlargement and odema of the udder. This gives appearance of complete milk letdown condition of the udder. Holding the teats with palm shows dryness of the teat skin. Animal feels pain while

milking or suckling by the calf and actual milk letdown is reduced (Fig.1). Further it progresses into tightness of the udder and shrinkage of teats. At this stage, there is difficulty in holding the teats with hand and milk letdown is almost negligible. Clots, flaks and pus like secretions are observed in such cases. Then starts bluish discoloration of the teats and some quarters may become blind at this stage (Fig.2). Finally there is loosening and wrinkling of the udder skin and whole of the udder gives bluish cyanotic appearance. At this stage, there occurs complete stoppage of milk. Udder becomes cold to touch and sloughing of the udder skin is rarely observed in such cases. It looks as if a leather bag has been tied over the udder and touching the skin reveals hard leathery skin covering a huge tight mass. (Fig.3). Sometimes either right half or left half of the udder are severely affected then the opposite site.(Tuteja *et al.* 2011)

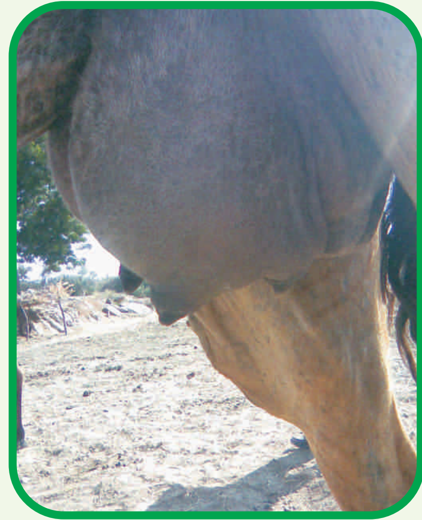


Fig.1: Initial persistence of udder odema

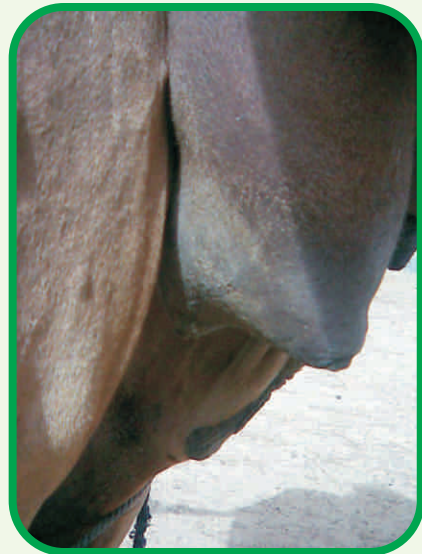


Fig.2: Advanced case with cessation of milk.

Fig.3: Advanced case with complete stoppage of milk.

(7b). Bacteriological examination: Majority of the clinical odematous mastitic quarters were infected with major mastitis pathogen i.e. *Staph aureus*. Whereas, fresh calvers were mostly clear from such organisms. This indicates that odema causes retention of milk, which subsequently results in entrance and precipitation of infectious agents. Further the toxins produced by *Staph aureus* results in damage of the udder tissue. Low level of infections observed in the middle of lactation in apparently healthy quarters may be due to slow entrance of infectious agents through the teat canal, level of these infections increases with the advancement of lactation (Table 17).

Table 17: Difference in infection level of fresh calvers, mid lactators and odematous mastitic quarters. (Tuteja *et al*. 2011)

Organisms	Odematous mastitis cases (40 quarters)	Fresh calvers (80 quarters)	Animals in their mid lactation (115 quarters)
	No of isolates	No of isolates	No of isolates
Staph aureus	38	2	1
Staph epidermidis	2	7	1
Corynebacterium spp.	-	4	1
Micrococcus spp.	-	2	-
Total no of isolates	40	15	3

8. Effectiveness of aqueous, crude and methanol extract of medicinal herbs against dromedary mastitis pathogens.

Table 18: *Invitro* antibacterial sensitivity with aqueous plant extracts.

Organism	No of isolates	No of isolates found sensitive					
		Salvadora oleoides	*Dalbergia sissoo*	*Albizia lebbeck*	*Prosopis juliflora*	*Murraya koenigii*	*Ailanthus excelsa*
Staph epidermidis	30	6	12	10	30	4	2
Staph aureus	19	0	0	9	18	3	1
Corynebacterium spp.	10	1	1	2	10	0	0
Micrococcus spp.	10	1	0	1	8	1	0
Bacillus spp.	9	0	0	0	9	0	2
Pseudomonas spp.	4	1	1	2	4	2	0
Proteus spp.	2	0	0	0	2	0	0
Streptococcus spp.	2	0	0	0	2	0	0
Klebisella spp.	2	0	0	1	2	0	0
E. coli	2	0	0	1	2	0	0
Total	90	9	14	26	87	10	5
Overall percent sensitivity		10	15.5	28.9	96.7	11.1	5.5

Table 19: Antibacterial sensitivity of crude and methanol extract of medicinal herbs.(Tuteja & Dixit,2009)

Organisms	No of isolates	No of isolates found sensitive																	
		Tulsi leaves		Ashwagandha leaves		Datura leaves		Peepal leaves		Anar leaves		Kiker leaves		Garlic bulb		Karela fruit		Ginger root	
		CT	MT	CAg	MAg	CD	MD	CP	MP	CA	MA	CK	MK	CGr	MGr	CKr	MKr	CGn	MGn
Staph epidermidis	34	12	1	34	30	34	0	1	34	34	34	34	34	28	8	13	7	11	0
Staph aureus	16	3	0	16	16	15	15	0	0	16	16	16	16	16	1	2	1	1	0
Corynebacterium spp.	9	6	1	9	9	9	9	0	0	9	9	9	9	9	1	6	4	2	1
Micrococcus spp.	4	2	0	4	4	4	4	0	0	4	4	4	4	4	0	3	2	0	0
Bacillus spp.	5	4	1	5	5	5	5	0	0	5	5	5	5	5	1	4	2	4	1
E. coli	8	0	0	0	0	0	0	8	0	8	8	8	8	8	1	0	0	0	
Total	76	27	3	68	64	65	67	8	1	76	76	76	76	70	12	28	16	18	2
Overall percentage		**35.52**	**3.95**	**89.47**	**84.21**	**85.53**	**88.16**	**10.52**	**1.31**	**100**	**100**	**100**	**100**	**92.10**	**15.78**	**36.84**	**21.05**	**23.68**	**2.63**

