Studies on therapeutic potential of *Ocimum sanctum* on subclinical mastitis in goats (*Capra hircus*)

बकरियों में उपनैदानिक थनैला रोग में तुलसी की चिकित्सकीय क्षमता का अध्ययन

DIKSHA SHARMA
B.V.Sc & A.H.

THESIS

MASTER OF VETERINARY SCIENCE

(**Veterinary Clinical Medicine**)

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Department of Clinical Veterinary Medicine, Ethics and Jurisprudence
College of Veterinary and Animal Science, Bikaner
Rajasthan University of Veterinary and Animal Sciences, Bikaner – 334 001 (Rajasthan)

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THESIS

Submitted to the
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(Veterinary Clinical Medicine)

FACULTY OF VETERINARY & ANIMAL SCIENCE

By

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2018

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This is to certify that **Ms. Diksha Sharma** had successfully completed the **comprehensive examination** held on ……as required under the regulation for the degree of Master of Veterinary Science.

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Place:
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ABBREVIATIONS

SCC	-	Somatic cell count
EC	-	Electrical Conductivity
PMN	-	Polymorphonuclear cells
BCP	-	Bromo cresol purple
BTB	-	Bromo thymol blue
CMT	-	California Mastitis test
MCMT	-	Modified California Mastitis test
SFMT	-	Surf Field Mastitis test
WHO	-	World Health Organisation
IDF	-	Indian Diary Federation
MDR	-	Multi Drug Resistent
MRSA	-	Methicillin-resistant S. aureus
MSSA	-	Methicillin-sensitive S. aureus
TBC	-	Total Bacterial Count
ORSA	-	Oxacillin resistant S. aureus
MIC	-	Minimum inhibitory Concentration
MBC	-	Minimum bactericidal Concentration
MT	-	Million Tonnes
CNS	-	Coagulase negative Staphlococci
COX-2	-	Cyclo-oxygenase-2
MWST	-	Modified White side test
DNMRT	-	Duncan' new mulitiple range test
LRS	-	Livestock Research Station
CVAS	-	College of Veterinary Science
IZ	-	Zone of inhibition
NMC	-	National Mastitis Council
IMI	-	Intra- mammary Infection
MSA	-	Mannitol Salt Agar
EMB	-	Eosine Methylene Blue

1. INTRODUCTION

Goats (*Capra hircus*) are the versatile animal. The goats are main source of meat and dairy products (Haenlein, 2004). Goat milk is cheap, wholesome, easily digestible and nutritious (Panicker *et al*., 2015). It is recommended for use in dyspepsia, peptic ulcer and pyloric stenosis. Therapeutic potential of goat milk for liver dysfunction, jaundice, biliary disorders, acidosis and insomnia have also been reported (Miranda *et al*., 2010).

According to Livestock census (2012) Rajasthan state has 21.66 million goats. Out of this population, Bikaner possesses 0.96 million goats. The State produces 13.23 million tonnes of milk per year, which is 10.86 per cent of total milk production in the country. Out of this, 1708.08 tonnes of milk is produced by goats (GOI, 2012). Mastitis is an economically important disease due to its high morbidity, loss of milk production and high cost of treatment (Blowey, 1986; Beck *et al.,* 1992). Current annual economic losses due to mastitis in India have been estimated to be Rs. 7165.51 crore that include Rs. 4151.16 and 3014.35 crore due to sub-clinical and clinical mastitis, respectively (Bansal and Gupta, 2009). There are more than three times of great losses due to sub-clinical mastitis as compared to the clinical form of mastitis (Singh and Singh, 1994).

Mastitis is the multi etiological complex disease, and is defined as inflammation of parenchyma of mammary gland with physical, chemical changes in milk and pathological changes in glandular tissue (Radostits *et al*., 2009). Udder is productive organ of dairy animals. Therefore for better production it should be healthy. However because of anatomical position of udder it is subjected to outside influences and prone to both inflammatory and non-inflammatory conditions (Sharma, 2007). Clinical mastitis is manifested by observable signs of inflammation of udder and gross abnormality in quantity and quality of milk along with fall in milk yield is usually referred to as individual health problem, but sub-clinical mastitis remains to be a herd problem, without observable clinical signs or grossly no changes in milk. The subclinical mastitis is detected by the various indirect tests like California

mastitis test (CMT), Somatic Cell Count (SCC), Electrical Conductivity (EC) and cultural examination. The occurrence of clinical mastitis would be propositional to the prevalence of sub-clinical mastitis, because an existing sub-clinical phase of intra mammary infection.

Sub-clinical mastitis denotes absence of apparent abnormalities in the mammary gland but with presence of chemical and bacteriological changes in the milk (Chakrabarti, 1996).

Goats are susceptible to variety of bacterial infections especially when rearing under high stocking density. Mastitis has been recognized as the most important economical factor affecting the dairy animals' worldwide (Ali *et al*., 2011). Major bacteria involved in etiology of dairy goat clinical or sub-clinical mastitis includes *Staphylococcus aureus, Escherichia coli, Streptococcus, Corynebacterium, Brucella, Bacillus and Pseudomonas* species. Among these *Staphylococcus* sp is at top rank in causing mastitis of dairy goats (Najeeb *et al*., 2013). However, prevalence and relative importance of different etiological agents of mastitis may differ in different geographical regions (Contreras *et al*., 1995). Diagnosis of clinical as well as sub-clinical cases largely depends on the presence of significantly higher leukocytes count in the milk from affected glands. In context of milk, these leukocytes are called as somatic cells. The sub-clinical mastitis is reasonable for greater pecuniary losses to the goat farmers than its clinical counterpart. The effective prevention and etiological therapy of this disease requires precise bacteriological diagnosis along with sensitivity testing of microbial agents against various antimicrobials (Malinowski *et al*., 2002).

Detection of sub-clinical mastitis is more difficult and it continues to have adverse effects on quality and quantity of milk without any apparent sign of illness. Loss of milk production is more in sub-clinical mastitis. It is more hazardous because of the lack of the perceptible symptoms of inflammation and no observable changes in the secreted milk. Sub-clinical mastitis, if diagnosed at an early stage, can be cured easily, minimizing milk loss, as well as treatment cost. So it is better to diagnose the disease when it is in sub-clinical phase.

The California Mastitis test (CMT) and Somatic Cell Count (SCC) of the milk are useful monitoring tools to detect the presence of sub-clinical mastitis in the mammary glands of dairy goats. The CMT is a simple rapid test for detecting mammary gland infection and irritation. It has wide acceptance and is used by veterinarians and dairymen in routine mastitis prevention and control programmes. Prescott and Breed (1910) introduced direct microscopic count method to detect sub-clinical mastitis in milk samples. It is one of the tests which have been universally accepted as screening test on bulk milk and in the milk of individual animal. Criteria accepted by the Indian Dairy Federation (IDF, 1999) for the diagnosis of sub-clinical mastitis is based on isolation of pathogen and cell counts more than 1000000/ml in the goat's milk. Several workers have given different cut off criteria about SCC. Still, it has been accepted as the best quantitative index of udder inflammation. It is used to evaluate the quality of the milk as well as to predict udder infection (Cremoux *et al*., 1994). Investigations are going continuously regarding the efficacy of these tests to detect sub-clinical mastitis.

Bacterial examination of milk samples is still a golden test for diagnosis of sub-clinical mastitis and selecting appropriate antibiotic according to sensitivity. Milk has many nutrients, which are required for bacterial growth. Simultaneously, it is endowed with several substances, which are inhibitory to bacterial growth. In the udder, Polymorphonuclear neutrophils (PMN) are capable of phagocytising a wide variety of particles like microbes, fat globules and casein. The milk fat globules and casein are perhaps significant deterrents to phagocytosis. These deterrents could be a major factor in establishment of infection in the mammary gland by the pathogens (Paape and Wergin, 1997).

Defence against bacterial infection is mediated first by the recognition of the invading organism, which is followed by the initiation of the inflammation favoring the mobilization of circulating, motile, phagocytic and bactericidal leukocytes such as neutrophils to eliminate the infectious agent. This inflammatory response comprises the first line of the immune defence against infection and is vital part of an innate immunity. However, the complement system is central to innate immunity as it is intimately involved in

the process of inflammation, and also eliminates the microorganisms either directly or through cooperation with phagocytic cells (Tomlinson 1983). Complement mediated recognition and ingestion of microorganisms by phagocytes was also appreciated and is an important mediator in the initiation and control of inflammation (Frank and Fries, 1991). The amount of the complement and the bacterial capacity to activate the alternative pathway may be limiting in normal milk but as inflammation progresses and exudation of plasma begins, enough complement becomes available in milk for opsonization both by alternative and classical pathways. An essential defence against mastitis is the phagocytosis of bacteria by polymorphonuclear neutrophils (Craven and Williams, 1985) and the complement system. Complement can contribute to phagocytosis by opsonization of bacteria, the attraction of phagocytes by phlogogenic fragments or complexes (through chemotactic activity) and the priming or activation of ingestion or intracellular killing of pathogens.

Appropriate clearance of the pathogens from the udder requires, along with optimum functioning of the immune cells, effectiveness of the drug also (Sordillo *et al.,* 1997), which depends largely till date on the use of antibiotics. However, antibiotic therapy of established mammary infection is moderately efficacious and requires prolonged milk withdrawal due to residues in milk (Daley and Hayes, 1992). Also, over and indiscriminate use of antibiotics has caused havoc by producing resistance in pathogens. The use of antimicrobials has, overtime, increased the number of antimicrobial-resistant microbes globally (Williams, 2000). Another concern is their effect on the manufacture of dairy products and the development of hypersensitivity syndromes in human beings. Further, the antibiotics used for the treatment of mastitis depress the activity of the polymorphonuclear cells (PMNs) that are considered primary cellular defences of the mammary gland (Hoeben *et al.,* 1997). Development of resistance after antimicrobial treatment of mastitis is an important consideration specially under the aspect of potential build-up of antibiotic-resistant organisms. Most developed countries tried to guarantee the food safety by the installation of drug residue detection systems for antibiotic residues in milk and milk products. In addition to the application of

milk quality regulation to reduce the potential risk of milk contamination to the consumer, there is a need to reduce the frequency of antibiotic treatment of mastitis cases.

For this reason, the concept of using non-antibiotic strategies for controlling mastitis is gaining attention. One such strategy is based on enhancement of the animal's natural defence mechanism by use of non-specific immuno-modulators such as plant materials. Medicinal plants (herbs) constitute a major source of alternative medicine and are used to treat diseases of man and animal since ancient times. The herbal medicines have gained importance due to their less toxicity, lesser side effects and being organic in nature. The herbal therapy generally does not pollute the milk and hence there is no milk withdrawal period like in antibiotic use. Even, World Health Organization (WHO) has emphasized on the use of medicinal plants as these are considered safe and effective than the synthetic drugs.

The *Ocimum sanctum* (Tulsi) is a valuable herbal medicine being used in wide spectrum of animal diseases. Chevallier (1996) proposed the key constituents of *O. sanctum,* which are volatile oil (Eugenol 80%), flavonoids and triterpine (Ursolic acid). The herbal extract of *O. sanctum* possesses immuno-modulatory properties in addition to anti-inflammatory properties as evident in several experiments carried by Sadekar *et al.* (1998). Prakash and Gupta (2005) found eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum*, to be largely responsible for its therapeutic potentials. *O. sanctum* seed oil appears to modulate both humoral and cell-mediated immune responsiveness and GABAergic pathways may mediate these immunomodulatory effects. (Mukherjee *et al.* 2005)

Shafi *et al.* (1916) evaluated immunotherapeutic potential of *Ocimum sanctum* in 10 cases of bovine subclinical mastitis. They found that oral administration of *Ocimum sanctum* leaf powder @ 600 mg/kg body weight daily divided in two doses for 7 days could eliminate 69.23% of intramammary infections and resulted a significant count reduction in somatic cell count.

Ocimum sanctum is powerful antioxidant, demonstrated as anti fungal, antibacterial, act as anti inflammatory immunomodulator, increase metabolism and lowers down stress hormones. (Shukla *et al*., 2013).Though some information is available on the activities of *Ocimum Sanctum* for therapy of mastits in dairy cattle but data regarding their use as antibacterial and in caprine mastitis is scanty. Therefore, present study entitled “Studies on therapeutic potential of *Ocimum sanctum* on sub-clinical mastitis in goats (*Capra hircus*)” was planned with the following objectives:

1. To diagnose sub-clinical mastitis in goats by various tests *viz.* electrical conductivity (EC), CMT, pH, SCC and cultural examination.
2. To evaluate *in vitro* antibacterial activity of aqueous and alcoholic extract of *Ocimum sanctum* (Tulsi) in subclinical mastitic goat milk.
3. To evaluate *in vivo* therapeutic potential of aqueous and alcoholic extract of *Ocimum sanctum* (Tulsi) in sub-clinical mastitis affected goats.

2. REVIEW OF LITERATURE

Lots of work has been carried out on sub-clinical mastitis in India and abroad but detection of sub-clinical mastitis is more difficult as it continues to have the adverse effects on quality and quantity of milk without any apparent sign of illness. Loss of milk production is more in sub-clinical mastitis. It is more hazardous because of the lack of the perceptible symptoms of inflammation and observable changes in the secreted milk. Early diagnosis may lead to effective cure of sub-clinical mastitis, thereby milk loss, as well as treatment cost.The pertinent literature is under mentioned:

Handique *et al*. (1988) defined sub-clinical mastitis as absence of apparent gross abnormalities in mammary gland but with recovery of bacteriological pathogens from the milk. Indirect tests like CMT, cell count etc. are required to make the diagnosis. The level of cell count regarded as significant varies with different workers. This type of mastitis is not frequently noticed by goat keepers.

Khan and Khan (2006) reported that sub-clinical mastitis is an extremely serious economic problem of the dairy industry, not only in India but throughout the world.

Sharif *et al. (*2009) reported that early diagnosis of mastitis with reliable tests facilitates successful treatment and control. The main control principles include: sound husbandry practices and sanitation, post milking teat dip, treatment of mastitis during non-lactating period, and culling of chronically infected animals

Koop *et al*. (2010) reported that sub-clinical mastitis (SCM) is one of the most challenging diseases in dairy goat herds because it has been linked to production loss, downgrading of milk quality and hygiene, increased replacement cost, and considerable veterinary expenses.

2.1 Prevalence

Majic *et al.* (1994) recorded the prevalence of subclinical mastitis in goats and out of 5598 milk samples, 1130(20%) had CMT positive and 261

(23%) of these were bacteriologically positive (mainly *Staphylococcus aureus*, *E. Coli*, and coliforms).

Varma *et al.* (2000) reported that out of 54 milk samples of goats raised under semi feral conditions , only 17(31.47%) were found positive on cultural examination

Contreras *et al.* (2007) reported that the prevalence of subclinical mastitis in small ruminants averaged 5-30%, but the annual incidence of clinical mastitis was generally lower than 5%.

Peixoto *et al.* (2010) evaluated the goat milk samples in Brazil and observed the prevalence of mastitis in goats varies from 22 to 75% with higher prevalence of subclinical cases.

Zhao *et al.* (2016) found 45.82 per cent prevalence of subclinical mastitis in goats on basis of CMT examination.

Bhanot *et al.* (2017) reported prevalence of clinical and subclinical mastitis as 6.4% and 66.6% in lactating goats using CMT from Ambala, Haryana.

2.1 Diagnostic tests for sub-clinical mastitis

Prescott and Breed (1910) for the first time determined the number of body cells (Leukocytes) in milk by a direct method.

Spancer and Simon (1960) found that California mastitis test (CMT) was most useful for field application.

Unlik (1960) reported that difference in pH between various quarters was indicative of udder infection.

Obiger (1961) stated that due to some environmental factors such as ration, temperature and storage, California Mastitis Test might detect normal milk as false positive and mastitic milk as false negative. This was associated with the growth of microflora in the milk which might alter the electrochemical environment.

Cruickshank *et al*. (1970) reported that the alkaline pH is very suitable for the multiplication of bacteria thus, change in milk pH is an another indication of sub-clinical mastitis.

Kapur and Singh (1977) made comparative study of somatic cell count and other indirect tests like California modified test (CMT), modified white side test (MWST) and bromocresol purple test (BCP) for the diagnosis of sub-clinical mastitis. They found that CMT was a good test with accuracy of 90.98, 97.20 and 77.50 per cent in cows, buffaloes and goats, respectively.

Wooding *et al*. (1977) reported that unlike cow milk, which is produced by alveolar merocrine secretion, goat milk is produced by apocrine secretion and contains fairly high somatic cell content compare to cow milk.

Blackshaw and McGrown (1978) examined 73 quarter samples for sub-clinical mastitis by simple electrical conductivity, modified whiteside test and a bacteriological examination. They found 61 samples positive (out of 73) by electrical conductivity, 16 by modified white side test and 14 by bacteriological examination. They concluded that mastitic detector is over sensitive for the diagnosis of sub-clinical mastitis.

Coel (1986) reported that the bromothymol blue and bromocresol purple tests are based on alteration of pH of milk, which in turn depends on salt concentration in exudates. These exudates increase the alkalinity of milk by alkaline salt so that the test may give positive values. Further, the normal milk pH is 6.4-6.9, whereas pH of affected samples is abnormally alkaline, may be as high as 7.4. The degree of alkalinity depends on the severity of inflammation. The milk pH has less diagnostic value in detecting the existence of udder inflammation.

Contreras *et al*. (1995) reported that among the indirect tests, CMT, WST, (Surf Field Mastitis Test) SFMT are commonly used for indirect somatic cell count as indicator of sub-clinical mastitis both in cows and does. California mastitis test (CMT) is a simple, market available, rapid screening test for sub-clinical mastitis, based upon the amount of cellular nuclear protein present in milk sample that react with CMT reagent.

Muhammad *et al.* (1995) reported that Surf Field Mastitis Test (SFMT) is another simple inexpensive indirect test for detection of Sub-clinical mastitis using 3% solutions of household detergent.

Milner *et al*. (1996) reported that electrical conductivity is now employed as a routines test for sub-clinical mastitis detection. EC is influenced by sodium, potassium, calcium, magnesium, chlorine and other ions. Electrical conductivity of the milk increases due to an increased concentration of sodium and chloride ion. However factors other than mastitis, like breed, lactation stage, milking interval and milk composition may affect milk EC.

Vihan and Rubino (1996) determined lysosomal enzyme activity, somatic cells, per cent fat and protein in sub-clinical caprine mastitis and found that mean values for uninfected and infected udder halves, respectively were; N-acetyl-beta-glucosaminiase (NAGase) activity (expressed as f moles p-nitrophenol per ml milk/ min) 0.099 and 0.43; somatic cell count, 3,22,000 and 7,08,000/ml; fat, 4.78 and 3.58 per cent and protein , 6.28 and 5.58 per cent.