8a. *Invitro* antibacterial sensitivity with aqueous extract: Testing of aqueous extract of different plants revealed overall maximum antibacterial activity by *Prosopis juliflora* (96.7%) followed by *Albizia lebbeck* (28.9%), *Dalbergia sissoo* (15.5%), *Murraya koenigii* (11.1%), *Salvadora oleoides* (10.0%) and *Ailanthus excelsa* (5.5%), as shown in table 18. (Tuteja and Dixit, 2010)

***Invitro* antibacterial sensitivity with crude and methanol extract**: Screening of nine medicinal herbs revealed 100 % sensitivity against crude and methanol extracts of anar and pardesi kiker leaves. Datura, ashawagandha and garlic were also found to possess a good antibacterial activity, whereas crude juice of peepal exhibited 100 % activity against *E. coli* isolates (Table 19).

(8b). Antibacterial activity after exposure to UV rays: Antibacterial activity of kiker and anar was unaffected by UV treated extract, whereas all other plants failed to show any antibacterial activity (Table 20).

(8c). Synergistic effect: No significant synergistic effect was observed by combining two plants extracts with any combination. All these combinations gave almost comparable zone of inhibition as observed with single plant extract.

(8d). In *vitro* minimum inhibitory concentrations: MIC of anar varied from 3.75 to 10 µl/ml and for pardesi kiker it varied from 1.25 to 8.75 µl/ml. whereas for oxytetracycline it varied from <1.25 to 5.0µg/ml. (Tuteja and Dixit, 2009)

Table 20: Antibacterial activity after exposure to UV rays (Tuteja & Dixit, 2009)

Plant	Organism (No. of isolates)			Total (8)
	Staph aureus (5)	*E. coli* (2)	*Pseudomonas* spp. (1)	
	No of isolates found sensitive			
Datura leaves	-	-	-	-
Anar leaves	5	2	1	8
Peepal leaves	-	-	-	-
Kiker leaves	5	2	1	8
Tulsi leaves	-	-	-	-
Ashawagandha leaves	-	-	-	-
Garlic bulb	-	-	-	-
Karela fruit	-	-	-	-
Ginger root	-	-	-	-

Comparison of antibacterial effects of plants with standard antibiotics: Antibacterial sensitivity results against 24 bacterial isolates comprising *Staph aureus* (16), *Staph epidermidis* (4), *E. coli* (2) and *Pseudomonas* spp. (1) were compared with the antibacterial effect of various plant extracts. The results revealed almost comparable effects of 10 µl anar and pardesi kiker extracts with tetracycline sensitivity, when a diameter of zone of inhibition of 10 mm was considered sensitive for these plants. Cloxacillin gave poor sensitivity against *Staph aureus* isolates. Four isolates of *Staph aureus* which were resistant to cloxacillin were found sensitive with anar and pardesi kiker extracts. (Tuteja and Dixit, 2009)

(8e). In *vivo* effectiveness of garlic *(Allium sativum)* juice: The overall clearance of infections was observed in 57.1% of the quarters with garlic juice and in rest of the treated quarters reduction in colony count was observed. In case of control gp, neither spontaneous recovery nor new infections were observed (Table 21). No adverse reactions were observed in the infused quarters.

Table 21: *In vivo* efficacy of *Allium sativum* against intramammary infections in camels.

Organism	*Allium sativum* solution		Untreated control	
	Quarters infected		Quarters infected	
	Before treatment	After treatment	Before treatment	After treatment
Staph aureus	3	2	2	2
Staph epidermidis	2	1	2	2
Streptococcus spp.	1	0	1	1
Corynebacterium spp.	-	-	2	2
Bacillus spp.	1	0	-	-
Overall	7	3	5	5
Percent clearance of infections	4/7= 57.14%		0/7= 0%	

DISCUSSION

Quarter infection rate of 39.72% was found slightly higher in comparison to the findings of Sena *et al* (2000). Whereas lower in comparison to the Egyptian camels (El Jakee, 1998). Predominance of *Staph epidermidis*/ coagulase -ve staphylococci is in agreement with the findings of other workers (Chaffer *et al,* 2000; El Jakee, 1998; Almaw and Molla, 2000; Bhatt *et al,* 2004; Abdurahman, 2006) but is contrary to the findings of Sena *et al* (2000) who reported predominance of *Str agalactiae*.

Increase in SCC with major mastitis pathogens (*Staph aureus* and *Str agalactiae*) occurs in cattle (Serieys, 1985) but here increase in SCC with all the infections might be due to the low milk yield (4-5 liter/day). The dilution of milk is a factor confusing SCC interpretation (Dentine and Mc Daniel, 1983; Miller and Paape, 1985). Management mishaps, such as accidental water or feed deprivation, results in drastic decreases in milk yield with corresponding proportional increases in SCC in cattle (Martin, 1973; Miller and Paape, 1985). Holmes *et al* (1996) observed that in Holstein Friesian cows, once daily milking caused a larger decrease in daily milk yield in cows with the high initial SCC. Camels in the present study were milked once daily. Wagner and Stott (1968) reported that increase in SCC might result from physiological stress factors without any inflammatory reaction in the udder.

Mean SCC was found to increase proportionately to CMT score. This is in agreement with the findings of Abdurahman (1984) and Sena *et al* (2000). No apparent difference was observed between fore and hind quarters as regards to both infection level as well as elevation of SCC. Increase in milk pH in clinical mastitis has also been reported by Sena *et al* (2000).

Several new species of staphylococci have been described from animals and man (Schleifer and Kloos, 1975; Kloos, 1980). In the present study among the 55 isolates of staphylococci obtained from intramammary infections in camels, 23 were coagulase +ve and 32 coagulase -ve. In all 10 species of staphylococci were identified. *Staph aureus* (30.91%) was the most frequent species. No published report about the characterization of staphylococci seems to be available on the basis of present classification in camels. *Staph aureus* and *Staph hyicus* have been reported to be the common isolates from milk samples in cows (Hodges *et al,* 1984; Hogan *et al,* 1986; Watts and Ownes, 1988; Watts and Washburn, 1991; Tuteja *et al,* 1993; Sharma and Kapur, 1996). Whereas, Tuteja (1999) found *Staph haemolyticus* and *Staph saprophyticus* to be the more frequent isolates from milk samples in buffaloes. Cox *et al* (1984) identified *Staph intermedius*, *Staph epidermidis*, *Staph aureus*, *Staph sciuri*, *Staph hyicus*

from selected clinical strains from animal infections, especially dogs.

Knowledge of species level distribution of staphylococci is important for disease control and epidemiological studies (Hogan *et al,* 1987; Watts and Owens, 1989). Species level identification is also necessary for recognition of pathogenic species of staphylococci (Watts, 1985). Among the coagulase +ve *staphy*lococci, identification of *Staph aureus*, a primary mastitis pathogen is necessary. *Staph intermedius* another coagulase +ve species is beta toxin producing isolated from carnivores (Cox *et al,* 1984). This is a predominant coagulase +ve organism isolated from dogs and has been infrequently isolated from bovine mastitis. Therefore, *Staph intermedius* should be distinguished from *Staph aureus* in veterinary diagnostic laboratories. Furthermore, distribution of other species such as *Staph aureus*, *Staph hyicus* and *Staph epidermidis* in different herds has been found to be associated with different control measures adopted (Watts and Owens, 1989).

Staph epidermidis, *Staph capitis*, *Staph saprophyticus*, *Staph warneri*, *Staph haemolyticus* and *Staph hominis* have also been reported from human infections (Swell *et al,* 1982; Hovelius *et al,* 1977). There is evidence in man that some of the coagulase -ve staphylococci may be more pathogenic and more resistant to antimicrobials than other species (Aldridge *et al,* 1983; Eng *et al,* 1982; Nicolle *et al,* 1983). As most of the research in veterinary field has been directed towards coagulase +ve staphylococci, the relative pathogenicity of coagulase -ve staphylococci still remains to be elucidated. The apparent increased virulence of human associated coagulase -ve staphylococci for the bovine mammary gland suggests an unnatural host parasite relationship (Watts, 1985). Species identification of coagulase -ve staphylococci may help us to learn more about the diversity, resistance pattern, epidemiology and virulence of the isolates, which were previously identified as *Staph epidermidis* (Watts and Washburn, 1991). The association of certain species with host animals will became apparent as more circumscribed species descriptions became available. However, host specificity does not preclude cross colonization particularly when close association occurs between host species.

In the present study all the species were associated with raised SCC (>500000/ml) of milk. Whereas in cattle (Tuteja *et al,* 1993) and buffaloes (Tuteja, 1999) few species were not associated with raised SCC of milk. The possible reasons for this could be the dilution effect, since camels are low milk yielders therefore the number of SCC per ml of milk were more.

Antimicrobial therapy remains a primary tool for treatment and control of

intramammary infections of dairy animals (Watts *et al,* 1995). Rarely does the veterinarian have the facility of microbial identification and susceptibility testing to guide initial therapy decisions. Development of resistant bacterial strains and improper treatment intervals and procedures may also play a role in treatment failures. Resistance patterns, particularly within the genus staphylococcus can vary greatly from one geographic location to another and from herd to herd (Watts and Owens, 1988). In general the choice of an antimicrobial agent is dictated by antibiotic sensitivity. Too often sensitivity patterns are expressed in quantitative terms such as sensitive, intermediate or resistant. At best this information can guide the practitioner as to which drugs are not to be used.

In the present study antibiotic sensitivity of *Staph aureus* strains was almost similar in cattle and buffaloes for neomycin and chloramphenicol (Jhala, 1976; Kapur *et al,* 1979; Tuteja,1999), gentamycin and cloxacillin (Kapur *et al,* 1979) low sensitivity pattern against penicillin (Jayappa *et al,* 1977; Babu *et al,* 1979; Kalorey *et al,* 1983; Tuteja *et al,* 1993), nitrofurantoin (Rahman and Baxi, 1984; Dahiya and Kapur, 1984), oxytetracycline and polymyxin-b (Tuteja *et al,* 1993) has been reported.

For the *Staph epidermidis* similar sensitivity in cattle and buffaloes against chloramphenicol and cloxacillin (Kapur *et al,* 1980; Tuteja *et al,* 1993; Tuteja, 1999), cloxacillin (Kapur *et al,* 1980), higher sensitivity against nitrofurantoin by Tuteja (1999) and low sensitivity against gentamycin (Shlke *et al,* 1998) has been observed.

Amongst *Str agalactiae* strains if compared in cattle and buffaloes similar sensitivity pattern to chloramphenicol (Jhala, 1976; Tuteja *et al,* 1993; Tuteja, 1999), amoxicillin and erythromycin (Bertoldini *et al,* 1985; Tuteja, 1999). Higher sensitivity to neomycin (Lafi and Hailat, 1998; Tuteja, 1999) has been observed.

Of the *Str dysgalactiae* strains similar sensitivity pattern against penicillin (Dahiya and Kapur, 1984), oxytetracycline, chloramphenicol, ampicillin, cloxacillin and higher sensitivity to neomycin (Dahiya and Kapur, 1984; Tuteja,1999) and low sensitivity to penicillin (Jhala, 1976) in cattle and buffaloes.

Unclassified streptococci of camel intramammary infections showed similar sensitivity pattern to chloramphenicol, oxytetracycline, higher sensitivity to erythromycin and neomycin (Jamkhedkar *et al,* 1969; Tuteja, 1999) and low sensitivity to penicillin (Jamkhedkar *et al,* 1969; Sharma *et al,* 1971; Jhala, 1976) reported from cattle and buffaloes.

Corynebacterium spp. tested were 100% sensitive to penicillin, chloramphenicol. Sharma *et al* (1971) reported 100% of the isolates resistant to penicillin. Tuteja (1999)

reported similar results for chloramphenicol in cattle and buffaloes.