Singh *et al.* (1998) reported that besides cellular content, compositional changes also occur during sub-clinical mastitis. In normal and mastitic milk, pH, lactose, chloride, cholesterol, whey protein content varies. Estimation of these variables was also employed as criteria for detection of sub-clinical mastitis.

Patel *et al.* (2000) reported higher reliability of CMT (85.69%) followed by MWST (79.74%).

Tale *et al.* (2001) compared milk pH and somatic cell count tests between normal and mastitic milk. In normal milk pH and SCC ranged between 6.45±0.02 to 6.49±0.02 and 3.25.

Robertson and muller (2005) reported that in the case of goat's milk, a considerable controversy exists as to the relationship between SCC and mastitic infection. The reason for this controversy is that milk secretion in the cow differs from that of the goat. In the cow, milk is squeezed out of the alveoli, while in the goat the alveoli actually burst open. Due to this apocrine

secretion, large numbers of cytoplasmic particles occur in normal goat's milk. These non-leucocytic cell particles do not contain deoxyribonucleic acid (DNA) or a nucleus as leucocytes do. They occur normally in the milk and their presence not only masks, but also complicates the interpretation of the leucocyte response to inflammation. Due to the presence of these cells the total SCC in goat's milk does not correlate well with the leucocyte count in the milk.

Min *et al*. (2007), discarding foremilk fractions, reported average SCC values ranging from 2,000 to 4,000 · 10^3 SC mL^{-1} in infected dairy goats and concluded that SCC in goat milk is not highly correlated to IMI.

Sharma *et al*. (2008) reported SCC as the most accurate test for the diagnosis of sub-clinical mastitis followed by the California Mastitis Test (CMT) and the modified white side test (MWST) while Patel *et al.* (2000) reported higher reliability of CMT (85.69%) followed by MWST (79.74%).

Tanja Stuhr and Karen Aulrich (2010) investigated the function of lysosomal enzymes as anti-inflammatory agents proposed β-Glucuronidase and N-acetyl-β-D-glucosaminidase (NAGase) and as possible as early indicators for SCM. The enzyme lactate dehydrogenase (LDH) is already in use for quality assurance of cow milk and was also investigated as a possible parameter for infection diseases in goats indicating differences in the state of infection. As even light infections of the udder lead to a change in milk composition, ingredients like lactose or lactoferrin (Lf) are also mentioned in studies regarding SCM in goats. Lactoferrin has bacteriostatic properties which could indicate an udder infection at an early stage.

Koop *et al*. (2011) using a composite SCC to detect mastitis by *Streptococcus aureus* in goats, proposed a cut-off value of 1,500 · 103 SC $mL^{-1,}$ with 0.9 and 0.95 sensitivity and specificity values, respectively. In this study, foremilk samples were collected from both udder halves for bacteriological culture.

Joshi *et al.* (2014) collected milk samples of 200 normal quarters from 50 apparently healthy buffaloes of different lactation stage and subjected to culture examination, SCC, EC and CMT to find out prevalence of SCM. The

prevalence of SCM on quarter basis was 40 per cent and 50 per cent on animal basis, based on cell count basis and 37 per cent. The mean ± SE values of SCC were 1.638 ±0.071 million cells/ml with range of 0.705 to 3.49 million cells /ml in sub-clinical mastitis affected milk samples whereas in normal milk samples mean ± SE values of SCC was 0.309 ±0.010 million cells/ml with the range of 0.105 to 0.494 million cells /ml. The mean ± SE value of Electrical conductivity was 6.14 ± 0.09 ms/cm with the range of 5.07-8.02 in sub-clinical mastitis affected milk sample and in normal milk sample mean ± SE value was 4.089 ± 0.064 with the range of 3.41-5.00 ms/cm.

Reddy *et al*. (2014) studied the comparative efficacy of commonly used diagnostic tests for detection of sub-clinical mastitis in dairy cattle of Andhra Pradesh using specificity, sensitivity and predictive value of different indirect tests like California mastitis test (CMT), electrical conductivity (EC) and somatic cell count (SCC) test and culture test as standard in sub-clinical mastitis affected cattle. Out of 135 quarter milk samples subjected to CMT, EC and SCC and cultural examination, the accuracies were found to be 73.33%, 70.37% and 71.00%, respectively. The false positive reactions were more in CMT (24.60 per cent) followed by SCC (23.70 per cent) and EC (20.40 per cent) where as the false negative reactions were highest in EC (34.90 per cent) followed by SCC (31.60 per cent) and CMT (28.60 per cent). The sensitivity, specificity and predictive value of different tests were studied and it was found that EC had the highest specificity (84.84 per cent) and predictive value (79.59 per cent) with lowest sensitivity (56.62 per cent) than compare with the other diagnostic methods for diagnosis of sub-clinical mastitis in cattle. Electrical conductivity can be used as the decision criteria to treat or to cull the animals in herds with high prevalence of sub-clinical mastitis.

Hanan *et al.(*2015) examined quarter milk samples obtained from 120 cows (480 quarter) using the California mastitis test (CMT) and Somatic cell count (SCC). A quarter was considered to have sub-clinical mastitis if it had a positive CMT and was subsequently confirmed to have a somatic cell count $150x10^3$-$<200x10^3$ cells/ml. Any cow with one or more quarters with sub-clinical mastitis was considered to have sub-clinical mastitis at cow level.

Johri (2016) examined milk samples from 96 quarters from 24 apparently healthy Rathi cows were subjected to various tests viz. modified california mastitis test (MCMT), total somatic cell count (TSCC), pH, electrical conductivity (EC) and cultural examination for the diagnosis of sub-clinical mastitis. It was reported that there was no significant change in electrical conductivity pre and post treatment and significant changes in the pH and TSCC pre- and post- treatment.

9.2 Changes in milk constituents

Agarwal and Narayanan (1976) examined the mastitic milk of Tharparkar cows for changes in their contents and noted that the average fat content of mastitic milk (2.81 per cent) was significantly lower than normal milk (4.89 per cent).

Harmon (1994) reported that mastitis leads to elevation of SCC is associated with a decrease in lactose, α-lactalbumin and fat in milk because of reduced synthetic activity in the mammary tissue.

Zitny *et al.* (1995) found correlation of somatic cell count (SCC) with changes in milk production and composition of cows milk and stated the significant correlation between somatic cell count (SCC) and yield of milk, milk fat, milk protein and lactose, respectively.

Auldist and Hubble (1998) examined the effect of mastitis on raw milk of cattle and reported that the decrease in fat, lactose and protein contents of mastitic milk as compare to normal healthy cattle milk.

Cooney *et al*. (2000) reported that as somatic cell count (SCC) increases, lactose and casein per centage decreases in mastitic milk.

Coulona *et al*. (2002) examined the effect of mastitis and their effect on milk composition (protein and lactose contents) and milk somatic cell count (SCC) in 501 milk quarter samples during two consecutive years in cows. Milk protein and mineral assays were performed in sub-samples of 128 milking cows. Major milk pathogens (*Staphylococcus aureus*, *Streptococcus uberis* or *E. coli*) associated with clinical signs of mastitis were accompanied by higher SCC (+1.6 $\log \cdot mL^{-1}$, $P<0.01$), lower lactose concentration (–7.6 $g \cdot kg^{-1}$, $P<0.01$), higher protein concentration (+3.3 $g \cdot kg^{-1}$, $P < 0.01$), hence there was

a sharp decrease in the casein/protein ratio (–10 per centage points, P<0.01). Changes were more marked when *E. coli* was present. *Corynebacterium bovis* did not alter milk chemical composition whereas coagulase-negative *Staphylococci* slightly reduced lactose concentration (–1.8 g/kg^{-1}) and increased SCC (+0.37 log/mL^{-1}).

Leitner *et al*. (2004a) examined twenty five Israeli goats of various crossbreeds with one udder half was naturally infected with identified coagulase negative staphylococci, and the contralateral gland was free of bacteria. the milk yield of the infected halves was significantly lower than that of the uninfected ones. Somatic cell count and *N* acetyl *β-D-* glucosaminidase activity were significantly higher in the infected halves. The lactose concentration in the infected glands was significantly lower than that in the uninfected ones, casein concentrations did not differ, and the whey protein and albumin concentrations were significantly higher in the infected glands.

Leitner *et al*. (2004b) examined 500 Israeli goats of 10 flocks of various breeds and crossbreeds and noted that Milk fat, protein and lactose concentrations ranged among flocks from 19.9 to 55.6 g/l for fat, 34.0 to 51.1 g/l for total protein and 47.4 to 51.1 g/l for lactose. Statistical analysis for the six goat herds that were included in the model showed that the mean total protein and fat were lower in uninfected halves than that in infected ones (39.1 vs. 39.9 g/l for protein and 37.5 vs. 42.0 g/l for fat), whereas lactose was higher in uninfected than in infected halves (49.6 vs. 47.2 g/l).

Merin *et al*. (2004a) conducted a study on goats which had one udder half infected with identified coagulase-negative staphyloccocci (CNS) species while the contra-lateral gland was free of bacteria and observed that milk yield of the infected halves was lower (P<0.001) than that of the uninfected halves in goats (1.38 *vs*. 1.96 kg/day). Only lactose concentration in the infected glands was lower (P < 0.004) than in the uninfected ones, while concentrations of fat, protein and casein did not differ between the uninfected and infected halves. Total whey protein and albumin concentrations were significantly higher in the infected than in the uninfected glands.

Bruckmaier *et al.* (2004) examined milk of dairy cows for sub-clinical mastitis and noted the effect of sub-clinical mastitis on milk protein and lactose concentration. They concluded that lactose content of normal and sub-clinical mastitic milk was 48.1 ± 0.6 and 43.8 ± 1.0 (g/lt), protein content of normal and sub-clinical mastitic milk was 34.4 ± 1.2 and 35.3 ± 1.17 (g/lt) respectively.

Uallah *et al*. (2005) conducted a study under field conditions to determine the effect of severity of mastitis on the milk protein and fat contents. They found that average protein (3.85 ± 0.76 per cent) and fat (5.01 ± 0.19 per cent) contents were maximum in the milk of buffaloes that were negative for mastitis. However, these contents decreased with the severity of mastitis from 3.56 ± 0.10 to 3.14 ± 0.10 per cent for protein and 4.91 ± 0.17 to 4.39 ± 0.15 per cent for fat.

Batavani *et al.* (2007) examined milk samples from quarters of 35 cows infected with sub-clinical mastitis, as well as from 37 healthy controls. Compared to the levels observed in milk from healthy quarters, milk from quarters with sub-clinical mastitis showed elevated sodium (91.97 vs. 52.93 mg/dl), chloride (>0.14 vs. <0.14 g/dl), pH (6.69 vs. 6.59), albumin (5.62 vs. 2.65 g/dl), lactate dehydrogenase (LDH) activity (1524.04 vs. 485.94 IU/L) and immunoglobulins (26.86 per cent vs. 7.43 per cent), respectively.

Ogola *et al*. (2007) analyzed milk samples of 396 quarters from cross-bred cows, fifty six per cent of these quarters were experiencing intra mammary infection, observed high SCC in infected quarters, the concentrations of non-casein fractions, sodium, chloride, and free fatty acid were higher ($p < 0.05$), while the casein content, lactose, casein-to-total protein, potassium, and calcium were lower ($p < 0.05$) compared to normal quarters.

Chen *et al*. (2010) found that milk composition (fat, protein, lactose, casein, and total solids), did not change when milk SCC varied from 214,000to 1,450 · 103 SC mL–1. However, total sensory scores and body and texture scores for cheeses made from high SCC milk were lower than those for cheeses made from low and medium SCC milks.

Tripaldi *et al*. (2010) observed the significant changes in lactose as 4.87, 4.80 and 4.64 per cent) and chloride content (0.650 mg/ml, 0.862 mg/ml and 0.882 mg/ml) along with increasing total somatic cell count (TSCC) values in mastitis in buffaloes.

Sharma *et al*. (2011) examined milk samples of dairy cows for sub-clinical mastitis and concluded that milk protein, fat and lactose contents decreased as somatic cell count increased. They found when SCC ($\times 10^3$cells/ml) ‧ 100 then concentration of lactose, casein and fat were 4.90, 2.81 and 3.74 per cent, when SCC ‧ 250 then concentration of lactose, casein and fat were 4.74, 2.79 and 3.69 per cent, when SCC 500-1000 then concentration of lactose, casein and fat were 4.60, 2.65 and 3.51 per cent and when SCC >1000 then concentration of lactose, casein and fat were 4.21, 2.25 and 3.13 per cent, respectively.

Dos-Reis *et al*. (2013) examined a total of 221 lactating Gyr cows from three commercial dairy farms for analysis of sub-clinical mastitis and concluded that the sub-clinical mastitis reduced lactose, solid not fat (SNF) and total solids content but no difference was found in the protein and fat content between infected and uninfected quarters.

Alemu *et al*. (2013) conducted cross sectional study which was undertaken from October, 2010 to June, 2011 to estimate prevalence of mastitis, to identify potential risk factors, and to assess impact of mastitis on chemical composition of bovine milk in and around Gondar town, Ethiopia. A total of 1,097 quarter milk samples collected from 290 local zebu and Holstein-zebu cross breed cows were examined; and overall prevalence of 46.9 and 24.3 per cent was observed at cow and quarter level, respectively. Clinical and sub-clinical mastitis were detected with prevalence of 9.7 and 37.2 per cent, respectively. Of all parameters, chemical composition of milk, statistically significant difference ($P < 0.05$) was observed in the mean fat composition among different mastitic milk.

Hassan (2013) carried out the study to evaluate the effect of sub-clinical mastitis on physio-chemical composition of raw milk of 45 samples for goats and revealed that there was a significantly difference ($P<0.05$) in fat and

lactose per centages for milk of goats. Fat per centage was (3.33 ±0.16) for milk of infected goats with sub-clinical mastitis as compared to (4.20 ± 0.02) for milk of uninfected animals while, the per centages of lactose is (4.30±0.01) for milk of goats as compared to (4.06±0.11) for milk of infected of the same animals.

Jagadeesh *et al.* (2016) determined the prevalence of sub-clinical mastitis (SCM) and effect on milk quality in cross-bred and desi dairy cattle of Hassan district, Karnataka. Overall, 70 out of 190 animals (37 per cent) were positive for sub-clinical mastitis. The milk pH, fat and solids not fat (SNF) exhibits slight variations between normal and sub-clinical mastitis positive animals. The fat per cent was 3.5 ± 0.1 in normal milk and 3.4 ± 0.1 in sub-clinical mastitic milk (SCM) and solids not fat (SNF) content was 8.8 ± 0.1 in normal milk and 8.6 ± 0.1 in sub-clinical mastitic milk, respectively.

9.2 Cultural examination

Shearer and Harris (1992) *Staphylococcus aureus* is the most important mastitic pathogen in most herds. Other organisms including several species of *Streptococci* (*Streptococcus agalactia*, *Streptococcus uberis*, and *Streptococcus dysgalactia*) are commonly isolated from infected udders. *Pasteurella haemolytica* is also isolated from mastitic glands and is believed to be associated with suckling kids. *Corynebacterium pseudotuberculosis* is often isolated from infected udders where there is a herd problem with abscesses. Additional organisms less commonly isolated from mastitic glands include coliforms and *Mycoplasma* spp.

Fox and Gay (1993) reported that the most important bacterial species responsible for mastitis is *S. aureus*, and its prevalence in dairy herds varied widely from 7 to 40%.

Byeng *et al.* (2007) reported that coagulase negative *Staphylococcus* (43.7%), *S. aureus* (35.4%), and *Pseudomonas aeruginosa* (12.4%) were the most prevalent pathogens in sub-clinical mastitis of goats.

Aydin *et al*. (2009) *Staphylococcus aureus*, *Streptococcus* spp. and *E. coli* revealed in milk collected from sub-clinical mastitis were 61, 15 and 5 per cent, respectively.

Islam *et al*. (2011) did a cross sectional study on 242 lactating Black Bengal does during January to August 2009 in Bangladesh. Clinical mastitis was detected by gross signs of udder infection during physical examination and abnormal milk whereas sub-clinical mastitis was recognized California Mastitis test (CMT). Milk samples of all clinical and sub-clinical mastitis goats were subjected to culture for isolation and identification of responsible bacterial pathogens.The overall prevalence of clinical mastitis and sub-clinical mastitis were 4.54% and 37.19%, respectively. Bacterial pathogens isolated were coagulase negative *Staphylococcus* (73.73%), *Staphylococcus aureus* (26.67%), *Streptococcus* sp. (20%), *Bacillus* sp. (70%) and *Escherichia coli* (6.67%).

Gebrewahid *et al*. (2012) conducted a study at Kafta Humera and Tanqua Abergelle Districts of Tigray, Ethopia from April to June, 2011 to assess the prevalence of sub-clinical mastitis in lactating small ruminants and identify bacterial causative agents. A total of 390 lactating animals comprising 255 goats and 135 sheep were randomly selected from population and screened for evidence of sub-clinical mastitis. The overall prevalence of sub-clinical mastitis was found to be 18.03% (46/255) and 28.14% (38/135) in goats and sheep, respectively. California mastitis test (CMT) positive milk samples were subjected to bacteriological examination and the following bacteria were isolated; coagulase negative *Staphylococcus* (44.7%), *Staphylococcus aureus* (27.7%), *Escherchia coli* (17.0%) and *Streptococci* (10.63%).

Koop *et al.* (2012) Subclinical mastitis in dairy goats is caused by a number of micro organisms and the most important bacterial genus is Staphylococcus, which accounts for more than 90% of all the isolated bacteria and is commonly divided into coagulase negative Staphylococci (CNS) and Staphylococcus aureus.

Bourbah *et al.* (2013) collected milk samples from 298 lactating goats for bacteriological and fungal analysis during March 2009 and February 2010. *Enterobacteria*ceae were the predominant bacterial isolated (54.02%). Whereas; *Aspergillus niger* was the predominant one among fungi. In conclusion, the present investigation reveals a weak impact of the sub-clinical

mastitis whereas the Coagulase negative *Staphylococci* is the causal agent of the majority of the mastitis.

Kumar *et al.* (2013) stated that in addition, fungal, virus or mycoplasma infection has occasionally been reported in goat mastitis, especially in clinical mastitis

Najeeb *et al*. (2013) examined 200 milk samples from 100 apparently healthy goats from D. G. Khan and Lahore districts, Punjab, Pakistan were screened with Whiteside Test (WST) for sub-clinical mastitis. Samples positive for mastitis were cultured for bacterial growth on blood agar. Bacterial growth was obtained in 45% milk samples (90/200). From WST positive milk samples, 146 bacterial isolates were identified on the basis of colonial, microscopic and biochemical profiles. Highest prevalence was of *Staphylococcus aureus* (61.64%) followed by *Escherichia coli* (10.96 %), *Streptococcus* spp. (9.59%), *Pseudomonas* spp., *Bacillus* spp. (6.85%, each) and *Corynebacterium spp*. (4.11%).