When the pH of milk is weakly acidic (pH 6.4 to 6.8), antimicrobial agents that are weak bases (*e.g.* polymyxin-b) are thought to be preferentially concentrated in the mammary gland by ion trapping. However milk from clinical mastitis cases can have a pH in the range of serum pH, so the antimicrobial agents that are weak acids (*e.g.* penicillins) may reach effective antimicrobial concentrations in milk (Cullor, 1993). Therefore for the treatment of subclinical and clinical mastitis different antibiotics should be considered depending upon the sensitivity and other factors.

Nickerson and Owens (1990) method of drug infusion may actually cause mastitis by inadvertently introducing bacteria through the teat duct. Full insertion of the conventional mastitis tube canula can result in temporary dilation of the teat sphincter muscle and the keratin plug that normally occludes the teat canal. In camel the most effective therapeutic method for treating intramammary infections may be via systemic administration. Soback (1987) suggested that the pharmacokinetic characteristics of a drug must be considered before rational decisions concerning therapy can be made. The ability of the drug to move from the blood into the mammary tissue and into leukocytes is a critical pharmacokinetic consideration for systemic drugs. Systemic therapy offers an alternate route for antibiotic to reach deep tissue foci of infection. Also, systemic therapy does not risk infection with organisms introduced via the teat duct during infusion. Intramammary infections in camels should not be undermined and there is immediate need to carry out pharmacokinetic studies of antibiotics administered via systemic route for the treatment.

Antibiotics in addition to their action on mastitis causing organisms may exert adverse direct effects on the phagocytic efficacy of PMN (Francis, 1989; Daley *et al,* 1992; Pyorala, 2002). The oxidative burst activity of bovine PMN can be altered after intramammary treatment, due to direct effect of the antimicrobials. Research has found that cloxacillin has no effect and enrofloxacin increases PMN activity. Conversely, neomycin, lincomycin, dihydrostreptomycin, doxycycline, oxytetracycline, danofloxacin, penicillin, ceftiofur, spiramycin, erythromycin and chloramphenicol reduce the oxidative burst activity of bovine PMN (Hoeben *et al,* 1998; Paape *et al,* 2003). Because intracellular mastitis causing organisms are not sensitive to antimicrobial action, dysfunctional PMNs may serve as a constant reservoir of protected mastitis causing organisms and thereby lead to a relapsed infection.

The treatment efficacy of enrofloxacin against clinical mastitis cases in camels

was 77.77%. Almost comparable results with enrofloxacin in bovine mastitis have been observed (Sharma and Prasad, 2003; Akhtar *et al,* 2003).

In all the treatments, cure rate for staphylococci was lower than other microbes. *In vitro* sensitivity testing showed staphylococci to be 100 % sensitive against amoxicillin and cloxacillin. So the *in vivo* results were not same as could be expected from *in vitro* testing results. Treatment efficacy for *Staph aureus* bovine mastitis during lactation has been reported by number of researchers. Results have been less than satisfactory with success rates ranging from 15 to 70% (Bramley and Dodd, 1984; Owens *et al,* 1988). Owens (1991) observed that the antibiotics do not easily kill certain population of the infecting *Staph aureus* even when concentrations above the *in vitro* minimum inhibitory concentrations were present.

Effect of oral supplementation of vitamin C, yielded a positive impact in the clearance of intramammary infections, in contrast to cows (Weiss *et al,* 2004) probably due to degradation in the rumen.

Mean serum Zn and Fe concentration in all the non clinical mastitic gps did not vary significantly in camels. Whereas, Cu and Co was found to have some effect either in terms of infection status or SCC in milk. Values reported by AbuDamir (1998) for levels of copper, zinc and iron in camel blood plasma do not correspond with present values. Recordings are partially matching to the reports of Noro *et al* (1992) who also failed to observe any significant difference in the serum Cu levels during lactation and dry period in the healthy and affected cows. Whereas, Sanders and Sanders (1983) observed that supplementation of Cu and reduction of the sulphur content of the water resulted in improved herd health, greater reproductivity, lower calf mortality and increased milk yield in dairy herd. Xin *et al* (1991) showed that neutrophils from Cu deficient steers had significantly lower capacity to kill *Staph aureus* than neutrophils from Cu supplemented animals.

The keratin lining of teat canals in susceptible quarters is thinner, less dense and detached from the epithelium compared with that in resistant quarters (McDonald, 1970); therefore, Zn supplementation decreases the exposure of teat ends to pathogens. Spain (1994) reported lower incidence of mastitis in cows receiving Zn in their ration. Incidence and severity of mastitis is high during early lactation. Such cows in early lactation develop borderline zinc deficiency, accentuated by high levels of calcium in diet (Miller, 1970). Moderate to severe blood lypozincemia during mastitis particularly acute coliform mastitis has been documented (Lohuis *et al,* 1990). Harmon and Torre (1994) also suggested a decrease in the percentage of infected quarters and reduction in

SCC at calving in cows supplemented with Zn as compared to untreated controls. Whitaker *et al* (1997) in paired dairy cows did not observe significant difference between the Zn supplemented and control gps in terms of clinical mastitis, mastitis caused by environmental organisms, new infection or recovery rate and SCC during the first 100 days of lactation.

Clinical studies are supported by experimental studies using other well known pathogenic bacteria. *E. coli* which on producing the mastitis in Holstein cows resulted in a decrease in the mean serum concentration of Zn, Fe, and Cu of 28, 35 and 52 % of prechallenge concentration, respectively (Erskine and Bartlett, 1993). Lactating ewes were inoculated through the teat canal with *Mycoplasma mycoides* var *mycoides* by Banga *et al* (1989), and on comparison with normal milk, mastitic milk showed increase in Cu and Fe and no changes in Co and Zn concentration. In udder tissues, concentration of Cu and Zn increased, whereas concentration of Fe and Co decreased. Endotoxin mastitis induced by Lappalainen *et al* (1988) to determine the effect of endotoxin induced inflammation exerted its effect on transfer of selective elements into milk during the course of inflammation and milk Cu and Fe closely paralleled the increase of milk BSA, indicating that these elements are transferred from blood to milk during inflammation. Shang-Chang Fa *et al,* (1996) analyzed the trace element content in hair samples from healthy and latent mastitic dairy cows. The contents of Zn, Cu and Co in the samples from the infected cattle were lower than those of the normal cows, but the Fe, Pb and Se levels of hair from the infected and normal cows did not vary significantly.

Feeding of Cu, Zn and Se was found effective in clearing the infections. Tuteja *et al* (2004) reported serum Cu concentration varied significantly between gps ($P<0.05$) in camels having different levels of SCC in milk. Xin *et al* (1991) showed that neutrophils from Cu deficient steers had significantly lower capacity to kill *Staph aureus* than neutrophils from Cu supplemented animals. Spain (1994) also suggested a reduction in SCC at calving in cows supplemented with Zn as compared to untreated controls.

The lower infection rate in milking animals as compared to drying off animals showed flushing action of milking. Since milking was reduced in the drying off period, there might be less flushing of the infections entering through the teat canal.

The highly vascular nature of the mammary gland makes the tissue more prone to develop localized odema due to an increase in blood and lymphatic flow. The developing bovine mammary gland undergoes extensive growth and physical changes during late gestation, which likely contributes to odema development. The

odematous and swollen udder is more prone to physical injury and damage as well. In cases where udder odema is severe or continues for long periods of time, damage to the suspensory apparatus of the udder can cause permanent damage, such as a pendulous udder, thus leading to a more chronic form of the disorder (Vestweber and Al-Ani, 1985). Dentine and McDaniel (1983) found that some degree of udder odema was noted in 97 % of cows around the time of parturition.

Mild to moderate cases of udder odema makes effective and complete milking difficult due to the swelling of the teats and other structural changes. Clinical mastitis in early lactation is more common in cows with udder odema than cows without odema. (Slettbakk *et al,* 1995; Waage *et al,* 2001). Udder odema was associated with clinical mastitis in the first 30 days postpartum (Van Dorp *et al,* 1999).

Regarding odematous mastitis in camels, almost similar clinical condition of mammary gland has been explained by Hawari and Hassawi (2008) that camels with obvious signs of inflamed udders had a mean lactation of about four months. The visible signs of inflammation included acute and odematous swelling of the udder and formation of pus in the mammary exudates resulting in a visible alteration of the milk. Congestion of udder at parturation is a physiological phenomenon but it may be sufficiently severe to cause the edema of the belly, udder and teats (Al-Ani and Vestweber, 1986). It can result due to compression of mammary vein by the large fetus, causing mammary or ventral edema in late pregnancy (Ibrahim *et al,* 1998). Muhammad *et al* (2005) reported parturient udder oedema in a 10 year old dromedary camel (*Camelus dromedarius*) with soft and cold swelling of the udder**.** Abera *et al* (2009) observed that taking clinical mastitis and blocked teats into account; only 57.9% of the camels have four teats for milk production. They concluded mastitis a major problem in traditionally managed camels and deserve further attention owning to its potential impact on milk production affecting food security. The period around calving, two weeks before calving and two weeks after calving, is often the highest risk period for mastitis infections to occur. Many of these infections can be prevented by implementing some simple management changes of cleanliness *etc*. Giving moderate exercise in terms of walking to relieve odema and subsequently development of mastitis in case of camels in the post calving periods may give fruitful results.

While treating mastitis with antibiotics in developing countries especially in unorganized herds, the milk withholding time recommended for human consumption is not strictly followed thereby it may further aggravate the development of resistant bacterial strains. Since most of plants are part of the food chain is either being

consumed directly by the human being or by the animals. Therefore efforts shall be directed towards development of antibiotic replacement treatment for mastitis from plants. *Ocimum sanctum* has versatile role in traditional medicine. In the present study some antibacterial activity against *Staph aureus* and no activity against *E. coli* were observed with crude extract of *Ocimum sanctum*. Gupta *et al* (2002) observed almost similar trend against these organisms with aqueous leaf extract of *Ocimum sanctum*. Higher content of linolenic acid in *Ocimum sanctum* fixed oil could contribute towards its antibacterial activity (Singh *et al,* 2005). Malondialdehyde is an aldehyde formed as a breakdown product of per oxidized polyunsaturated lipids (Hall *et al,* 1997). Therefore, linoleic acid and linolenic acid on oxidation could give malondialdehyde. Malondialdehyde is a cross linker and initiates oxidation reaction in which undesirable bonds form between nucleic acids (Leuzaj and Skrzydlewska, 2003). The probable result is inhibition of DNA replication. In addition, malondialdehyde could also crosslink amino group of bacterial enzymes and thereby inhibit the growth. The therapeutic efficacy of *Ocimum sanctum* fixed oil against mastitis suggests that it has the potential to replace the steroid (if not both antibiotic and steroid) and offer a cheaper therapy for the disease (Singh *et al,* 1995).

Potent antibacterial activity exhibited by withania against staphylococcus organisms is in comparison to but no activity against *E. coli* is contrary to findings of Abdulmoniem *et al* (2006), they reported extracts from *Withania somenifera* active against some Gram +ve and Gram -ve pathogenic bacteria. Medically withania leaves are used internally for fever and hemorrhoids. Externally for wounds, hemorrhoids, tumors, tuberculous glands, anthrax pustules, syphilitic sores, erysipelas, and ophthalmitis (Kirtikar and Basu, 1991; Varrier, 1996).

Datura medically has a wide range of applications, including in the treatment of epilepsy, hysteria, insanity, heart diseases, fever with catarrh, diarrhoea, skin diseases *etc.* (Chopra *et al,* 1986). Obi *et al* (2001) reported marked antibacterial activities with the roots and stems of *Datura stramonium* against Gram +ve and Gram-ve bacteria of medical importance. Similar activity against Gram+ve organisms was observed in the present investigation with *Datura metel* leaves.