Zhao *et al*. (2015) examined 683 dairy goats for subclinical mastitis by using California mastitis test in China and found 313(45.82%) goats positive. Among these positive goats, 209 milk samples were used to identify the causing agents by multiplex PCR assay, and results were as follows: Coagulase- negative *Staphylococci* (59.52%) *Staphylococcus aureus* (15.24%) *E. Coli* (11.43%) and *Streptococcus spp*. (10.95%)

Kumar *et al.* (2016) collected milk samples from 397 udder halves of 200 lactating goats. Of all udder half samples, 115 (28.97%) samples were culture positive and 282 (71.03%) samples yield no bacterial growth. Isolates from positive cases were 87 (75.65%) coagulase negative *Staphylococci* (CNS), 22 (19.1%) coagulase positive *Staphylococci* (CNS) and 6 (5.22%) *Streptococcus* spp. Most of the sub-clinical cases were due to coagulase negative *Staphylococci* (CNS).

Pirzada *et al*. (2016) collected 200 milk samples from two hundred quarters of goats from surroundings of Tandojam, Pakistan and analysed for sub-clinical mastitis using California Mastitis Test (CMT) and conventional bacterial culture technique. A total of 76 (38%) milk samples were found

positive by CMT, those also showed bacterial growth on culture media. Among those 43 (56.58%) were from right quarters whereas remaining 33 (43.42%) were detected with sub-clinical mastitis from left quarters. Of these positive samples, 60 (78.94%) were recorded as pure (one bacterial species, whereas 16 (21.05%) samples were found contaminated with mixed bacterial species. A total of nine bacterial species were isolated from the milk samples. The incidence of *Staphylococcus aureus* was found highest (36.84%) in both right and left quarters of sub-clinical mastitis, followed by *Bacillus subtilis* (18.42%), *Bacillus cereus* (10.52%), *Proteus vulgaris* (9.21%), *Citrobacter* species (6.57%), *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus epidermidis* (5.26% each) and *Streptococcus agalactiae* (2.63%).

Constable *et al.* (2017) cited that *Staphylococcus aureus* and *E.coli* are the most common causes of clinical and subclinical mastits in goats. Other infectious agents include *Pseudomonas spp*. , *Streptococcus dysgalactiae*, *Streptococcus pyogenes* and *Bacillus* spp. In a study over 600 does from 18 herds bacteria were found in 23.3% glands.

9.3 Therapeutic studies

Treatment of diseases through herbs/plants alone or/and in combination from ancient era to till today are common practices in many parts of the world. Herbs are eco-friendly with least side effect and no or least residual effects. Countries having rich natural medicinal resources have started rethinking on issues of exploitation of their available bulk in favor of animals so as to avoid/minimize the hazardous effect of so called latent allopathic drugs (Dixit *et al*., 2006). The active constituent, Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), present in *Ocimum sanctum L*. has been found to be largely responsible for the therapeutic potentials of the tulsi (Ekta *et al*., 2012).

Also, aqueous extract, alcoholic extract and seed oil of Tulsi showed antimicrobial properties against enteric pathogens (Geeta *et al*., 2001; Singh *et al*., 2005). It also exhibited significant antimicrobial activities against some of the clinical isolates and multi-drug resistant *Neisseria gonorrhoeae* (Shokeen *et al*., 2008).

Nair *et al.* (1982) reported the phenolic compounds viz. cirsilineol, cirimaritin, isothymusin, apigenin and rosmarinic acid, and appreciable quantities of eugenol (a major component of the volatile oil) from *Ocimum sanctum* extract of fresh leaves and stems possessing good antioxidant activity.

Sinha *et al*. (1990) reported that antimicrobial activity of *Ocimum sanctum* was found to be higher as compared to commonly available other species of *Ocimum* (i.e. *O. canum*, *O. gratissimum*, *and O. basilicum*) in India.

Kelm *et al*. (2000) reported that rosmarinic acid in *Ocimum sanctum* is natural phenol antioxidant carboxylic acid. It possesses anti viral, anti bacterial, anti-inflammatory and anti oxidant properties. They reported that Eugenol, an active constituent of *Ocimum sanctum* has significant anti oxidant activity and cyclooxygenase activity. The activity was comparable to ibuprofen, naproxen and aspirin at 10, 10, 1000 microM concentration, respectively. This supports traditional use of *O. sanctum* for its anti inflammatory activity.

Vasudevan *et al*. (2001) reported that aqueous extract of *Ocimum sanctum* showed growth inhibition for *Klebsiella, E. coli, Proteus* and *Staphylococcus aureus* while alcoholic extract of *O. sanctum* showed growth inhibition for *Vibrio cholera*.

Aqil *et al*. (2005) reported that the ethanolic extracts have ability to inhibit clinical isolates of β-lactamase producing methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin–sensitive Staphylococcus aureus [MSSA].

Mukherjee *et al*. (2005) aqueous extract of *O. sanctum* showed immunotherapeutic potential in bovine sub-clinical mastitis. Polymorphonuclear cells (PMNs) are the primary cellular defence cells of the mammary glands of the bovines and they are depressed during peri parturient period. Use of antibiotics to treat mastitis further depresses the activity of PMNs. Use of 100 mg/teat/day aqueous extract infusion of *O. sanctum* for seven days reduced total bacterial count (TBC) in the milk and increased neutrophil and lymphocyte counts with enhanced phagocytic activities and

phagocytic index. Similarly, lysozyme content of the milk PMNs were also enhanced significantly in animals pretreated with *O. sanctum*. It was suggested that the bioactive constituents could be urosolic acid, oleanolic acid and sarigenin, which may possess immunomodulatory potential indicated by per centage increase in lymphocyte, enhanced activity of the phagocytosis of PMN cells in the bovine mammary gland, and the reduction in TBC in the milk.

Prakash and Gupta (2005) mentioned the important constituents of *O. sanctum* like eugenol, ursolic acid and carvacrol which also has antimicrobial activity.

Edeoga (2006) reported that *O. gratissimum* plants contain crude protein, crude fibre, ash and crude lipid. The oil is reported to possess antibacterial and insecticidial properties.

Singh *et al*. (2007) reported that the presence of linolenic acid in the oil of *Ocimum sanctum* imparts antibacterial activity against *S. aureus*. The oil alone or in combination with Cloxacillin, a β -lactamase resistant penicillin, has found to be beneficial in bovine mastitis, an inflammatory disorder resulting from staphylococcal infection.

Chandra et *al*. (2011) reported that *Ocimum sanctum* (Tulsi) was found to be the most effective against fungal strain in comparison to bacterial strain.

Mishra and Mishra (2011) studied the antibacterial activity of the aqueous, alcoholic, chloroform extract and oil obtained from leaves of *O. sanctum* against *E. coli, P. aeruginosa*, and *S. aureus*. Extract obtained from *O. sanctum* were observed equally effective against pathogenic gram positive and gram negative bacteria.

Moghaddam *et al.* (2011) studied the antibacterial properties of basil essential oil on the standard gram-negative bacteria including *Escherichia coli , Pseudomonas aeruginosa*, and gram-positive ones including *Bacillus cereus , Staphylococcus aureus*, using agar disk diffusion and detected minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC).The results of agar disk diffusion tests showed the inhibition zones as follow: *S. aureus* : 29.20-30.56 mm, *B. cereus* :10.66-16.11 mm, *E. coli* :17.48-23.58 mm and for *P. aeruginosa* the maximum

inhibition zones were seen. The results of this study showed the presence of bacteriostatic effects of basil essential oil on all the test bacteria. The MICs for gram-positive bacteria were as: *B. cereus* ranging 36-18 µg/mL, *S. aureus* 18 µg/mL, and for Gram-negative bacteria of *E. coli* and *P. aeruginosa* were 18-9 µg/mL.

Bhatt *et al*. (2012) reported that the methanolic extract of *O. sanctum* possess antimicrobial activity against gram positive and gram negative bacteria and also showed antifungal activity against *Aspergillus niger.*

Mahima *et al*. (2012) reported that Tulsi (*Ocimum sanctum*) possess analgesic, antipyretic and anti-inflammatory properties, which is proven by various researchers.

Sharma *et al*. (2012) evaluated the anti microbial activity of *Ocimum tenuiflorum* against selected gram positive, gram negative bacterial and fungal strains. They used the crude ethyl acetate and methanol extract of *O. tenuiflorum* (stem, leaves and root) to assess their antimicrobial activity using disc diffusion method. The inhibition zone (IZ) ranged from 6 mm- 28 mm and activity index (AI) ranged from 0.17-1.47 mm. The most susceptible bacteria and fungi are *E. coli* (IZ= 17 mm and AI=0.89) and *A. niger* (IZ= 7mm and AI=0.19), respectively.

Shukla *et al.* (2013) reported that *Ocimum sanctum* is powerful antioxidant, demonstrated as anti fungal, anti bacterial, act as anti inflammatory immunomodulator, increase metabolism and lowering down stress hormones.

Ali *et al.* (2014) reported that *Ocimum sanctum* (tulsi) is known to be an important medicinal plant from ancient period in India. Ursolic acid (UA), Oleanic acid (OA) and Betulinic acid (BA), the derivatives of triterpenoid saponins, acts as a bioactive compounds and reported to possess a potent anti-bacterial activity against the pathogenic bacteria. They reported that the methanolic extract showed maximium antibacterial activity followed by aqueous and diethyl extracts against *S. aureus, P*. *aeruginosa, E. coli, S. mutans* and *K. pneumonia* etc. However, the extracts were found ineffective against *K. pneumonia.*

Jiyauddin *et al*. (2015) concluded that *Ocimum tenuiflorum* showed antibacterial activity towards *S.aureus, P.aeruginosa* and *E.coli* at the concentration of 100mg/mL which were 12mm, 10 mm and 10mm respectively. They used ethanolic extract of *Ocimum tenuiflorum* and the antimicrobial activity was evaluated by using disc diffusion method on the gram positive and gram negative bacteria.

Sarah and Lamia (2015) focused the phytochemical study of aqueous extracts (cold and hot) and ethanolic extract (70%) of *Ocimum sanctum* L. that are collected from local markets in Iraq. The investigation of phytochemical constituents involved the qualitative and quantitative studies. The results for qualitative analysis explained that the aqueous hot extract and ethanolic extract contains more amounts of active components such as flavonoids, saponins, tannins, alkaloids, terpenoids, glycosides and amino acids or primary and secondary amines rather than cold extract, also the quantitative analysis illustrated that the aqueous hot and ethanolic extracts contains more amounts of flavonoids, saponins, tannins and alkaloids because the aqueous hot and ethanolic extracts still have high yield from all active components and this results may be due to the role of heating in the hot extract and the type of solvent in the ethanolic extract of these active components that cannot be extracted by cold water. Biological activity was also analyzed for the aqueous and ethanolic extracts of Ocimum Sanctum L. by using four different bacterial strains (*Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi* and *Klebsiella)* and using agar diffusion method. The results showed that ethanolic extract has the biggest zone of inhibition for *Staphylococcus aureus, Streptococcus pyogenes* and *Klebsiella* while the aqueous cold and hot extracts given less results of inhibition zone.

Shafi *et al*. (2016) conducted an experiment to explore the immunotherapeutic potential of an herb, *Ocimum sanctum*, in mastitis in dairy cows. The study involved twenty HF × Sahiwal lactating dairy cows, each identified with at least one specific sub-clinical mastitis quarter as per International Dairy Federation criteria. The cows were divided into two equal groups: a control group and a group administered *O. sanctum* leaf powder at 600 mg/kg body weight daily divided into two doses orally for 7 days. The

treatment could eliminate 69.23% of intramammary infections ($\chi 2 = 5.07$; $P \leq 0.5$) and resulted in a significant reduction in somatic cell count and ceruloplasmin concentration, thus subsiding udder inflammation and improving milk quality. The herb was also found to possess immunomodulation potential, as evidenced by the significant increase in phagocytic activity of milk neutrophils and enhanced lactoperoxidase and myeloperoxidase activities. Thus, the results indicated the immunotherapeutic potential of *O. sanctum* in treating bovine-specific sub-clinical mastitis.

Johri (2016) used the aqueous extract of *Ocimum sanctum* at the dose rate of 150 mg/kg body weight for 7 days orally in Rathi cattle and observed the efficacy of this extract to be 72%.

3. MATERIALS AND METHODS

3.1 Source of animals and milk samples

In the present investigation, milk samples of 192 quarters from 96 apparently healthy goats were collected from individual holdings of Bikaner and were subjected to California Mastitis Test (CMT), Electrical conductivity (EC), pH, Somatic Cell Count (SCC) and bacterial cultural examination for the diagnosis of sub-clinical mastitis. The milk samples were collected from goats who had parturated 1 month earlier. The present study was carried out from November 2016 to February 2017.

Based on CMT, SCC, EC, pH and cultural examination of goat milk, 12 goats suffering from subclinical mastitis were taken out of 96 goats for therapeutic evaluation of aqueous and alcoholic extract of *Ocimum sanctum* (Tulsi) and six goats as control group. The milk samples were collected aseptically for cultural and sensitivity examination by using standard procedure as per Cowan and Steel (1975).

3.2 Collection of milk sample

Milk sample for culture were collected before milking in the morning that was most convenient under the management conditions of the individual holdings. Strict aseptic procedures were followed while collecting the milk samples in order to prevent the contamination with microorganisms present on the skin of goat's flanks, udder, teats, and hands of the sampler. To collect the milk samples aseptically, udder and teats were washed with water and air-dried. Then teat opening of each udder half was wiped off by spirit swab. Two-three stripping of fore milk were discarded. Approximately 30 ml of fore milk from each udder half was collected in 50 ml sterilized test tube. Care was taken to avoid any type of contamination in the milk. All the samples of milk were brought to the laboratory and kept in refrigeration (4^0C) until analyzed.

3.3 Diagnostic tests for the detection of sub-clinical mastitis

3.3.1 California Mastitis test

The principle of the test is the reactive reagent which reacts with the DNA of the somatic cell nuclei after the dissolution of their outer wall and the

nucleus cell wall with the formation of filamentous mass which is proportional with the somatic cell count (Sheldrake *et al*., 1983). A higher concentration in the somatic cell count leads to a higher CMT score. California mastitis test scores are directly related to the average somatic cell counts (Browning, 2008).

The California mastitis test have been developed by Schalm and Noorlander (1957).

The composition of the CMT reagent was as follows:

Teepol = 2.0 ml

Sodium hydroxide (AR) = 4.5 g

Bromo cresol purple = 10.00 mg

Distilled water = 1000 ml

Procedure

The test was carried out with 3 ml of milk from each udder half into the respective two cups in plastic paddle. An equal amount of the above test reagent was added in each cup and gently mixed by circular movement of the paddle in horizontal plane. The total cell count is reflected by the degree of precipitation or gel formation that occurs. The pH change associated with abnormal milk is indicated by a colour reaction with bromocresol purple. This test has specificity for leucocytes in the milk. (Figure 1).

In the present study, California Mastitis test reactions were scored as follows.

Table 1 Interpretation California mastitis test (CMT) scores on goat milk.

CMT Score	**Somatic cell range**	Interpretation
Negative	700000-1000000	Healthy Quarter
Trace	1000000-1200000	Subclinical mastitis
1	1200000-1300000	Subclinical mastitis
2	1300000-2000000	Subclinical mastitis
3	2000000-5000000	Clinical mastitis

3.32 Electrical conductivity

This was determined by Pen type EC-035(ATC) Conductivity meter of ERMA instruments using the standard procedure as mentioned by the user manual of the product.

3.3.3 pH reaction

pH of the milk was determined immediately using single electrode Pen type digital pH meter (PH-035 (ATC) of ERMA instruments) .

3.3.4 Somatic cell count

Preparation of milk smear

Before preparation of milk smear, milk sample was mixed thoroughly so as to obtain uniform distribution of cells. The sample was allowed to stand for five minutes to permit air bubbles to rise and foam to disappear.

0.01 ml (10µl) of milk was withdrawn by micropipette and spread evenly on a grease free glass slide in 1 square cm area. The smear was dried in air. Thereafter, a few drops of xylene were poured over the milk smear and kept for 2 minutes to dissolve the fat globules of milk.

The smear was then air dried and fixed with 99 per cent methanol for 2 minutes and washed with distilled water. After fixing smear, it was stained with Giemsa stain for 30 minutes as used by Mansson *et. al.* (2016). After staining, the smear was kept in phosphate buffer solution (pH 7.0) in coupling jar for 5 minutes and bloat dried. This smear was used for somatic cell count under oil immersion.

Counting of somatic cells

Examination of milk smear was done at random. One square cm area of smear was divided into four equal parts by dividing it at the right angle. The smear was examined under oil immersion. Cells were counted in five fields from each divided area. Thus the cells were counted in total 20 fields. The average number of cells per sq cm area was calculated. For counting of cells per ml of milk the average numbers of cells per field were multiplied by microscopic factor.

Derivation of common microscopic factor

Common microscopic factor was determined as per Prescott and Breed (1910) as under:

Diameter of an oil immersion microscopic field of Nikon microscope used in study = 190µ or 0.019 cm.

Radius of oil immersion microscopic factor = 0.0095 cm.

Area of oil immersion microscopic field = πr^2

$= 22/7 \times (0.0095)^2 \text{ cm}^2$

$= 3.1428 \times 0.0095 \times 0.0095 \text{ cm}^2$

$= 0.0002833 \times 10^{-5} \text{ cm}^2$

$= 28.33 \times 10^{-5} \text{ cm}^2$

$$\text{No. of fields in 1 cm}^2 \text{ area} = \frac{1\text{cm}}{\text{Area of an oil immersion microscopic field}}$$

$$= \frac{1}{28.33 \times 10^{-5}}$$

$$= 3529.82$$

Number of cells counted in 20 fields = X

Then total numbers of cells in 1 cm^2 area are

$$= \frac{X}{20} \times 3529.82 \text{ in 0.01 ml of milk}$$

$$\text{So number of cells in 1 ml of milk} = \frac{X \times 3529.82 \times 100}{20}$$

$$= \frac{X}{20} \times 352982 \text{ (multiplication factor)}$$

Here $\frac{X}{20}$ = Average No. of cells in 20 field of 1 cm^2 area

Total number of cells in 1 ml of milk = Avg. No. of cells in 20 fields of 1 cm^2 area × 352982.

3.3.4 Changes in milk constituents

The mastitis leads to changes in composition of milk protein, fat, lactose etc. Changes in composition of mastitic milk is usually depends on the severity of damage of udder tissue by pathogenic microbes. These were determined by milkoScan - The milk analyzer (Figure 2). Measurement of changes in composition of milk was done by passing milk through the milk analyzer.