Potent antibacterial activity against all isolates of *E. coli* tested was shown by *Ficus religiosa* leaves. Odunbaku *et al* (2008) reported ethanolic leaf extract of *Ficus exasperata* showed a mic of 300 mg per ml for *E. coli.* Extracts of *Ficus religiosa* leaves also demonstrated some antibacterial activity (Farrukh and Iqbal, 2003).

Significant antibacterial activity against all the test organisms was observed with

Punica granatum leaves. Negi and Jayaprakasha (2003) reported antioxidant and antibacterial activities of *Punica granatum* peel extracts. The aqueous extract of leaf of punica was able to inhibit the growth of *Bacillus subtilis* and *Staph aureus* (Nair and Chanda, 2005).

In the present study significant antibacterial activity observed with *Prosopis Juliflora* support the findings of Aqeel (1991).Antimicrobial activity of the juliflorine, julifloricine and a benzene insoluble alkaloidal fraction isolated from *Prosopis juliflora* were found to posses remarkable effect against Gram +ve bacteria as well as Gram -ve *Campylobacter* spp. Qazi (1987) found juliflorine more effective than penicillin, sulphamethoxazole and erythromycin against *Staph aureus*. Caceres *et al* (1995) studied plants popularly used in Guatemala for the treatment of gonorrhea and found *Prosopis juliflora* to posses' *in vitro* activity against *Neisseria gonorrhoeae* using strains isolated from symptomatic patients.

In India, garlic has been used to prevent wound infection and food spoilage (Arora and Kaur, 1999). In Ireland, garlic was used to combat pulmonary infection and was reported to be effective against mycobacterium (Delaha and Garagusi, 1985). Garlic has antimicrobial properties against range of bacteria; *Staph aureus* (Deresse, 2010), *E. coli*, *Salmonella* (Johnson and Vaughn, 1969), *Klebsiella* (Jezowa *et al,* 1966), *Micrococcus*, *Bacillus subtulis* (Sharma *et al,* 1977), *Clostridium* (De Witt *et al,* 1979) and *Helicobacter* (O'Gara *et al,* 2000). Good antibacterial activity against Gram +ve bacteria and 100 % against *E. coli* isolates with *Allium sativum* supports the therapeutic efficacy against *E. coli* infection in chickens (Rao *et al,* 1983). Indu *et al* (2006) showed excellent antibacterial activity at all concentrations (100%, 75%, 50% and 25%) of garlic extract to various serogroups of *E. coli* tested. Siegers *et al* (1999) suggested that the alliin metabolite allicin might be responsible for the oxygen scavenging properties of *Allium sativum*. It is postulated that the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of thiol group. *In vivo* the results of the present study, although preliminary, do indicate the potential of garlic as an antimicrobial agent, which may be exploited in the treatment of mastitis.

Leaf extracts of *Momordica charantia* have clinically demonstrated broad-spectrum antimicrobial activity. Water, ethanol and methanol extracts of the leaves have demonstrated *in vitro* antibacterial activities against *E. coli*, staphylococcus, pseudomonas, salmonella, streptobacillus and streptococcus (Omoregbe *et al,* 1996; Khan and Omoloso, 1998). In another study, a fruit extract has demonstrated activity against the stomach ulcer- causing bacteria *Helicobacter pylori* (Yesilada *et al,* 1999). In this study it failed to show antibacterial activity against *E. coli* tested.

Zingiber officinale was not found sensitive against *E. coli* tested, which is contrary to the findings of Cohen (1992) that ginger extract was found to have moderate antibacterial properties against *E. coli* serogroups O^8 and O^{88}. Ekwenye and Elegalam (2005) observed that the solvent of extraction affected the degree of antibacterial activity of the extracts. It was observed that the ethanolic extract of ginger gave the widest zone of inhibition (22mm) using the concentration of $0.8 gml^{-1.}$

High antibacterial activity observed in some of the plants with crude extract compared to methanol extract suggests either all the active components are not soluble in methanol or some of the compounds get degraded with storage.

Salvadora plant branchlets are being used as chew sticks throughout South Africa for tooth cleaning to maintain oral hygiene and to prevent dental carries (Memory, 2003).

Little antibacterial activity observed with other plants suggests the traditional use of these plants, in certain areas depending upon availability of these plants and the knowledge of traditional users in that particular area. The water extract of *Albizia lebbeck* has been used as a traditional remedy for bronchitis, leprosy, gum inflammations and helminth infections (Chopra *et al*, 1956).

Traditionally the mattress made of *Ailanthus excelsa* leaves is used as bed for children suffering from fever. It is also used to cure wounds and skin eruptions (Kirtikar and Basu, 1995). In Chinese system of medicine bark of *Ailanthus excelsa* is used to treat diarrhea and dysentery (Chopra *et al*, 1958). In Africa the plant is used to treat cramps, gonorrhea, epilepsy, tape warm infestation and high blood pressure (Sharma, 1996). Shrimali *et al* (2001) studied antibacterial activity of different fractions of a methanol extract obtained from the dried stem bark of *Ailanthus excelsa* using different bacterial strains. The ethyl acetate fraction inhibited the growth of test bacteria. The minimal concentration of ethyl acetate fraction was found to be 6mg/disc.

Strong odiferous oil occurs in the leaves and the seeds of *Murraya koenigii*. Gautam and Purohit (1974) reported that this essential oil exhibited a strong antibacterial and antifungal activity. An alkaloid, murrayacinine is also found in this plant.

Dalbergia sissoo is a folk remedy for excoriations, gonorrhea and skin ailments (Duke and Wain, 1981). Traditionally wood and bark is being used for dysentery, boils, eruptions, leprosy and nausea (Kirtikar and Basu, 1975).

In the present study no significant synergistic effect was observed by combining

methanol extract of two plants. Sensitivity with standard antibiotics revealed comparable effects of 10µl anar and pardesi kiker extracts with tetracycline sensitivity. Whereas cloxacillin gave poor sensitivity against *Staph aureus* isolates. Even four isolates of *Staph aureus*, which were resistant to cloxacillin, were found sensitive with anar and pardesi kiker extracts.

Synthetic pharmaceuticals are based upon single chemicals, the benefits of phytomedicines often result from synergistic actions of multiple active chemicals acting at single or multiple target sites associated with a physiological process. This synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body (Tyler, 1999). Multiple chemicals acting in an additive or synergistic manner likely has its origin in the functional role of secondary products in promoting plant survival (Kaufman *et al*, 1999).The role of secondary products as defense chemicals, a mixture of chemicals having additive or synergistic effects at multiple target sites would not only ensure effectiveness against a wide range of herbivores or pathogens but would also decrease the chances of these organisms developing resistance or adaptive responses (Kaufman *et al*, 1999; Wink, 1999). Further antibacterial activity against pathogenic organisms like *Staph aureus*, *E. coli* and *Pseudomonas* spp. observed with *Prosopis juliflora* and *Punica granatum* may be of value for considering these plants for the treatment of certain infectious diseases, after evaluation of cytotoxicity, storage stability and excretion of these compounds after degradation or as such from the body.

CONCLUSIONS

Cultural examination and SCC revealed 34.40 % (97/282) of the quarters having 'subclinical mastitis' as per international Dairy federation criteria (isolation of organism and SCC > 500000/ml of milk).

Mean pH of quarter milk samples was within the normal range (6.32-6.45) in all the non-clinical infected quarters whereas in case of clinically infected quarters there was a significant rise in the mean pH (7.19).

Staph epidermidis was the most predominant (27.83%) organism followed by *Staph aureus* (20.0%), *Str agalactiae* (10.43%), *Str dysgalactiae* (10.43%), *Corynebacterium* spp. (9.57%) and *Bacillus* spp. (0.87%) amongst commonly encountered intramammary infections in camels.

A total of 55 isolates of staphylococci including 23 coagulase +ve isolates from

camel intramammary infections were characterized by different biochemical tests. The different species of staphylococci identified in order of their frequency were *Staph aureus* (30.91%), *Staph hyicus* (10.91%), *Staph intermedius* (7.27%), *Staph haemolyticus* (7.27%), *Staph auricularis* (7.27%), *Staph sciuri* (7.27%), *Staph hominis* (5.45%), *Staph epidermidis* (3.64%), *Staph capitis* (1.82%) and *Staph warneri* (1.82%). All of these species were associated with raised SCC of milk.

Variable chemotherapeutic sensitivity pattern was observed for different species of organisms. In considering overall *in vitro* antibacterial efficacy, irrespective of the species of the organisms, 100% of the isolates were sensitive to chloramphenicol, cephalexin, amoxicillin and amoxyclav. More than 90% were sensitive to tetracycline, oxytetracyciline, cloxacillin, gentamicin, ciprofloxacin, lincomycin and penicillin. Sensitivity to kanamycin, polymyxin-b, nitrofurantoin, neomycin and ampicillin was more than 80%. Whereas 79.38, 76.3 and 72.8% of the isolates were sensitive to spiramycin, erythromycin and furazolidone, respectively. In the treatment of apparently healthy but culturally +ve quarters, combination of amoxicillin and cloxacillin resulted in 69.23% clearance of infections and its efficacy was improved to 84.61% when given along with vitamin–E and selenium. Whereas the overall efficacy of treatment with ascorbic acid was 46.1 %. In the treatment of culturally +ve and apparently clinical quarters enrofloxacin resulted in 77.77 % clearance of infections.

There was significant variation in mean Co concentrations among negative, sub clinical, non-specific and clinical gps. to be 1.78± 0.12, 1.34± 0.18, 1.26± 0.10 and 0.70± 0.41µg/ml, respectively, However Cu concentration varied significantly with SCC of milk ($P < 0.05$). However, daily feeding of Cu, Zn and Se for 30 days resulted in almost 40 % lower infections.

Increase in sodium concentration in the milk of clinically infected quarters (171.92± 29.525 ppm) compared to milk of healthy quarters (65.56± 3.221 ppm) shows increase permeability of sodium due to inflammation of the secretary tissue.

The lower infection rate in milking animals as compared to drying off animals shows flushing action of milking, since milking is reduced in the drying off period there occurs less flushing of the infections entering through the teat canal. So the she camels shall be dried of slowly from milking.

Odematous mastitis in camels is a manage mental problem, due to inadequate exercise after calving. This inadequate exercise results in persistent post parturient udder odema for longer periods and interferes with the normal milk let down and milking process. In some cases, it leads to a chronic odematous condition of udder with

cessation of milk. In very advanced cases, it leads to flabby udder with loosening and wrinkling of udder skin and there is complete stoppage of milk. Examination of 40 clinical odematous mastitis quarters revealed, infections with major mastitis pathogen *i.e. Staph aureus*. Most of the fresh calvers were clear from such infections. In the apparently healthy animals increase in level of intramammary infections, occurs with the advancement of lactation.

Screening of nine medicinal herbs *viz*. Tulsi (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Datura (*Datura metel*), Peepal (*Ficus religiosa*), Pardesi Kiker (*Prosopis juliflora*), Anar (*Punica granatum*) leaves, Garlic (*Allium sativum*) bulb, Karela (*Momordica charantia*) fruit, Ginger (*Zingiber officinale*) root for antibacterial activity against 76 bacterial isolates from camel intramammary infections, which comprised of *Staph epidermidis* (34), *Staph aureus* (16), *Corynebacterium* spp. (9), *Micrococcus* spp. (4), *Bacillus* spp. (5) and *E coli* (8) revealed 100 % sensitivity against crude and methanol extract of anar and pardesi kiker leaves. Datura, ashawagandha and garlic were also found to possess good antibacterial activity, whereas crude juice of peepal exhibited 100 % activity against *E. coli* isolates. On exposure of methanol extract of these plants to UV rays, antibacterial activity of kiker and anar was unaffected whereas all other plants failed to show any antibacterial activity. Further aqueous extract of *Prosopis juliflora* was too effective against majority of the isolates tested. *Invitro* mic of methanol extract of anar leaves varied from 3.75 to 10 µl/ml and for pardesi kiker from 1.25 to 8.75 µl/ml. No significant synergistic effect was observed by combining two plants extract. Further antibacterial activity against pathogenic organisms like *Staph aureus, E. coli* and *Pseudomonas* spp. observed with *Prosopis juliflora* and *Punica granatum* may be of value for considering these plants for the treatment of certain infectious diseases, after evaluation of cytotoxicity, storage stability and excretion of the compounds after degradation or as such from the body.