Procedure

The MilkoScan was turned ON, the option of other milk was selected. Milk sample which to be tested taken in to receptacle which was provided along with the milkoScan. The receptacle was brought below the suction pipe of the milkoScan and ran the instrument by pressing enter key. Results were displayed within 1 minute. Then the exit key was pressed to remove residual milk from milkoScan and at last thorough cleaning of milkoScan with acidic and alkaline solution was done.

3.3.5 Cultural examination

Each milk sample was screened for the presence of bacteria by cultivation, isolation and identification using standard procedure as per Cowan and Steel (1975)

Cultivation and isolation of bacteria

The milk samples collected aseptically were shaken thoroughly. With the help of a four mm diameter platinum loop 0.01 ml of the milk sample was streaked on five per cent sheep blood agar, Nutrient agar plate and Mannitol salt agar/Mac-Conkey agar plates in primary, secondary and tertiary fashion in order to obtain isolated colonies of bacteria. These petri dishes were incubated for 24 hours at 37^{o}C and in case colonies did not appear or were found small, the plates were incubated for further 24 hours. Following incubation, the plates were observed for colonial characteristics and haemolytic zones on blood agar plates. If more than one type of colonies appeared on the agar plates, the different colonies were fished out and sub cultured separately for obtaining the pure culture of the bacterial isolates.

Mannitol salt agar culture plates were observed for appearance of *Staphylococci* and *Micrococci* colonies as it is selective media for Gram positive bacteria *Staphylococci* and *Micrococci*, as high level of NaCl is inhibitory to most other bacteria. In Mannitol salt agar *Staphylococcus aureus* (Mannitol fermenting) produced yellow colonies with yellow zones, whereas other coagulase-negative *Staphylococci* e.g. *S. epidermidis* (Non mannitol fermenting) produce small pink or red colonies with no colour change to the medium.

Mac-Conkey agar culture media plates were also observed for the appearance of colonies. The colonies were further examined for fermentation reaction of lactose. The lactose fermenting colonies were distinguished by their red or pink colour and non lactose fermenting colonies were colourless. Now, pure lactose fermenting culture were streaked on Eosine Methylene Blue (EMB) agar plates and incubated for 24 hours. The cultures that gave metallic sheen were considered as having *E. coli*. This appearance of metallic sheen on culture was due to the formation of eosinate from eosine.

The smears were prepared from pure colonies of bacteria, fixed by gentle heating and stained by Gram's method. The stained smears were examined under oil immersion objective for determining the type of Gram's reaction, morphological characters and to ascertain homogenicity of organisms. The pure isolates were taken on the nutrient agar slants and preserved in refrigeration at 4^{o}C after proper sealing and numbering was done for reference. The other bacterial growth slants were subjected to further characterization.

3.3.6 Identification of bacteria

Identification of mastitis causing bacteria was done as per following procedures:

1. Morphology

Colonies of bacteria on nutrient agar plates were purified and bacteria were observed for their shape, arrangement, sporulation, capsulation and presence of any other distinctive feature.

2. **Motility:** Motility was studied in hanging drop preparation of broth culture of bacteria.
3. **Growth in air:** Growth in air was studied to confirm whether the bacterial isolates were able to grow under aerobic or anaerobic condition.
4. **Gram's reaction:** Smears of young culture of bacterial isolates were stained by modified Gram's method of staining described by Hucker and Cohan (1923). The results of staining were noted as Gram's positive (+) staining blue of primary stain and Gram's negative (-ve) staining pink of counter stain.
5. **Spore formation:** Spore formation was observed in smear prepared from bacterial colonies
6. **Catalase activity:** Catalase is an enzyme, which is produced by bacteria. Catalase activity was tested as per the technique of Thomas (1963) for the confirmation of bacteria producing catalase. Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water. One drop of 3 per cent solution of hydrogen peroxide was placed on a clean glass slide. Pure culture was picked up from the nutrient agar slant with an inoculation straight wire in front of flame and placed on the drop of hydrogen peroxide on glass slide. Culture was properly emulsified and coverslip was placed. The production of gas bubbles confirms a positive reaction.
7. **Oxidation reaction test/oxidase test:** It confirms the production of cytochrome oxidase by certain bacteria. The culture from nutrient agar slant was picked up with an inoculating loop and rubbed on the filter paper. Simultaneously a drop of oxidase reagent (N, N, N, N. –p-phenylenediamine dihydrochloride) was added. Colonies producing oxidase give coloured reaction. The colour on the filter paper turns purplish brown in few seconds.
8. **Oxidation and fermentation test:** this test was used to differentiate the oxidative bacteria from fermenters. The test demonstrates the breakdown of sugars by oxidation or fermentation as per the technique of Hugh and Leifson (1953).

   Hugh and Leifson medium was used containing glucose and bromothymol blue as an indicators. Semisolid medium was prepared in pairs for culture of bacteria to be tested. One tube of the pair was kept open while the other tube was covered with 1-2 mm layer of sterilized paraffin to provide anaerobic condition. The tubes were incubated for 24 hours.

Those bacteria that oxidized the sugar showed acid production and yellow discoloration of the medium in open tube (*E. coli*) while the bacteria that ferment sugar (Staphylococcus) showed acid production and yellow discolouration of the medium in both the paired tubes.

3.4 Prepration of aqueous and alcoholic extract of *Ocimum sanctum*

Procedure

Dried *Ocimum sanctum* leaves were purchased from the local market. The dried leaves were finely grounded and were placed the thimble made up of strong filter paper which was placed in the extractor of soxhlet apparatus. The extraction was carried out as proposed by Handa (2008) using distilled water as a medium in aqueous extract and absolute ethanol in alcoholic extract.

Preparation of disc of aqueous and alcoholic extract of *Ocimum sanctum* for antibacterial activities

Steriled Whatman filter paper No 1, discs of 5 mm diameter were soaked in the aqueous and alcoholic extracts, respectively. The prepared discs were dried in controlled temperature to remove excess of solvent and used for study.

Cefotaxime antibiotic disc was taken to compare the zone of inhibition with the disc produced by both aqueous and alcoholic extracts of *Ocimum sanctum* (Tulsi).

3.5 Antibacterial sensitivity test of aqueous and alcoholic extracts of *Ocimum sanctum* (Tulsi)

Antibacterial activity of both aqueous and alcoholic extracts were evaluated by using paper disc agar diffusion method. This is also known as Kirby-Bauer disk diffusion method (Bauer *et al.,* 1966).

Testing of antibacterial activity of prepared aqueous and alcoholic extract disc, using disc diffusion method-

Mueller Hinton agar plate was used for the antibacterial activity testing. Mueller Hinton agar was prepared by mixing 28 g of Mueller Hinton powder with 100mL distilled water which was then autoclaved at 121^{o}C 15 psi for 15

minutes. Under aseptic conditions, 15 ml of Nutrient agar medium was dispensed into pre-sterilized petri dishes to yield a uniform depth of 5 mm and inoculated by the bacteria. The sterile discs (5 mm diameter) were impregnated with the aqueous and alcoholic extracts of *Ocimum sanctum* (Tulsi). The freshly prepared inoculum was swabbed all over the surface of Muller Hinton agar plate using the cotton swab. The dried discs were placed on the agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. The discs were spaced far enough to avoid reflections wave from the edges of the petri dishes and overlapping rings of inhibition. The agar plate was then incubated for 24 hours at the temperature of 37 ℃. The diameter of zone of inhibition as ind icated by clear area which was devoid of growth of microbes was measured and recorded.

3.5 Treatment trials

For evaluation of *In vivo* therapeutic potential of aqueous and alcoholic extracts of *Ocimum sanctum* (Tulsi), 12 goats suffering from sub-clinical mastitis were selected out of 96 goats. These goats were divided into two groups i.e. group I and group II comprising of six animals in each group, for two different therapeutic regimens. The dose of aqueous and alcoholic extract of *Ocimum sanctum* (Tulsi) @ 150 and 125mg/kg body weight was considered as suggested by Shafi *et al.* (2016).

Group I goats were administered aqueous extract of *Ocimum sanctum* @ of 150 mg/kg body weight, orally daily with jaggery/water for 7 days.

Group II goats were administered alcoholic extract of *Ocimum sanctum* @ of 125 mg/kg body weight, orally daily with jaggery for 7 days.

After administration in both groups for 7 days, evaluation of milk samples of group I and II goats was again done with CMT, SCC, pH, EC and cultural examination on 8^{th} day to see the therapeutic efficacy of the *Ocimum Sanctum* (Tulsi).

3.6 Statistical methods

All numerical data were processed via SPSS 16.0 for Windows. Analysis of parametric data was conducted by using ANOVA, and when the main effect was significant then Duncan’s multiple range test was performed.

4. RESULTS AND DISCUSSION

In the present investigation, milk samples of 192 quarters from 96 apparently healthy goats were collected from individual holdings and subjected to California Mastitis Test (CMT), Electrical conductivity (EC), pH, Somatic Cell Count (SCC) and cultural examination for the diagnosis of sub-clinical mastitis.

The result of CMT, SCC, EC, pH and cultural examination of 192 quarters from 96 apparently healthy goats are shown in appendix-I.

The 12 goats were found suffering from subclinical mastitis based on CMT, SCC, EC, and pH and cultural examination. These 12 goats were divided into 2 groups – Group I and Group II comprising of 6 goats in each group for 2 different therapeutic regimens. Six goats were kept as control out of 96 goats based on CMT, SCC, EC, pH and cultural examination.

The criteria for selecting subclinical mastitis goat include distinct CMT test (++), positive cultural examination, SCC (≥1.3million cells/ml of milk), pH (>6.6) and EC (>3.84 mS/cm).

The criteria for selecting six control group goats was negative CMT and bacterial cultural examination, somatic cell count less than 1.0 million, electrical conductivity and pH less than 3.84 and 6.8, respectively.

4.1 Prevalence of Subclinical Mastitis

The prevalence of subclinical mastitis in goats was found 12.50 per cent (12/96) on animal basis in the present study.

Majic *et al.* (1994) reported prevalence of subclinical mastitis in goats as 20% and 23% on the basis of CMT and cultural examination.

Varma *et al.* (2000) found 31.47% prevalence of subclinical mastitis in goats based on cultural examination and whereas Contreras *et al.* (2007) recorded 5-30% prevalence of subclinical mastitis in goats.

Bhanot *et al.* (2017) found prevalence of clinical and subclinical mastitis as 6.4% and 66.6% in lactating goats using CMT from Ambala, Haryana.

In our study the prevalence was less as compare to other authors. This might be due to the fact that milk samples were collected from individual holdings rather than herd. The management and hygienic conditions are better in individual holdings in comparison to herd. The personel care is taken by the owner in case of individual holdings. Further the environmental conditions are dry in Bikaner which is unfavourable for maintenance and transmission of mastitis causing bacteria as reported by Megersa *et al*., (2010).

4.1 Diagnostic tests

4.1.1 California mastitis test (CMT)

In the present study, out of 96 goats milk samples, 12 goats were selected whose both right and left half gives ++ results on CMT examination.

The results of CMT and SCC were compared and samples with CMT results of weak positive (+) were considered as having total somatic cell count between 1.2 million to 1.3 million cells/ml, while samples with CMT results of distinct positive (++) were considered as having total somatic cell count between 1.3 to 1.5 million/ml as per Radostits *et al*. (2009). All the positive quarters were having CMT results of distinct positive (++).

Out of 192 quarters weak positive reaction on CMT examination was recorded in 42 quarters.

CMT always gives strong positive reaction in the first week after calving and in last stages of lactation as reported by Constable *et al*., (2017).

Zhao *et al.* (2016) found 45.82 per cent prevalence of subclinical mastitis in goats on basis of CMT examination and also reported that CMT should be used as a standard test as it is cheap and rapid to use.

Bhanot *et al.* (2017) reported prevalence of clinical and subclinical mastitis as 6.4% and 66.6% in lactating goats using CMT from Ambala, Haryana.

In goats, SCC are more in healthy quarter as well as in subclinical mastitis in comparison to Cattle. SCC less than 1.0 million/ml of milk were considered as healthy quarter in goats.

4.1. 2 Electrical conductivity

The mean ± SE values of electrical conductivity of control, group I and group II of sub-clinical mastitis affected milk of goats have been presented in Table 2.

The mean ± SE value of electrical conductivity in milk of control group goats, was 2.97 ± 0.23 mS/cm which ranged between 2.11 - 3.57 mS/cm. The mean ± SE values of electrical conductivity in sub-clinically affected quarter milk in group I and group II were 3.84 ± 0.19 and 3.99 ± 0.29 mS/cm, respectively and ranged between 2.80-4.49 and 2.09-4.65 mS/cm, respectively.

The EC of milk of goats of group I and group II were higher than control group goats. The higher electrical conductivity in goats of group I and group II is due to high concentration of Na^+ and Cl^- ions.

Table 2: Mean ± SE values of Electrical conductivity (EC) in control, Group I and Group II with sub-clinical mastitis.

Group	Mean ± SE	Range
Control	2.97 ± 0.23	2.11 - 3.57
Group I	3.84 ± 0.19	2.80-4.49
Group II	3.99 ±0.29	2.09-4.65

The mean ± SE values of EC recorded in Group I and Group II in goats showed no significant difference when compared to mean ± SE value of control group goats. The similar findings were also recorded by Milner *et al.* (1996) in goats and Reddy *et al*. (2014), Johri (2016) and Savita (2017) in cattle.

The EC of milk is mostly determined by the concentration of anions and cations, with Na^+, K^+, and Cl^- is the most important. The sodium pumps located on the baso-lateral membrane of the secretory cells of the mammary gland, pumping Na^+ into the extracellular fluid and K^+ into the cells, while Na^+ and K^+ are transported passively between the secretory cells and the milk, across the apical membrane. In addition to it, a paracellular pathway is also present across the epithelium (tight junctions), where Na^+ and Cl^- are moving

into the milk and K^+ and lactose are moving into the extracellular fluid. At an instance when an animal is exposed to an intramammary infection, the EC of the milk increases due to an increased concentration of Na^+ and Cl^- in the milk. This increase is caused by destruction of tight junctions and the active ion-pumping system. As a result of the cell damage, Na^+ and Cl^- leak into the lumen of the alveolus, and K^+ and lactose move together out of the milk. However, factors other than mastitis, like breed parity, lactation stage milking interval and milk composition may affect EC of milk (Sloth and Lovendahl, 2004; Norberg, 2005).

Oshima (1978) and Fernando *et al.* (1991) reported that there were many factors such as lactation, breed, parity, herd difference and amount of fat in milk, which influences EC of milk.

4.1.3 Milk pH

The mean ± SE values of pH of control, group I and group II of sub-clinical mastitis affected quarter milk of goats have been presented in Table 3.

The mean ± SE value of pH of milk of control group goats was 6.29 ± 0.068 which ranged between 6.07-6.47. The mean ± SE value of pH in sub-clinically affected quarter milk in group I and group II were 6.85 ± 0.07 and 6.60 ± 0.08, respectively and ranged between 6.63-7.2 and 7.09-7.45, respectively.

Table 3: Mean ± SE values of pH of Control, Group I and Group II goats with sub-clinical mastitis.

Group	Mean ± SE	Range
Control	6.29 ±0.068	6.07-6.47
Group I	6.85±0.07	6.63-7.2
Group II	6.60±0.08	7.09-7.45

The mean ± SE values of pH recorded in Group I and Group II showed no significant difference when compared to mean ± SE value of apparently healthy quarter milk of goats. The similar findings were also recorded by Cruickshank *et al.* (1970) and Coel (1986) in goats and Patel *et al.* (2000), Tale *et al.* (2001), Johri (2016) and Savita (2017) in cattle.

The bromothymol blue and bromocresol purple tests are based on alteration of pH of milk, which in turn depends on salt concentration in exudates. These exudates increase the alkalinity of milk by alkaline salt so that the test may give positive values. Further, the normal milk pH is 6.4-6.9, whereas pH of affected samples is abnormally alkaline, may be as high as 7.4. The degree of alkalinity depends on the severity of inflammation. The milk pH has less diagnostic value in detecting the existence of udder inflammation (Coel, 1986)**.**

4.1.4 Somatic cell count (SCC)

The mean ± SE values of somatic cell counts of control group and group I and II of sub-clinical mastitis affected quarter milk of goats have been presented in Table 4.

The mean ± SE value of total somatic cell count from control group was 0.88 ± 0.29 million/ml which ranged between 0.78-0.94 million/ml. The mean± SE value of total somatic cell counts in sub-clinically affected quarter milk in group I and group II were 1.58 ± 0.74 and 1.61 ± 0.98 million/ml, respectively and ranged between 1.40-1.86 and 1.17-2.00 million/ml, respectively. The threshold value for total somatic cell count to detect sub-clinical mastitis was considered 10, 00,000 cells/ml of milk and above. All the sub-clinically affected milk samples showed higher TSCC.

Table 4: Mean ± SE values of somatic cell count (SCC) million/ml milk of control group, Group I and Group II Goats.

Group	Mean ± SE	Range
Control	0.88^{a} ± 0.29	0.78-0.94
Group I	1.58^{c}±0.74	1.40-1.86
Group II	1.61^{c}±0.98	1.17-2.00

The International Dairy Federation (IDF) recommends the criterion for sub-clinically mastitis as quarter infected with pathogenic organisms and having somatic cell counts more than 10, 00,000 cells/ml. The mean ± SE values of SCC recorded in Group I and Group II showed high ($P \leq 0.05$) significant difference when compared to mean ± SE value of healthy quarter milk of goats. Similar findings were also recorded by Vihan and Rubino (1996), Robertson and Muller (2005), Min *et al.* (2007), Koop *et al*. (2011) in goats and NMC (1999), Tale *et al*. (2001), Tuteja *et al.* (2003), Johri (2016) and Savita (2017) in cattle.

Somatic cell count could be influenced by the type of pathogen and season as reported by Salsberg *et al.* (1984), Guillemette *et al*. (1996) and Joshi (2012).

Robertson and Muller (2005) reported that in the case of goat's milk, a considerable controversy exists as to the relationship between SCC and mastitic infection. The reason for this controversy is that milk secretion in the cow differs from that of the goat. In the cow, milk is squeezed out of the alveoli, while in the goat the alveoli actually burst open. Due to this apocrine secretion, large numbers of cytoplasmic particles occur in normal goat's milk. Due to the presence of these cells the total SCC in goat's milk does not correlate well with the leucocyte count in the milk. Discarding foremilk fractions, Min *et al*. (2007) reported average SCC values ranging from 2,000 to 4,000 × 10^3 SC mL^{-1} in infected dairy goats and concluded that SCC in goat milk is not highly correlated to IMI. Similarly Joshi (2012) also reported that variation in somatic cell count in milk influenced by milking time, stage of lactation, parity and season, whereas Serieys (1985) observed that SCC tends to increase as lactation progressed, but these counts were not correlated with daily milk yield. TSCC increased with lactational age and stage of lactation (Giesecke and Vanden Heever, 1974). Singh and Ludri (2001) and Joshi (2012) also reported that variation in somatic cell count in milk influenced by milking time, stage of lactation, parity and season.

4.2 Changes in milk constituents

The mean ± SE values of fat, protein and lactose of control group, group I and group II of goats have been presented in Table 5 and Appendix XI, XII, XIII, XV, XVII and XVIII.

The mean ± SE value of fat, protein and lactose content in control group were 4.2 ± 0.031, 3.43 ± 0.017 and 5.11 ± 0.010 (ranged between 3.5 - 4.9, 3.1 - 3.9 and 4.8 - 5.3), and mean ± SE values of fat, protein and lactose content in sub-clinically affected Group I were 3.89 ± 0.038, 3.20 ± 0.024 and 4.756 ± 0.029 (ranged between 2.8 - 4.5, 2.6 - 3.6 and 4.1 – 5.1) and group II were 3.76 ± 0.031, 3.17 ± 0.020 and 4.549 ± 0.036 (ranged between 2.6 - 4.4, 2.9 - 3.5 and 4.0 – 4.9, respectively.

Table 5: Mean ± SE values of various milk constituents of control Group, Group I and Group II of goats.

Group	Milk constituents					
	Fat (%)		Protein (%)		Lactose (%)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Control	4.2 ± 0.031	3.5 - 4.9	3.43 ± 0.017	3.1 - 3.9	5.11 ± 0.010	4.8 - 5.3
Group I	3.89 ± 0.038	2.8 - 4.5	3.20 ± 0.024	2.6 - 3.6	4.756 ± 0.029	4.1 - 5.1
Group II	3.76 ± 0.031	2.6 - 4.4	3.17 ± 0.020	2.9 - 3.5	4.549 ± 0.036	4.0 – 4.9

Similar findings were also observed by Leitner *et al*. (2004a), Leitner *et al*. (2004b), Merin *et al*. (2004) and Hassan (2013) in goats and Sharma *et al*. (2011), Alemu *et al*. (2013), Jagadeesh *et al.* (2016) and Savita (2017) in cattle.

Leitner *et al*. (2004b) examined 500 Israeli goats of 10 flocks of various breeds and crossbreeds and noted that Milk fat, protein and lactose concentrations ranged among flocks from 19.9 to 55.6 g/l for fat, 34.0 to 51.1 g/l for total protein and 47.4 to 51.1 g/l for lactose. Statistical analysis for the six goat herds that were included in the model showed that the mean total protein and fat were lower in uninfected halves than that in infected ones (39.1vs. 39.9 g/l for protein and 37.5 vs. 42. 0 g/l for fat), whereas lactose was higher in uninfected than in infected halves (49.6 vs. 47. 2 g/l).

Merin *et al*. (2004) conducted a study on goats which had one udder half infected with identified coagulase-negative *staphyloccocci* (CNS) species while the contra-lateral gland was free of bacteria and observed that milk yield of the infected halves was lower ($P < 0.001$) than that of the uninfected halves in goats (1.38 *vs*. 1.96 kg/day). Only lactose concentration in the infected glands was lower ($P < 0.004$) than in the uninfected ones, while concentrations of fat, protein and casein did not differ between the uninfected and infected halves. Total whey protein and albumin concentrations were significantly higher in the infected than in the uninfected glands.

4.2 Cultural examination

Out of 24 quarters of 12 goats, 16 (66.67%) quarters were found positive for bacterial organisms. *Staphylococcus* spp. was found in 7 (43.75%) quarters and 9 (56.25%) quarters showed mixed infection of bacteria in different combinations *viz*. *Staphylococcus* spp., *E. coli, Streptococcus* spp. and *Klebsiella* spp. The bacteria were identified up to genus only.

The *Staphylococcus* spp. was the most frequent organism, accounting for 7 of the total 16 isolates (43.75 %) followed by mixed infections by various bacteria in different combinations. (Table 6 and Figure 12).

Table 6: Relative frequency of different types of bacterial isolates in sub-clinical mastitic milk of goats

S.No.	Bacteria isolates	No. of infected quarters	Per cent
1.	*Staphylococcus* spp.	7	43.75
2.	Mixed infection (*E. coli* and *Staphylococcus*)	4	25
3.	Mixed infection (*Staphylococcus* and *Klebsiella*)	2	12.5
4.	Mixed infection (*Staphylococcus* and *Streptococcus*)	3	18.75

Mixed infection was recorded in 9 quarters. The organisms isolated in mixed infection were *Staphylococcus* spp. and *E. coli* spp. recorded in 4 (25%) quarter, combination of *Klebsiella* spp. and *Staphylococcus* spp. in 2 (12.5%) quarter and *Staphylococcus* spp. and *Streptococcus* in 3 (18.75%) quarters. The mixed infections in quarters have been depicted in Table 7.

Similar findings were observed by Shearer and Harris (1992), Islam *et al*. (2011), Gebrewahid *et al*. (2012), Bourbah *et al.* (2013), Najeeb *et al*. (2013) and Pirzada *et al*. (2016) in goats and Aydin *et al*. (2009); Yuan Yuan *et al*. (2012); Hanan *et al*. *(*2015); Mahenthiran *et al*. (2015); Johri (2016) and Savita (2017) in cattle.

In the present study, *Staphylococcus* spp. was found as most common isolate in subclinical mastitic milk samples of goats.

According to Shearer and Harris (1992) *Staphylococcus aureus* is the most important mastitic pathogen in most herds. Other organisms including several species of Streptococci (*Streptococcus agalactia*, *Streptococcus uberis*, and *Streptococcus dysgalactia*) are commonly isolated from infected udders. *Pasteurella haemolytica* is also isolated from mastitic glands and is believed to be associated with suckling kids. *Corynebacterium pseudotuberculosis* is often isolated from infected udders where there is a herd problem with abscesses. Additional organisms less commonly isolated from mastitic glands include coliforms and *Mycoplasma* spp.

Najeeb *et al*. (2013) examined 200 milk samples from 100 apparently healthy goats from D. G. Khan and Lahore districts, Punjab, Pakistan. Milk samples were screened with Whiteside Test (WST) for sub-clinical mastitis. Samples positive for mastitis were cultured for bacterial growth on blood agar. Bacterial growth was obtained in 45% milk samples (90/200). From WST positive milk samples, 146 bacterial isolates were identified on the basis of colonial, microscopic and biochemical profiles. Highest prevalence was of *Staphylococcus aureus* (61.64%) followed by *Escherichia coli* (10.96%), *Streptococcus* spp. (9.59%), *Pseudomonas* spp., *Bacillus* spp. (6.85%, each) and *Corynebacterium* spp. (4.11%).

Group I

In Group I, 12 quarters milk samples of 6 goats were subjected to culture and sensitivity tests. Positive milk samples were inoculated on Nutrient agar, Mac Conkey agar and Mannitol salt agar plates. These were incubated at 37℃ for 24 hours under aerobic condition. The b acterial growth was preliminary identified as *Staphylococcus* spp. and *E. coli* on the basis of

colonial characteristics, morphology, Gram's reaction, catalase and oxidase activities. The suspected *Staphylococcus* spp*. and E. coli* infected colonies were fished out and pure cultures were obtained.

Table 7: Quarter-wise frequency of bacterial isolates in group I of goats

S. No.	Bacteria isolates	No. of infected quarters (12)	Per cent
1.	*Staphylococcus* spp*.*	7	58.33
2.	Mixed infections *(E. coli* and *Staphylococcus* spp*.)*	2	16.66
3.	Mixed infections (*Klebsiella* spp. and *Staphylococcus* spp.)	3	25.00

Out of 12 quarters, *Staphylococcus* spp. was isolated from 7 quarters (58.33%), and mixed infection in combination of *Staphylococcus* spp. and *E. coli* infection was isolated from 2 quarter (16.66%) and 3 quarters showed (25%) mixed infection in combination of *Klebsiella* spp. and *Staphylococcus* spp.

Group II

In Group II, 12 quarters milk samples of 6 goats were subjected to culture and sensitivity. Positive milk samples were inoculated on Nutrient agar, Mac Conkey agar and Mannitol salt agar plates. These were incubated at 37℃ for 24 hours under aerobic condition. The b acterial growth was preliminary identified as *Staphylococcus* spp*.* and *E. coli* on the basis of colonial characteristics, morphology, Gram's reaction, catalase and oxidase activities. The suspected *Staphylococcus* spp*. and E. coli* infected colonies were fished out and pure cultures were obtained.

Table 8: Quarter-wise frequency of bacterial isolates in group II of goats

S. No.	Bacteria isolates	No. of infected quarters (12)	Per cent
1.	*Staphylococcus* spp*.*	3	25.00
2.	Mixed infections (*E. coli* and *Staphylococcus* spp.)	5	41.66
3.	Mixed infections (*Streptococcus* spp. + *Staphylococcus* spp.)	4	33.33

Out of 12 quarters, *Staphylococcus* spp. was isolated from 3 quarters (25.00%) and mixed infection in combination of *Staphylococcus* spp. and *E. coli* infection was isolated from 5 quarters (41.66%) and 4 quarters showed (33.33%) mixed infection of *Streptococcus* spp. and *Staphylococcus* spp.

Similar finding of the present study is also corborated by Shukla *et al.* (1998) who reported high prevalence of *Staphylococcus* spp. This may be due to the fact that *Staphylococcus* spp. survives better in the environment than other microorganisms. It is present in large numbers in different body sites, such as teat orifice and surfaces (Prabhakar *et al*., 1988). Further the prevalence of *Streptococcus* spp*.* in milk was found to be lower than *Staphylococcus* spp. as the *Streptococcus* spp*.* are unable to survive for longer periods in environment outside the body (Schalm *et al.,* 1971). *E. coli* is generally pathogenic for udder and causes some mild infections (Schalm and Lasmanis, 1968). According to Michael *et al*. (1991) the invasive property of *Staphylococcus* spp. enables it to survive in udder tissues and during cyclic infection, inefficient phagocytosis and killing contributes the relapse of infection. The low incidence of Gram negative rods may be due to their destruction by mammary glands (Waage *et al*., 1998).

4.3 *In vitro* antibacterial activity sensitivity of *Ocimum sanctum*

The study was undertaken to evaluate the *in vitro* antibacterial activity of *O. sanctum* (Tulsi) leaves against the common mastitis pathogens viz. *Staphylococcus* spp*., E. coli, Klebsiella* spp. and *Streptococcus* spp. Antibacterial activity of the extracts was evaluated by using paper disc agar diffusion method. This is also known as Kirby-Bauer disk diffusion method (Bauer *et al.,* 1966).

4.3.2 Inhibition of bacterial growth

The antibacterial activity of the Tulsi against particular mastitis pathogens was determined as proportion of zones of growth inhibition formed by the *Ocimum sanctum* and the standard antibiotic i.e. cefotaxime depicted as percentage. The zones of inhibition produced by aqueous extract of *O. sanctum*, and their antibacterial activity for different bacteria in terms of zones of inhibition formed by cefotaxime have been depicted in Table 9. In the

present study, the average zone of inhibition shown by aqueous extract of *O. sanctum* against *Staphylococcus* spp. was 16.75 mm and 14.15 mm against *E. coli*.

Table 9: Mean values of Zones of Inhibition (mm) of aqueous extract of leaves of *O. sanctum* against sub-clinical mastitis pathogens of Group I and Group II of goats.

Pathogen	Group II average zones of Inhibition (mm)		% Sensitivity
	O. sanctum	**Cefotaxime**	
Staphylococcus spp.	16.75	23.50	71.27
E.coli	14.15	24.00	58.95

Table 10: Mean values of Zones of Inhibition (mm) of alcoholic extract of leaves of *O. sanctum* against sub-clinical mastitis pathogens of Group I and Group II of goats.

Pathogen	Group II average zones of Inhibition (mm)		% Sensitivity
	O. sanctum	**Cefotaxime**	
Staphylococcus spp.	18.95	23.50	80.64
E.coli	17.05	24.00	71.04

In the present study, the average zone of inhibition shown by aqueous extract of *O. sanctum* against *Staphylococcus* spp. was 16.75 mm and 14.15 mm against *E. coli*. The zone of inhibition shown by control antibiotic cefotaxime against *Staphylococcus* spp. was 23.5 mm, 24 mm against *E. coli*.

The alcoholic extract of *O. sanctum* showed higher antibacterial activity against *Staphylococcus* spp. and *E. coli* as 80.64% and 71.04%, respectively as compared to aqueous extract of *O. sanctum* which showed antibacterial activity against *Staphylococcus* spp. and *E. coli* as 71.27% and 58.95%, respectively.

Mukherjee (2006) also observed similar zones of inhibition ranging from 12.5 to 18.3 mm for aqueous extract of *O. sanctum* leaves against *S. aureus*, *S. agalactiae* and *E. coli.* Vasudevan *et al.* (2001) observed appreciable antibacterial activity of aqueous and alcoholic extracts of *O. sanctum* against enteric pathogens. Harikrishnan and Balasundaram (2008)

observed *in vitro* and *in vivo* antimicrobial activity of aqueous and ethanolic extracts of *O. sanctum* against *Aeromonas hydrophila*, a ubiquitous and opportunistic bacterial pathogen that produces ulcerative dermatitis under stress conditions. Ali and Dixit (2012) observed the zones of inhibition for flavanoids of *O. sanctum* as 20.12, 20.75, 20.95, 19.55 and 20.1 mm against *E. coli*, *Proteus*, *S. aureus*, *Staphylococcus cohni* and *Klebsiella pneumonia,* respectively. Gupta (2010) reported that at a concentration of 100 mg/ml, the hydro-alcoholic (1:1) extract of *O. sanctum* leaves*,* exhibited the antibacterial activity against *S. aureus, S. agalactiae* and *E. coli.* Prakash and Gupta (2005) found eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum*, to be largely responsible for its therapeutic potential. Singh *et al.* (2005) observed that *O. sanctum* fixed oil has good antibacterial activity against *Bacillus pumilus*, *P. aeruginosa* and *S. aureus,* and concluded that higher content of linolenic acid in *O. sanctum* fixed oil could contribute towards its antibacterial activity.

4.4 Treatment trial

For evaluation of *in vivo* therapeutic potential of *Ocimum sanctum* extract in sub-clinical mastitis in goats, 12 goats were divided into two groups comprising of 6 animals in each group, for two different therapeutic regimens. The treatment was started soon after confirmation of sub-clinical mastitis. The aqueous and alcoholic extract were administered accordingly in each group. The therapeutic efficacy was recorded after observing the results of California mastitis test (CMT), pH, electric conductivity (EC) test and total somatic cell count (TSCC) (Table 11).

Table 11: Pre and Post- treatment mean ± SE values of EC (mS/cm), pH, SCC (million/ml) in Control, Group I and II of goats.

Group		Diagnostic Test		
		EC (mS/cm) Mean ± SE	pH Mean ± SE	SCC(million/ml) Mean ± SE
Control group	After 7th day	2.97± 0.23	6.29± 0.06	0.88± 0.23
I group	Pre-treatment	3.84±0.19	6.85±0.06	1.58± 0.75
	Post-treatment	3.24±0.17	6.35±0.07	1.24± 0.96
II group	Pre-treatment	3.99±0.29	6.60± 0.08	1.608±0.98
	Post-treatment	3.39 ±0.23	6.13± 0.03	1.19±0.96

Table 12: Pre and Post – treatment mean ± SE values of lactose (%), fat (%) and protein (%) in control, Group I and II goats.

Group		Milk Constituents		
		Fat (%) Mean ± SE	Protein (%) Mean ± SE	Lactose (%) Mean ± SE
Control group		4.2 ± 0.031	3.43 ± 0.017	5.11 ± 0.010
group I	Pre-treatment	3.89 ± 0.038	3.20 ± 0.024	4.756 ± 0.029
	Post-treatment	3.99± 0.17	3.63± 0.12	4.831± 0.16
group II	Pre-treatment	3.76 ± 0.031	3.17 ± 0.020	4.549± 0.036
	Post-treatment	3.91 ± 0.25	3.94 ± 0.14	4.99 ±0.13

Group I

Goats of group I were administered prepared aqueous extract of *Ocimum sanctum* @ 150 mg/kg body weight, orally daily for 7 days. To determine the therapeutic efficacy of prepared aqueous extract of *Ocimum sanctum*; samples were collected on day 0 as pre-treatment and on 8th day as post-treatment to record the changes in CMT, electric conductivity (EC), milk pH and somatic cell count (SCC) and cultural examination.

There was no significant increase in mean ± SE pre-treatment value (3.84±0.19) of electrical conductivity in sub-clinical mastitic goats in group I as compared to control group (2.97±0.19). The post- treatment mean ± SE value of electrical conductivity in group I was (3.24±0.17) (Table 12), (Figure 18), (Appendix III). There was no changes in post-treatment mean± SE value of electric conductivity as compared to its pre-treatment level in group I on giving aqueous extract of *Ocimum sanctum* @ 150 mg/kg bwt.

There was no significant increase in mean ± SE pre-treatment value (6.85±0.06) of milk pH in sub-clinical mastitic goats in group I as compared to control group (6.29± 0.06). The post- treatment mean ± SE value of milk pH in group I was (6.35± 0.07) (Table 13), (Figure 19), (Appendix VI). There was no significant decrease in post-treatment mean± SE value of pH as compared to its pre-treatment level in group I on giving aqueous extract of *Ocimum*

sanctum @ 150 mg/kg bw which was more or less nearer to the mean± SE values of healthy udders. There was significant (P<0.05) increase in mean ± SE pre-treatment value (15.8 ± 0.75) of SCC in sub-clinical mastitic goats in group I as compared to healthy goats (8.85±0.23). The post- treatment mean ± SE value of SCC in group I was (12.4 ± 0.96) (Table 13), (Figure 20), (Appendix IX). There was significant (P<0.05) decrease in post-treatment mean± SE value of SCC as compared to its pre-treatment level in group I on giving aqueous extract of *Ocimum sanctum* @ 150 mg/kg bwt. which was more or less nearer to the mean± SE values of healthy udders.

There was significant decrease in mean ± SE pre-treatment value (of fat content in sub-clinical mastitic goats in group I as compared to control group. The post- treatment mean ± SE value of fat content in group I was (3.99 ± 0.17) (Table 13), (Fig. 21), (Appendix XV). There was significant increase in post-treatment mean± SE value of fat content as compared to its pre-treatment level in group I on giving aqueous extract of *Ocimum sanctum* @ 150 mg/kg bwt.

On post treatment bacterial cultural examination revealed that three goats were negative for cultural examination in group I.

There was decrease in mean ± SE pre-treatment value of protein content in sub-clinical mastitic goats in group I as compared to control group. The post- treatment mean ± SE value of protein content was higher in group I (Table 13), (Fig. 22) and (Appendix XVIII). There was significant (P<0.05) increase in post-treatment mean± SE value of protein content as compared to its pre-treatment level in group I on giving aqueous extract of *Ocimum sanctum* @ 150 mg/kg bwt.