RECOMMENDATIONS

Mastitis broadly may be described as subclinical or clinical

Subclinical mastitis is the presence of an infection without apparent signs of local inflammation or systemic involvement. Although transient episodes of abnormal milk or udder inflammation may appear, these infections are for the most part asymptomatic and if the infection persists for at least two months are termed chronic. Once established, many of these infections persist for entire lactation.

Clinical mastitis is an inflammatory response to infection causing visibly abnormal milk in the form of color, fibrin clots *etc.* As the extent of the inflammation

increases, changes in the udder in the form of swelling, heat, pain, redness may also be apparent. Clinical cases that include local signs only are referred to as mild or moderate. If the inflammatory response includes systemic involvement like fever, anorexia, shock, the case is termed severe. If the onset is very rapid, as often occurs with severe clinical cases, it is termed an acute case of severe mastitis. The presence of odematous gangrenous type of mastitis in camels is a managemental problem and may be due to one of the most common factor, inadequate exercise of the animals.

Diagnosis:

1. In case of clinical mastitis by clinical signs of changes in the gross abnormality in the shape and size of the udder, swelling, heat, pain, redness and abnormal milk in the form of color, fibrin clots etc.

2. Cultural examination: Cultural examination is considered to be the golden test in order to establish any opinion about infection status of the udder may be clinically or sub clinically infected.

Detection of subclinical mastitis is difficult and depends upon various test procedures aimed at detecting the cause or the products of inflammation in milk. Various indirect tests are based upon detection of products of inflammation or changes in milk and have a well established role as screening test for predicting disease status of mammary glands in cattle but their relevance for application to the camel is less known. These inflammatory markers can be reliable and easy source for detection of subclinical mastitis in camels also, these tests are:

3. Electrical Conductivity: The major anions and cations present in milk, which have been studied in relation to secretary disorder in the mammary glands are Na^+, K^+ and Cl^-. The normal secretion of Na^+ and K^+ is controlled by active pumping systems on the basal and lateral membranes of the secretary cell. Bacterial infection of the udder results in damage to the ductal and secretary epithelium, opening up of tight junction between secretary cells and increased permeability of the blood capillaries. Thus Na^+ and Cl^- (which are high in extra cellular fluid) pour into the lumen of the alveolus and in order to maintain osmolarity, K^+ level decreases proportionately. The increase in concentrations of sodium and chloride and decrease in potassium therefore result into increase in the electrical conductivity (EC) of milk. The value of EC of normal milk of camel was found to be higher than cow milk, in which EC value of 6.0 and above can be regarded as a clear indication of subclinical mastitis. The higher basal value of EC in camel milk could be due to its higher chloride content (168 mg/dl) in comparison to cow milk (110 mg/dl) as the EC of milk mainly depends upon the concentration of

chloride ions in milk.

4. Somatic cell count: SCC are positively correlated with the presence of infection. Likewise, higher the SCC in a herd bulk tank, the higher the prevalence of infection in the herd.

While counting somatic cells, the important finding observed was the anucleated particles the 'cell fragments', the presence of which has been reported to be a predominant and constant feature in camel milk. These round or ovoidal particles are comparable to the fragments found in goat milk, containing many vacuoles with endoplasmic reticulam and mitochondria. These cell fragments may constitute upto 95 % of total particles in milk. The origin of these fragments has not been studied; however their similarities with goat milk suggest an apocrine secretion, as in goats. The finding of these cell fragments in camel milk has an important practical implication as being similar in size; they may be counted as somatic cells in direct microscopic cell count, making both enumeration and differentiation of somatic cells difficult.

5. California Mastitis test: Grading of the test is based on the number of somatic cells in milk, since breakage of cell leads to gel formation which is easy tool to interpret. Plastic paddle having four shallow cups is used. Equal amount (3 ml) of milk and the reagent are put into each cup of the paddle and the contents are mixed by a gentle circular motion of the paddle in a horizontal plane.

6. H^+ ion concentration: pH reaction of milk is determined immediately after milking using digital pH meter. pH of normal camel milk is approx. 6.2, if this increases to 6.4 or more is indicative of sub clinical mastitis.

Treatment and control:

1. Hygenic Measures: Besides maintenance of overall cleanliness and body parts including udder *etc*. Maintenance of milking hygiene by milking healthy animals first and infected/diseased later. Regular removal of dung for maintaining bedding hygiene. Milking animals shall be kept away from flies, insects *etc*. Regular treatment of udder trauma, injury *etc*. Since most of the camels are left loose in the jungle for browsing *etc*. these practices can be easily followed at any place.

2. Antimicrobial Therapy: Due to wide and indiscriminate use of anti-microbials, there are chances of emergence of resistant bacterial strains and changes in their sensitivity pattern. Selection of antibiotic should be done by *in vitro* chemotherapeutic sensitivity testing. If such facilities of cultural examination and antibiotic sensitivity are not available in the field conditions, veterinarians must

consult recently published antibiotic sensitivity charts specific to that area. In our two recent studies tetracycline is still found to be drug of choice against camel mastitis.

3. Antioxidant Therapy: Vitamin E and Se therapy has given good results. Daily feeding of Zn, Cu and Se for one month in camels resulted in 40 % lower infections. So the farmers shall be advised for mineral feeding.

4. Supportive Therapy: Oxytocin can be given for clearing milk if milk let down is incomplete due to pain *etc*. Analgesics can be given to relieve pain. Anti-inflammatory drugs can be given to relieve inflammation. B-complex therapy can be given as supportive therapy. Ascorbic acid feeding resulted in clearance of subclinical infections.

5. Exercise: Giving moderate post calving exercise in terms of walking to relieve odema and subsequently development of mastitis may give fruitful results in terms of controlling the infections.

6. Drying of the Animals: Animals from milking shall never be abruptly dried at once rather animals shall be dried slowly by reducing the frequency of milking.

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