There was decrease in mean ± SE pre-treatment value of lactose content in sub-clinical mastitic goats in group I as compared to control group goats. The post- treatment mean ± SE value of lactose content in group I was (4.831± 0.16) (Table 13), (Fig 23), (Appendix XII). There was increase in post-treatment mean ± SE value of lactose content as compared to its pre-treatment level in group I on giving aqueous extract of *Ocimum sanctum* @ 150 mg/kg bwt.

Group II

Group II animals were administered prepared alcoholic extract of *Ocimum sanctum* @ 125 mg/kg body weight orally daily for 7 days. To determine the therapeutic efficacy of prepared herbal extract of leaves of *Ocimum sanctum*; samples were collected on day 0 as pre-treatment and on 8^{th} day as post-treatment to record the changes in electric conductivity (EC), milk pH, somatic cell count (SCC) and cultural examination.

There was no significant increase in mean ± SE pre-treatment value (3.99±0.29) of electric conductivity in sub-clinical mastitic goats in group II as compared to healthy goats (2.97± 0.23). The post- treatment mean ± SE value of electric conductivity in group II was (3.39 ±0.23) (Table 13), (Figure 18) and (Appendix IV). There was no significant changes in post-treatment mean± SE value of electrical conductivity as compared to its pre-treatment level in group IV on giving alcoholic extract of *Ocimum sanctum* @ 125 mg/kg bwt.

There was no significant increase in mean ± SE pre-treatment value (6.60 ± 0.08) of milk pH in sub-clinical mastitic goats in group II as compared to control group goats (6.29 ± 0.06). The post- treatment mean ± SE value of milk pH in group II was (6.13 ± 0.03) (Table 13), (Fig. 19) and (Appendix VI). There was no decrease in post-treatment mean± SE value of pH as compared to its pre-treatment level in group II on giving alcoholic extract *Ocimum sanctum* @ 125 mg/kg bwt. which was more or less nearer to the mean± SE values of healthy udders.

There was significant ($P<0.05$) increase in mean ± SE pre-treatment value (16.08 ± 0.98) of SCC in sub-clinical mastitic goats in group II as compared to control group goats (8.85 ± 0.23). The post- treatment mean ± SE value of SCC in group II was (11.9 ± 0.96) (Table 13), (Fig 20) and (Appendix IX). There was significant ($P<0.05$) decrease in post-treatment mean± SE value of SCC as compared to its pre-treatment level in group II on giving alcoholic extract *Ocimum sanctum* @ 125 mg/kg bwt. which was more or less nearer to the mean± SE values of healthy udders.

There was decrease in mean ± SE pre-treatment value of fat content in sub-clinical mastitic goats in group II as compared with control group. The post- treatment mean ± SE value of fat content in group II was (3.91± 0.25) (Table 13) and (Fig. 21), (Appendix XV). There was increase in post-treatment mean ± SE value of fat content as compared to its pre-treatment level in group II on giving alcoholic extract of *Ocimum sanctum* @ 125 mg/kg bwt.

There was decrease in mean ± SE pre-treatment value of protein content in sub-clinical mastitic goats in group II as compared to control group. The post- treatment mean ± SE value of protein content in group II was (3.94 ± 0.14) (Table 13) and (Fig 22), (Appendix XVIII). There was increase in post-treatment mean ± SE value of protein content as compared to its pre-treatment level in group I on giving alcoholic extract of *Ocimum sanctum* @ 125 mg/kg bwt.

There was decrease in mean ± SE pre-treatment value (3.74 ± 0.17) of lactose content in sub-clinical mastitic goats in group II as compared to control group. The post- treatment mean ± SE value of lactose content in group II was (4.99 ±0.13) (Table 13) and (Fig 23), (Appendix XII). There was increase in post-treatment mean± SE value of lactose content as compared to its pre-treatment level in group II on giving alcoholic extract of *Ocimum sanctum* @ 125 mg/kg bwt.

On post treatment bacterial cultural examination revealed that four goats were negative for cultural examination in group II. The therapeutic efficacy of aqueous and alcoholic extract of Tulsi (*Ocimum sanctum*) in subclinical mastitis in goats were recorded as 50% and 66.66%, respectively.

The extract of *O. sanctum* possessed immuno-modulatory properties in addition to anti-inflammatory properties as evident in several experiments carried by Sadekar *et al.* (1998). Mukherjee *et al.* (2005a) reported that *O. sanctum* seed oil appears to modulate both humoral and cell-mediated immune responsiveness and GABAnergic pathways may mediate these immunomodulatory effects. The active constituent, Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), present in *Ocimum sanctum L*. has been found to

be largely responsible for the therapeutic potentials of the tulsi (Singh *et al*., 2012)

Nair *et al.* (1982) reported the phenolic compounds, viz., cirsilineol, cirimaritin, isothymusin, apigenin and rosmarinic acid, and appreciable quantities of eugenol (a major component of the volatile oil) from *Ocimum sanctum* extract of fresh leaves and stems possessing good antioxidant activity. Mastitis could induce the increase of free radicals formation in milk and leading to oxidative stress (Gu *et al.,* 2009), especially during the early lactation period of dairy cows (Sordillo *et al.,* 2007). Both CM and SCM are associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk (Atakisi *et al.,* 2010). Although, role of antioxidant vitamins and trace elements in mastitis in dairy cows was already established by Yang and Li (2015).

5. SUMMARY

In the present study, Milk samples of 192 quarters of 96 apparently healthy goats were collected from individual holdings and were subjected to California Mastitis Test (CMT), Electrical conductivity (EC), pH, Somatic Cell Count (SCC) and cultural examination for the diagnosis of sub-clinical mastitis. The prevalence of subclinical mastitis was 12.50% based on CMT, SCC, pH, EC and cultural examination. The study was carried out from From November 2016 to February 2017, Twelve goats suffering from sub-clinical mastitis were selected for evaluation of *in vivo* therapeutic potential of Aqueous and alcoholic extracts of *Ocimum sanctum*. These 12 goats were divided into 2 groups – Group I and Group II comprising of 6 goats in each group for 2 different therapeutic regimens. Out of 96 goats, 6 goats which were negative for CMT, SCC less than 10 lakh cells/ml of milk, EC less than 3.8 mS/cm, pH less than 6.6 and having cultural examination of milk served as control group.

Group I animals were administered aqueous extract of *Ocimum sanctum* @150 mg/kg body weight, orally daily for 7 days. Group II goats were administered alcoholic extract of *Ocimum sanctum* @125 mg/kg body weight, orally daily for 7 days. After administration of treatment in all the groups for 7 days, milk samples were evaluated on 8^{th} day to note the efficacy of the drug.

After study there was no significant change ($P>0.05$) in Electrical conductivity (EC) and pH in both the two groups between pre and at post-treatment stage but total Somatic Cell Count showed significant change ($P>0.05$) in both the groups between pre and post-treatment stage. The somatic cell count values as recorded in pre-treatment stage were significantly higher than the post-treatment stage of both the groups and these post treatment values were approximately same as compared to the apparently healthy goats indicating the efficacy of *Ocimum sanctum* in all goats. 12 goats was negative after post treatment and increased somatic cell count at pre treatment stage in group I and group II reduced to almost normal

values of control group. Milkoscan parameters showed increase in protein, fat and lactose content in post treatment milk samples.

Post treatment cultural examination of milk of goats of group I and II revealed that three goats were negative in group I and four goats were negative in group II for bacterial cultural examination, Therapeutic efficacy of aqueous and alcoholic extract of Tulsi (*Ocimum sanctum*) was recorded as 50% and 66.66%, respectively.

There were increase in milk constituents viz. fat, protein and lactose in both the group I and Group II was recorded between pre and post-treatment stage and revealed slight increase in protein, fat, lactose contents of milk on post treatment examination as compare to pre treatment examination.

It was observed that aqueous and alcoholic extracts of *Ocimum sanctum* showed wide zone of inhibition against *Staphylococci* and *E. coli* in sub-clinical mastitis in goats as both the aqueous and alcoholic extracts worked well but alcoholic extract of *O. sanctum* was better than aqueous extract of *O. sanctum.*

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Studies on therapeutic potential of *Ocimum sanctum* on sub-clinical mastitis in goats (*Capra hircus*)

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ABSTRACT

In the present investigation, milk samples of 192 quarters from 96 apparently healthy goats were collected from individual holdings and were subjected to California Mastitis Test (CMT), Electrical conductivity (EC), pH, Somatic Cell Count (SCC) and cultural examination for the diagnosis of sub-clinical mastitis. The samples were collected from goats after 1 month of parturition. The aim of the present investigation was to evaluate the therapeutic potential of one medicinal plant *viz., Ocimum sanctum* (Tulsi) known to possess anti-inflammatory, immuno-modulatory and antibacterial properties was selected for evaluation in the therapy of sub-clinical mastitis *in vitro* and *in vivo*. The 12 goats were divided into two groups having 6 goats in each *viz.* Group I (aqueous extract @150 mg/kg b.wt.) and Group II (alcoholic extract @125 mg/kg b.wt.). After 8^{th} day post- treatment, There was no significant change ($P>0.05$) in Electrical conductivity (EC) in both the two groups Post-treatment. There was no significant change ($P>0.05$) in pH in both the two groups Post–treatment. Similarly, there was significant changes ($P>0.05$) in total Somatic Cell Count (TSCC) in both the groups post-treatment. There were increases in values milk constituents viz. fat, protein and lactose in both the two groups in post-treatment examination. Thus, the results indicated that the aqueous and alcoholic extracts have shown therapeutic potential in treating sub-clinical mastitis in caprines.

बकरियों में उपनैदानिक थनैला रोग में तुलसी की चिकित्सकीय क्षमता का अध्ययन

स्नातकोत्तर शोध ग्रन्थ
पशु औषध, नीति एवं न्याय शास्त्र विभाग
पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय
राजस्थान पशुचिकित्सा और पशु विज्ञान विश्वविद्यालय, बीकानेर–334001

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मुख्य उपादेष्टा डॉ. ए.पी. सिंह

अनुक्षेपण

वर्तमान जांच में बीकानेर की व्यक्तिगत होल्डिंगस् (झुंड) से 96 स्वस्थ बकरियों के 192 थनों के दूध के नमूने उपनैदानिक थनैला रोग के लिए एकत्रित किए गए है। जहां से 12 बकरियों के 24 थनों के दूध में उपनैदानिक थनैला रोग पाया गया। इस जांच में मोडिफाईड केलिफोर्निया थनैला रोग जांच, कुल दैहिक कोशिका गिनती, pH, विधुत चालकता एवं कल्चरल सेन्स्टीविटी टेस्ट का प्रयोग किया गया। वर्तमान जांच का उद्देश्य एक चिकित्सकीय पौधें– तुलसी का उपनैदानिक थनैला रोग में उपचार का पता लगाना है। वर्तमान अध्ययन उक्त बकरियों में से 12 बकरियों को 2 समूह (समूह–1 एवं समूह–2) में बांटा गया। प्रत्येक समूह में 6 बकरियां ली गई है। अध्ययन हेतु समूह–1 में जलीय अर्क तुलसी @ 150 mg/शारीरिक भार के अनुसार मुंह से रोजाना 7 दिन तक दिया गया। इसी प्रकार समूह–2 में मद्यव्यकनी अर्क @ 125 mg/शारीरिक भार के अनुसार मुंह से रोजाना 7 दिन तक दिया गया। उपचार के आठ दिन पश्चात् दोनों समूहों की विद्युत चालकता में कोई विशेष परिवर्तन नहीं पाया गया। इसी तरह से दोनों समूहों की pH में कोई विशेष परिवर्तन नहीं पाया गया। जबकि दोनों समूह में कुल दैहिक कोशिका गिनती में विशेष परिवर्तन पाया गया। इसी तरह से दोनों समूह में औसत वसा, प्रोटीन एवं लेक्टोस में विशेष परिवर्तन पाया गया।

Appendices

Appendix-I

CMT Score, Somatic cell count, milk pH, Electrical Conductivity and Bacterial cultural examination of milk of 96 lactating goats.

Goat Number	CMT Score		Somatic cell count (Million cells/ml)		Milk pH		Electrical Conductivity (mS/cm)		Bacterial cultural examination	
	Left half	Right half	Left half	Right half	Left half	Right half	Left half	Right half	Left half	Right half
1.	-	-	0.94	0.78	6.60	6.20	3.64	3.48	-	-
2.	++	++	1.24	1.36	6.85	7.01	3.68	3.98	+ve	+ve
3.	-	-	.74	.81	6.02	6.05	3.20	3.54	-	-
4.	-	-	.69	.77	6.50	6.61	3.01	3.66	-	-
5.	+	-	.79	.89	6.55	6.52	2.94	3.29	-	-
6.	-	-	.71	.83	6.46	6.39	2.61	3.02	-	-
7.	-	-	.77	.72	6.36	6.40	3.66	3.26	-	-
8.	-	+	.84	.73	6.34	6.39	3.33	3.12	-	-
9.	++	++	1.46	1.38	7.18	6.82	3.40	3.50	+ve	+ve
10.	-	-	0.88	0.96	6.02	6.07	2.72	2.80	-	-
11.	-	-	.93	.89	6.21	6.26	3.24	3.59	-	-
12.	-	-	.87	.77	6.68	6.62	3.49	3.06	-	-
13.	-	-	.70	.78	6.54	6.61	3.17	3.84	-	-
14.	-	-	.88	.71	6.34	6.39	3.29	3.68	-	-
15.	-	-	.77	.95	6.42	6.40	3.11	3.69	-	-
16.	-	-	.69	.76	6.58	6.50	3.20	3.13	-	-
17.	+	+	.83	.73	6.41	6.54	3.16	3.00	-	-
18.	++	++	1.20	1.16	6.71	6.73	2.00	2.18	+ve	+ve
19.	-	-	.71	.77	6.46	6.60	3.11	3.26	-	-
20.	-	-	.79	.73	6.39	6.45	3.24	3.54	-	-
21.	-	-	.97	.88	6.28	6.36	3.88	3.49	-	-
22.	-	-	.73	.77	6.64	6.59	3.26	3.14	-	-
23.	+	+	.74	.82	6.29	6.34	3.47	3.26	-	-
24.	-	-	.89	.78	6.38	6.37	3.22	3.08	-	-
25.	-	-	.71	.81	6.50	6.46	3.20	3.46	-	-
26.	+	-	.89	.79	6.42	6.49	3.69	3.00	-	-
27.	-	-	0.95	0.93	6.39	6.45	2.72	2.62	-	-
28.	-	-	.77	.92	6.58	6.63	2.88	3.17	-	-
29.	-	-	.71	.88	6.20	6.26	3.03	3.20	-	-
30.	-	-	.94	.89	6.32	6.39	3.69	3.39	-	-
31.	-	+	.73	.81	6.28	6.36	4.00	4.15	-	-
32.	+	-	.91	.71	6.66	6.54	3.93	3.54	-	-
33.	-	-	.77	.88	6.29	6.26	4.01	4.08	-	-
34.	++	++	1.67	1.59	6.23	6.21	3.88	3.76	+ve	+ve
35.	-	-	.81	.85	6.46	6.54	3.90	3.97	-	-

36.	-	-	.79	.91	6.16	6.20	3.46	3.95	-	-
37.	-	-	.92	.87	6.29	6.38	3.98	3.90	-	-
38.	-	-	.89	.86	6.59	6.52	3.26	3.19	-	-
39.	-	-	.71	.81	6.42	6.40	3.06	3.21	-	-
40.	++	++	1.56	1.84	6.77	6.88	2.91	2.99	+ve	+ve
41.	+	-	.73	.89	6.42	6.40	3.34	2.96	-	-
42.	-	-	.74	.86	6.36	6.39	2.97	3.17	-	-
43.	-	-	.88	.77	6.60	6.54	3.26	3.41	-	-
44.	-	-	.89	.79	6.49	6.46	3.90	3.26	-	-
45.	-	-	.88	.92	6.42	6.47	3.09	3.21	-	-
46.	-	-	.71	.81	6.50	6.53	3.06	3.28	-	-
47.	-	-	.73	.84	6.59	6.62	3.91	3.46	-	-
48.	-	+	.87	.77	6.40	6.44	2.94	2.66	-	-
49.	-	-	.73	.83	6.38	6.41	3.19	3.21	-	-
50.	++	++	1.54	1.46	6.59	6.57	4.54	4.46	+ve	+ve
51.	-	-	.89	.77	6.46	6.54	2.82	2.59	-	-
52.	-	-	.93	.89	6.34	6.33	3.86	3.21	-	-
53.	-	-	.73	.86	6.29	6.36	3.02	3.66	-	-
54.	-	-	.89	.72	6.65	6.39	3.18	3.24	-	-
55.	++	++	1.95	1.77	6.78	6.65	4.39	4.27	+ve	+ve
56.	-	-	.71	.92	6.26	6.39	2.99	3.36	-	-
57.	-	-	.93	.89	6.29	6.33	2.94	3.36	-	-
58.	+	+	.95	.86	6.30	6.46	4.00	3.84	-	-
59.	-	-	.77	.79	6.35	6.50	3.14	3.96	-	-
60.	-	-	.81	.71	6.64	6.49	3.21	2.86	-	-
61.	-	-	.88	.76	6.32	6.52	3.57	3.24	-	-
62.	+	-	.73	.81	6.36	6.45	3.36	3.71	-	-
63.	-	-	0.91	0.87	6.32	6.29	3.67	3.47	-	-
64.	-	-	.89	.79	6.64	6.37	4.00	4.02	-	-
65.	-	+	.77	.92	6.61	6.41	3.86	3.50	-	-
66.	-	-	.93	.81	6.52	6.42	3.91	3.84	-	-
67.	-	-	.91	.71	6.49	6.66	3.21	3.00	-	-
68.	++	++	1.86	1.90	6.51	6.57	4.30	4.50	+ve	+ve
69.	-	-	.73	.93	6.29	6.39	2.99	3.41	-	-
70.	-	-	.74	.82	6.32	6.42	3.53	3.91	-	-
71.	-	-	.86	.76	6.39	6.42	3.22	3.40	-	-
72.	++	++	1.79	1.91	6.62	6.64	4.15	4.29	+ve	+ve
73.	-	-	.79	.85	6.55	6.39	3.02	3.19	-	-
74.	-	-	.80	.93	6.37	6.47	3.12	3.50	-	-
75.	+	+	.89	.76	6.42	6.52	3.22	3.01	-	-
76.	-	-	.94	.79	6.50	6.42	3.78	3.51	-	-
77.	-	-	.85	.77	6.32	6.41	3.06	3.12	-	-
78.	-	-	.89	.94	6.50	6.42	2.99	2.86	-	-
79.	++	++	1.49	1.55	6.65	6.45	3.79	3.99	+ve	+ve
80.	-	-	.87	.73	6.67	6.49	3.41	3.39	-	-
81.	-	-	0.72	0.76	6.68	6.38	2.88	3.92	-	-
82.	++	++	2.06	1.94	6.47	6.43	3.99	4.05	+ve	+ve
83.	-	-	0.79	0.83	6.65	6.31	3.18	3.66	-	-

84.	-	-	0.83	0.92	6.55	6.46	4.24	3.99	-	-
85.	-	+	.89	.94	6.47	6.57	3.88	3.93	-	-
86.	-	-	.97	.77	6.46	6.56	3.99	4.21	-	-
87.	-	-	.73	.94	6.32	6.42	2.66	3.19	-	-
88.	-	-	.83	.95	6.41	6.59	2.32	2.13	-	-
89.	-	-	.89	.76	6.39	6.63	3.26	3.19	-	-
90.	-	-	0.80	0.76	6.1	6.24	2.10	2.12	-	-
91.	-	-	.96	.89	6.62	6.56	3.86	3.29	-	-
92.	+	+	.78	.95	6.55	6.59	2.88	3.26	-	-
93.	-	+	.77	.83	6.50	6.47	3.22	3.13	-	-
94.	-	-	.79	.89	6.59	6.39	2.80	2.96	-	-
95.	-	-	0.81	0.83	6.46	6.50	3.41	3.61	-	-
96.	++	++	1.55	1.63	6.75	6.89	3.82	3.98	+ve	+ve
97.	-	-	.95	.85	6.29	6.32	3.76	3.02	-	-
98.	-	-	.96	.89	6.45	6.36	3.97	3.66	-	-
99.	-	-	.76	.83	6.5	6.41	3.44	3.86	-	-
100.	+	-	.79	.89	6.36	6.62	3.56	3.89	-	-
101.	-	-	.71	.80	6.41	6.33	3.38	3.46	-	-
102.	+	-	.83	.86	6.5	6.46	3.61	3.53	-	-
103.	-	-	.75	.81	6.49	6.37	3.76	3.52	-	-
104.	-	-	.91	.82	6.32	6.45	3.27	3.41	-	-
105.	+	+	.79	.86	6.50	6.39	3.85	3.59	-	-
106.	-	-	.82	.87	6.46	6.51	3.73	3.50	-	-
107.	-	-	.80	.78	6.48	6.41	3.78	3.67	-	-
108.	+	-	.75	.71	6.31	6.29	3.46	3.62	-	-
109.	-	-	.78	.86	6.52	6.46	3.50	3.66	-	-
110.	-	-	.72	.83	6.29	6.51	3.57	3.43	-	-
111.	-	+	.84	.79	6.38	6.54	3.49	3.51	-	-
112.	-	-	.81	.85	6.42	6.37	3.45	3.57	-	-
113.	-	-	.74	.79	6.43	6.36	3.32	3.46	-	-
114.	+	-	.80	.85	6.39	6.43	3.49	3.54	-	-
115.	-	-	.82	.87	6.53	6.59	3.51	3.57	-	-
116.	-	-	.79	.73	6.47	6.52	3.43	3.52	-	-
117.	-	-	.75	.78	6.25	6.32	3.55	3.61	-	-
118.	-	-	.83	.79	6.39	6.28	3.46	3.51	-	-
119.	-	-	.87	.80	6.42	6.40	3.48	3.57	-	-
120.	-	-	.75	.82	6.46	6.49	3.37	3.51	-	-
121.	-	-	.72	.81	6.53	6.42	3.41	3.53	-	-
122.	-	-	.70	.74	6.40	6.31	3.34	3.42	-	-
123.	-	-	.72	.81	6.36	6.47	3.28	3.36	-	-
124.	+	+	.89	.84	6.41	6.54	3.24	3.21	-	-
125.	-	-	.82	.89	6.42	6.50	3.35	3.39	-	-
126.	-	-	.86	.78	6.46	6.58	3.60	3.57	-	-
127.	-	-	.80	.75	6.37	6.42	3.53	3.42	-	-
128.	+	-	.73	.78	6.23	6.32	3.20	3.43	-	-
129.	-	-	.90	.73	6.01	6.15	3.17	3.33	-	-
130.	-	+	.75	.86	6.24	6.08	3.22	3.51	-	-
131.	-	-	.81	.92	6.06	6.23	3.27	3.18	-	-

132.	-	-	.74	.79	6.19	6.28	3.36	3.39	-	-
133.	-	-	.85	.90	6.26	6.17	3.35	3.36	-	-
134.	-	-	.72	.70	6.14	6.08	3.30	3.38	-	-
135.	+	-	.77	.71	6.30	6.24	3.27	3.21	-	-
136.	-	-	.91	.86	6.39	6.41	3.33	3.60	-	-
137.	-	-	.83	.89	6.25	6.20	3.46	3.28	-	-
138.	+	-	.78	.73	6.04	6.00	3.50	3.42	-	-
139.	-	-	.84	.80	6.27	6.19	3.38	3.40	-	-
140.	-	-	.81	.88	6.07	6.23	3.52	3.21	-	-
141.	-	-	.84	.80	6.05	6.02	3.24	3.32	-	-
142.	-	+	.76	.72	6.26	6.15	3.20	3.19	-	-
143.	-	-	.74	.79	6.17	6.25	3.28	3.33	-	-
144.	-	-	.90	.83	6.11	6.19	3.30	3.45	-	-
145.	+	-	.92	.81	6.23	6.28	3.34	3.28	-	-
146.	-	-	.80	.76	6.08	6.21	3.56	3.37	-	-
147.	-	-	.72	.78	6.26	6.34	3.52	3.42	-	-
148.	-	-	.71	.83	6.24	6.02	3.40	3.29	-	-
149.	-	-	.90	.87	6.07	6.24	3.36	3.31	-	-
150.	-	-	.77	.72	6.23	6.37	3.47	3.32	-	-
151.	-	-	.82	.89	6.05	6.29	3.28	3.24	-	-
152.	-	-	.74	.83	6.11	6.28	3.34	3.30	-	-
153.	-	-	.71	.69	6.24	6.34	3.17	3.26	-	-
154.	-	+	.92	.87	6.36	6.39	3.41	3.35	-	-
155.	-	-	.75	.79	6.07	6.18	3.28	3.39	-	-
156.	+	-	.91	.93	6.40	6.37	3.39	3.27	-	-
157.	-	-	.74	.78	6.17	6.28	3.26	3.35	-	-
158.	-	-	.79	.69	6.23	6.19	3.32	3.39	-	-
159.	-	-	.71	.83	6.29	6.36	3.28	3.34	-	-
160.	-	-	.85	.80	6.41	6.36	3.34	3.30	-	-
161.	-	-	.74	.78	6.05	6.17	3.28	3.26	-	-
162.	-	+	.83	.91	6.37	6.42	3.40	3.45	-	-
163.	-	-	.75	.81	6.19	6.24	3.37	3.39	-	-
164.	-	-	.72	.70	6.04	6.16	3.28	3.32	-	-
165.	-	-	.82	.88	6.30	6.23	3.34	3.39	-	-
166.	-	-	.71	.79	6.06	6.15	3.28	3.31	-	-
167.	-	+	.81	.93	6.19	6.24	3.29	3.36	-	-
168.	-	-	.83	.87	6.22	6.26	3.42	3.35	-	-
169.	-	-	.72	.75	6.02	6.09	3.26	3.34	-	-
170.	-	-	.85	.79	6.38	6.27	3.32	3.38	-	-
171.	-	-	.77	.72	6.04	6.15	3.25	3.21	-	-
172.	-	-	.73	.76	6.09	6.21	3.37	3.24	-	-
173.	-	-	.83	.86	6.28	6.35	3.34	3.39	-	-
174.	-	-	.85	.75	6.36	6.23	3.37	3.24	-	-
175.	-	-	.68	.70	6.02	6.09	3.23	3.31	-	-
176.	-	-	.82	.87	6.31	6.36	3.40	3.37	-	-
177.	-	-	.74	.70	6.17	6.23	3.24	3.32	-	-
178.	-	-	.78	.75	6.28	6.16	3.31	3.36	-	-
179.	-	+	.84	.93	6.24	6.37	3.11	3.29	-	-

180.	-	-	.72	.78	6.03	6.17	3.27	3.34	-	-
181.	-	-	.74	.84	6.31	6.23	3.19	3.25	-	-
182.	-	-	.69	.73	6.04	6.25	3.17	3.23	-	-
183.	-	-	.79	.81	6.07	6.03	3.26	3.22	-	-
184.	-	-	.83	.88	6.32	6.26	3.38	3.31	-	-
185.	-	+	.80	.87	6.25	6.38	3.29	3.35	-	-
186.	-	-	.72	.78	6.06	6.18	3.34	3.30	-	-
187.	-	-	.68	.74	6.02	6.25	3.29	3.36	-	-
188.	-	-	.82	.79	6.32	6.27	3.35	3.26	-	-
189.	-	-	.75	.78	6.36	6.39	3.38	3.40	-	-
190.	-	-	.73	.71	6.28	6.16	3.24	3.28	-	-
191.	+	-	.84	.87	6.34	6.39	3.21	3.33	-	-
192.	-	-	.74	.79	6.37	6.40	3.42	3.36	-	-

Appendix II: Overall mean ± SE values of EC of control group goats.

S.No.	EC (mS/cm)
1.	3.56
2.	2.76
3.	2.67
4.	3.15
5.	3.57
6.	2.11
Mean ± SE	2.97± 0.23

Appendix III: Overall mean ± SE values of EC of pre-treatment stages of Group I and Group II goats.

S. No.	EC(mS/cm) Group I	EC(mS/cm) Group II
1.	3.88	2.09
2.	3.45	3.82
3.	2.95	4.50
4.	4.33	4.40
5.	4.22	3.89
6.	3.90	4.02
Mean ±SE	3.84 ±0.19	3.99±0.29

Appendix IV: Overall mean ± SE values of EC of post-treatment stages of Group I and Group II goats.

S. No.	EC(mS/cm) Group I	EC(mS/cm) Group II
1.	3.23	1.99
2.	3.10	3.51
3.	2.50	4.15
4.	3.87	3.22
5.	3.11	3.18
6.	3.02	3.56
Mean ±SE	3.24± 0.17	3.99± 0.29

Appendix V: Overall mean ± SE values of pH of control group goats.

S. No.	pH
1	6.4
2	6.07
3	6.39
4	6.47
5	6.32
6	6.1
Mean ±SE	6.29 ±0.07

Appendix VI: Overall mean ± SE values pH of pre-treatment stages of Group I and Group II goats.

S. No.	pH of Group I	pH of Group II
1	6.93	6.72
2	7.00	6.22
3	6.83	6.58
4	6.73	6.85
5	6.63	6.83
6	6.82	6.45
Mean ±SE	6.85± 0.07	6.60± 0.08

Appendix VII: Overall mean ± SE values of pH of post-treatment stages of Group I and Group II goats.

S. No.	pH of Group I	pH of Group II
1.	6.5	6.13
2.	6.65	6.2
3.	6.47	6.01
4.	6.32	6.21
5.	6.1	6.16
6.	6.02	6.11
Mean ±SE	6.35±0.08	6.13±0.03

Appendix VIII: Overall mean ± SE values of SCC of control group goats.

S. No.	SCC(million/ml)
1.	.86
2.	.92
3	.94
4	.90
5	.89
6	.78
Mean ± SE	$.88^{a}$±0.23

Appendix IX: Overall mean ± SE values of SCC of Pre-treatment Group I and II goats.

S. No.	SCC(million/ml) Group I	SCC(million/ml) Group II
1.	1.30	1.18
2.	1.42	1.63
3	1.70	1.50
4	1.86	1.83
5	1.52	1.34
6	1.59	2.00
Mean ± SE	1.58^{c}± 0.74	1.61^{c}± 0.98

Appendix X: Overall mean ± SE values of SCC of post-treatment stages of Group I and Group II goats.

S. No.	SCC(million/ml) Group I	SCC(million/ml) Group II
1.	.87	.85
2.	1.02	1.13
3	1.40	1.04
4	1.61	1.50
5	1.15	.96
6	1.24	1.60
Mean ± SE	1.24^{b}± 0.95	1.19^{b}± 0.96

Appendix XI: Overall mean ± SE values of lactose of control group goats.

S No	Lactose (%)
1.	5.1
2.	5.3
3	4.8
4	4.9
5	5.2
6	5.0
Mean ± SE	5.11^{b}±0.010

Appendix XII: Overall mean ± SE values of lactose of pre-treatment stages of Group I and Group II goats.

S No	Lactose (%) Group I	Lactose (%) Group II
1.	5.1	4.0
2.	4.7	4.5
3	4.3	4.7
4	4.5	4.2
5	4.6	4.1
6	4.9	4.9
Mean ± SE	4.756^{a}± 0.029	4.549^{a}± 0.036

Appendix XIII: Overall mean ± SE values of lactose of post-treatment stages of Group I and Group II goats.

S No	Lactose (%) Group I	Lactose (%) Group II
1.	5.3	4.4
2.	4.9	4.8
3	4.4	4.9
4	4.7	4.6
5	4.9	4.5
6	5.1	5.1
Mean ± SE	4.831^{b}±0.16	4.99^{b}±0.13

Appendix XIV: Overall mean ± SE values of fat of control group goats.

S No	Fat (%)
1.	3.7
2.	4.9
3.	3.5
4.	4.2
5.	4.0
6.	4.1
Mean ± SE	4.2^{a}±0.031

Appendix XV: Overall mean ± SE values of fat of pre-treatment stages of Group I and Group II goats.

S No	Fat (%) Group I	Fat (%) Group II
1.	3.7	3.9
2.	3.0	3.7
3	4.5	4.0
4	2.8	3.4
5	3.4	3.8
6	4.2	2.6
Mean ± SE	3.89^{b} ±0.038	3.76^{c} ±0.031

Appendix XVI: Overall mean ± SE values of fat of post-treatment stages of Group I and Group II goats.

S No	Fat (%) Group I	Fat (%) Group II
1.	4.1	4.0
2.	3.5	3.7
3	4.6	4.1
4	3.0	3.5
5	3.6	3.9
6	4.3	2.8
Mean ± SE	3.99^{a}±0.17	3.91^{a}±0.25

Appendix XVII: Overall mean ± SE values of protein of control group goats.

S No	Protein (%)
1.	3.7
2.	3.1
3	3.9
4	3.6
5	3.4
6	3.3
Mean ± SE	3.43^{a}± 0.017

Appendix XVIII: Overall mean ± SE values of protein of pre treatment stages of Group I and Group II goats.

S No	Protein (%) Group I	Protein (%) Group II
1.	3.2	3.1
2.	2.7	2.9
3.	2.8	3.2
4.	3.6	3.5
5.	2.6	3.0
6.	3.3	3.1
Mean ± SE	3.20^{a}±0.024	3.17^{a}±0.020

Appendix XIX: Overall mean ± SE values of protein of post-treatment stages of Group I and Group II goats.

S No	Protein (%) Group I	Protein (%) Group II
1.	4.1	3.4
2.	4.3	3.3
3	4.9	3.5
4	4.7	3.7
5	4.2	3.0
6	3.9	3.2
Mean ± SE	3.63^{b}±0.12	3.94^{b}±0.14

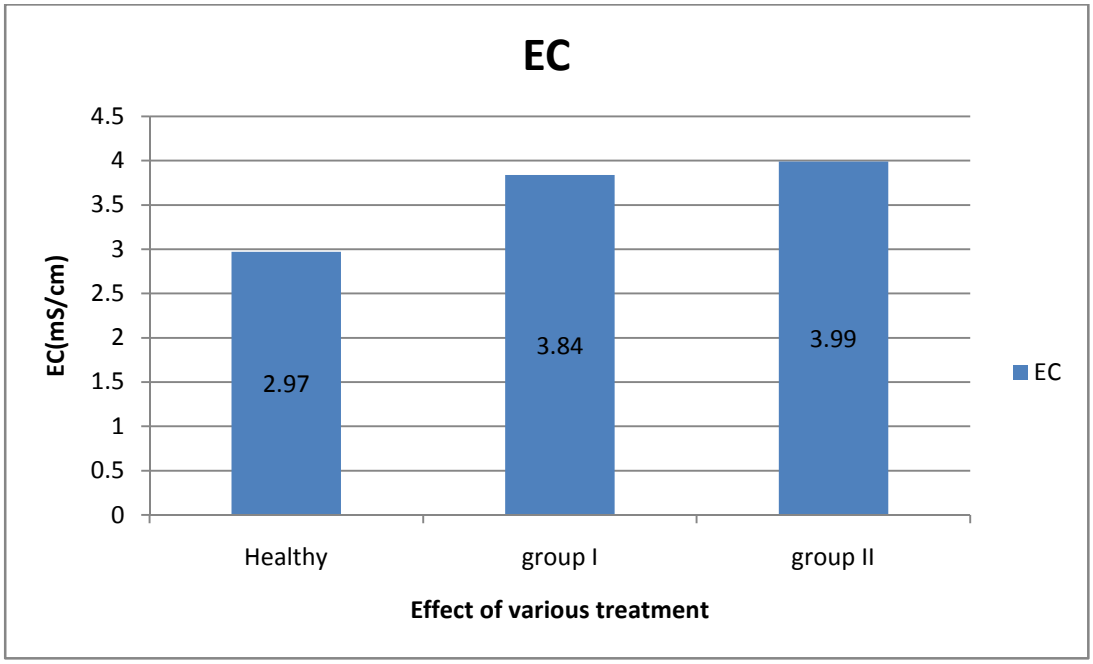

Figure 7: Mean ± SE values of Electrical conductivity (EC) mS/cm of control group, Group I and Group II of Goats.

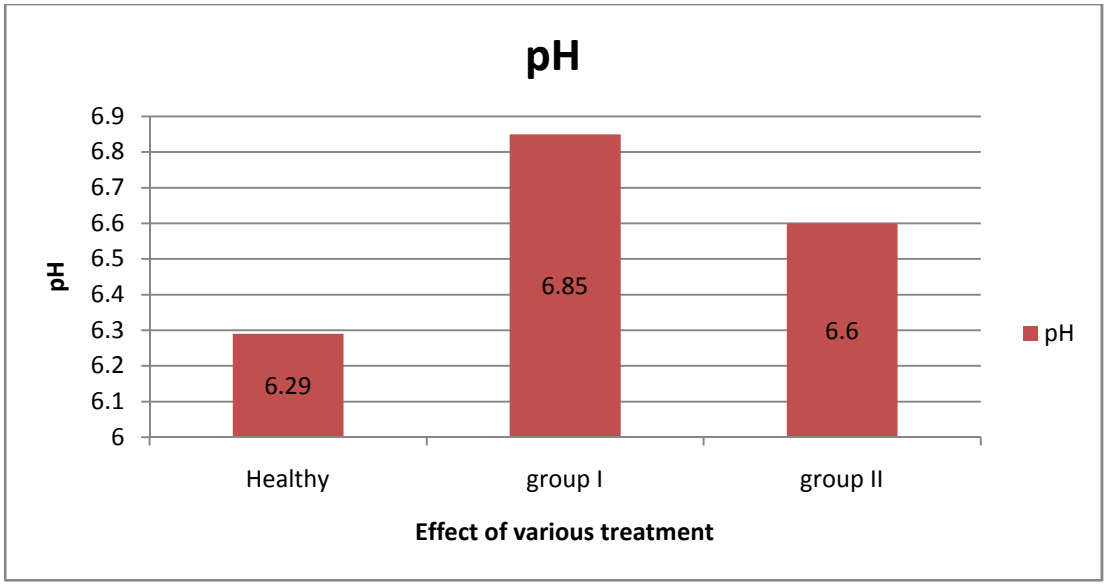

Figure 8: Mean ± SE values of pH of control group, Group I and Group II of goats.

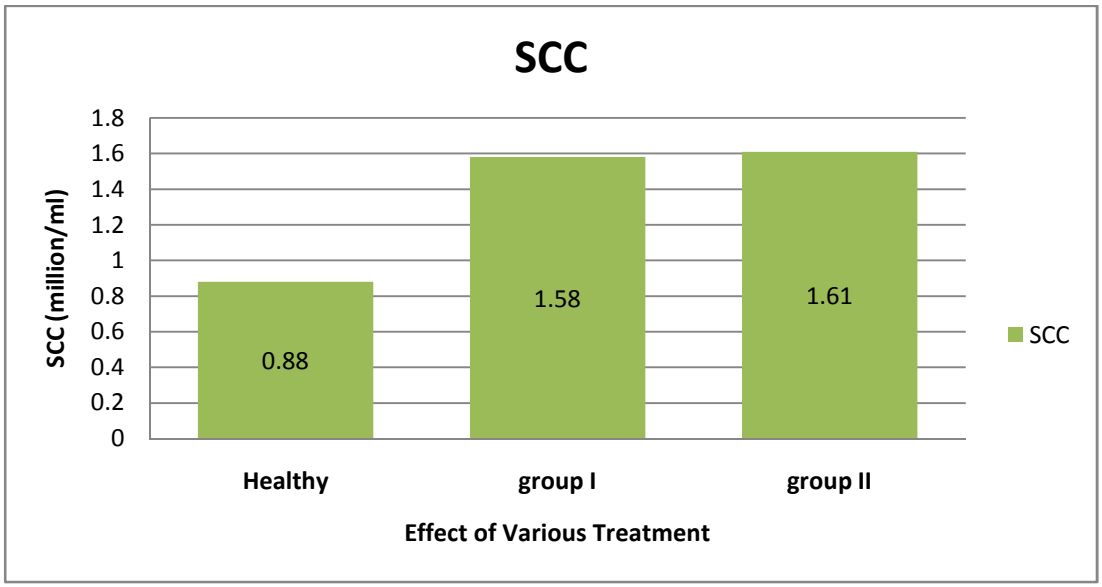

Figure 10: Mean ± SE values of somatic cell count (SCC) million/ml of control group, Group I and Group II of Goats.

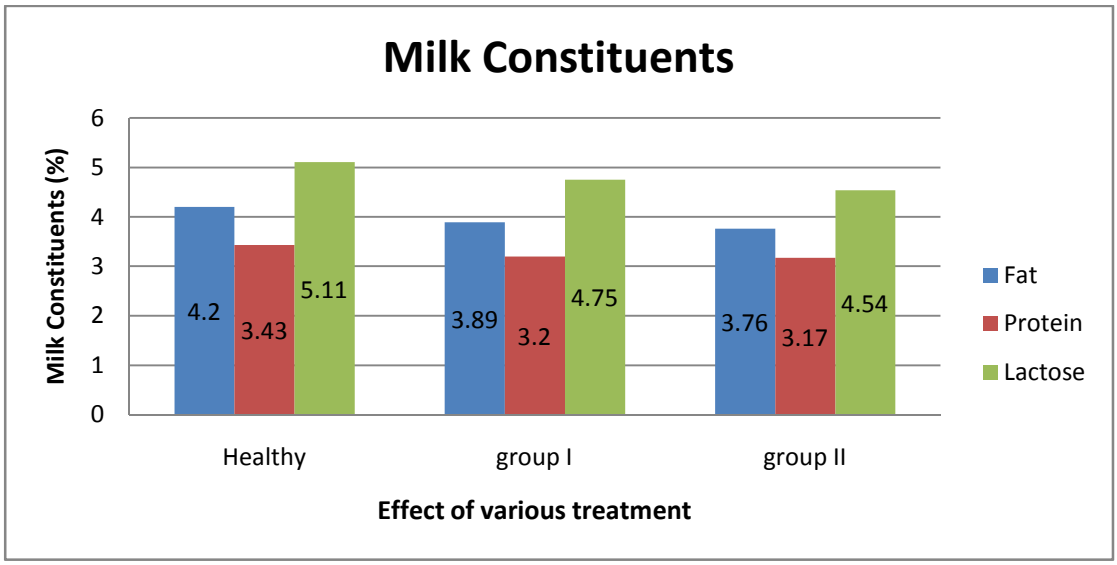

Figure 11: Mean ± SE values of various milk constituents % of control group, Group I and Group II of Goats.

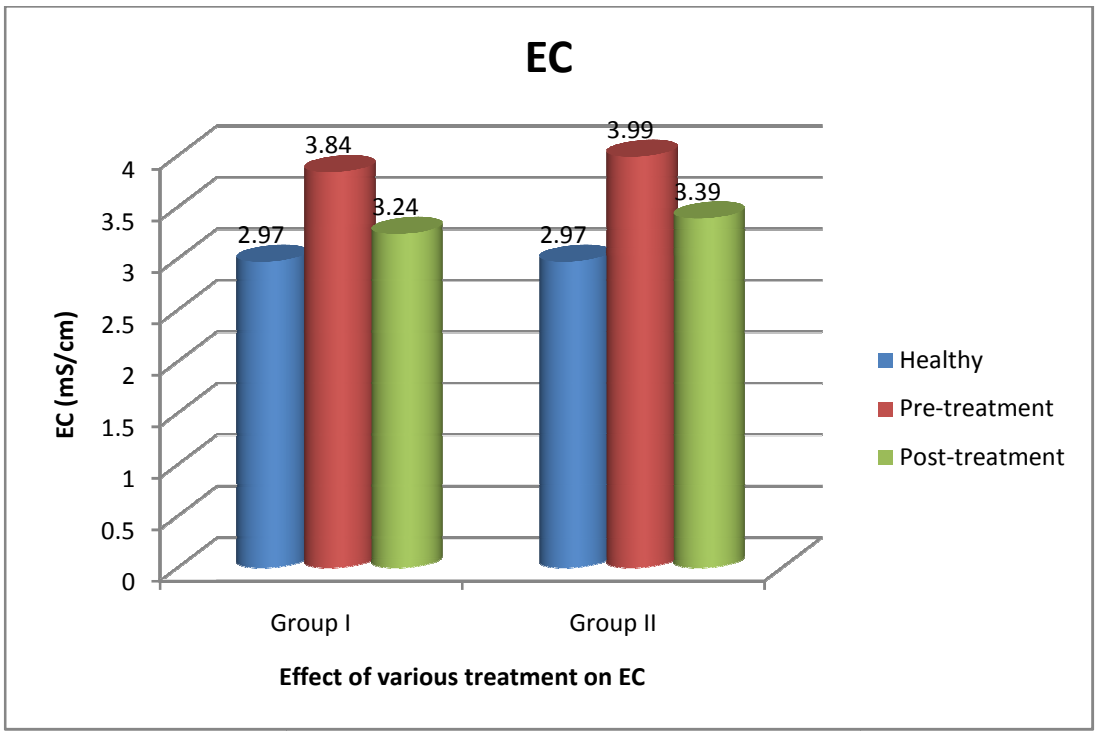

Figure 18: Pre- and post treatment mean ± SE values of Electrical conductivity (EC) mS/cm of control, Group I and Group II goats.

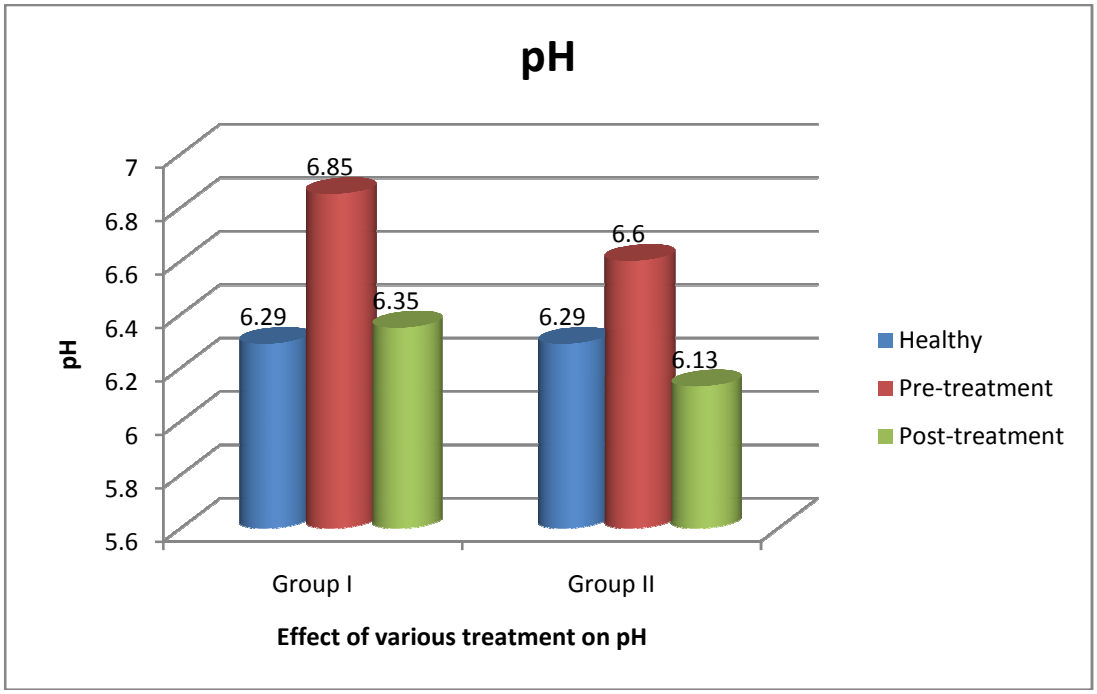

Figure 19: Pre- and post treatment mean ± SE values of pH of control group, Group I and Group II goats.

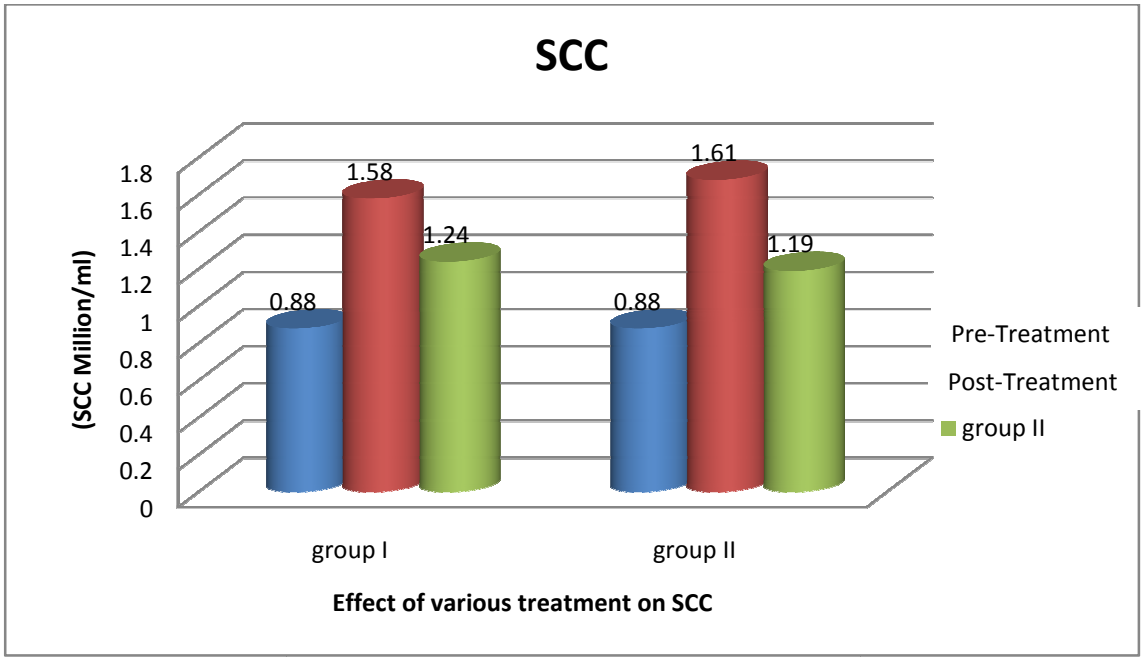

Figure 20: Pre- and post treatment mean ± SE values of somatic cell count (SCC) million/ml of control, Group I and Group II goats.

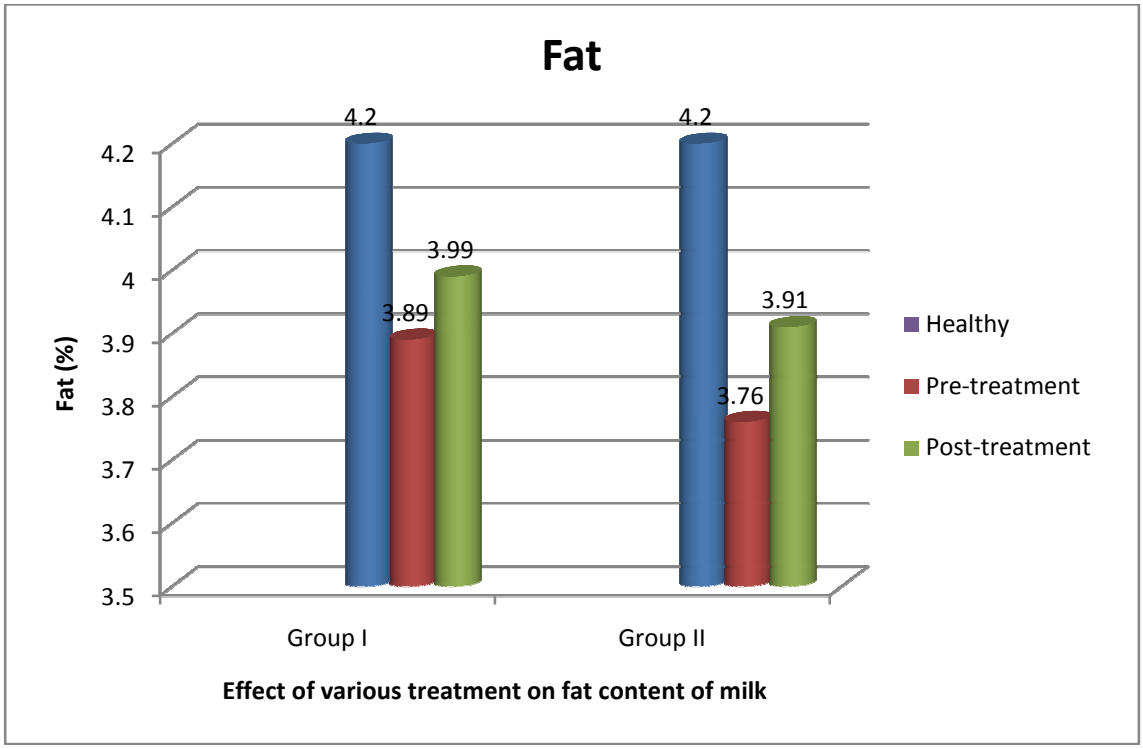

Figure 21: Pre- and post treatment mean ± SE values of fat content of control group, Group I and Group II goats.

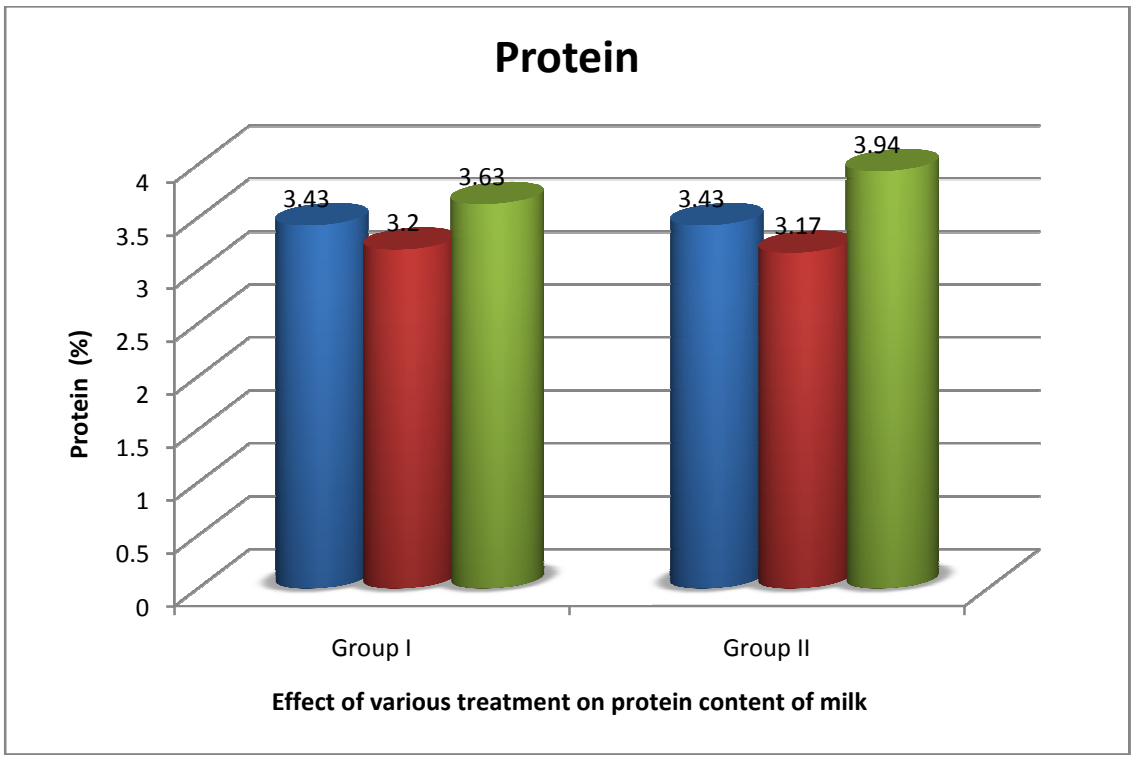

Figure 22: Pre- and post treatment mean ± SE values of protein content of control group, Group I and Group II goats.

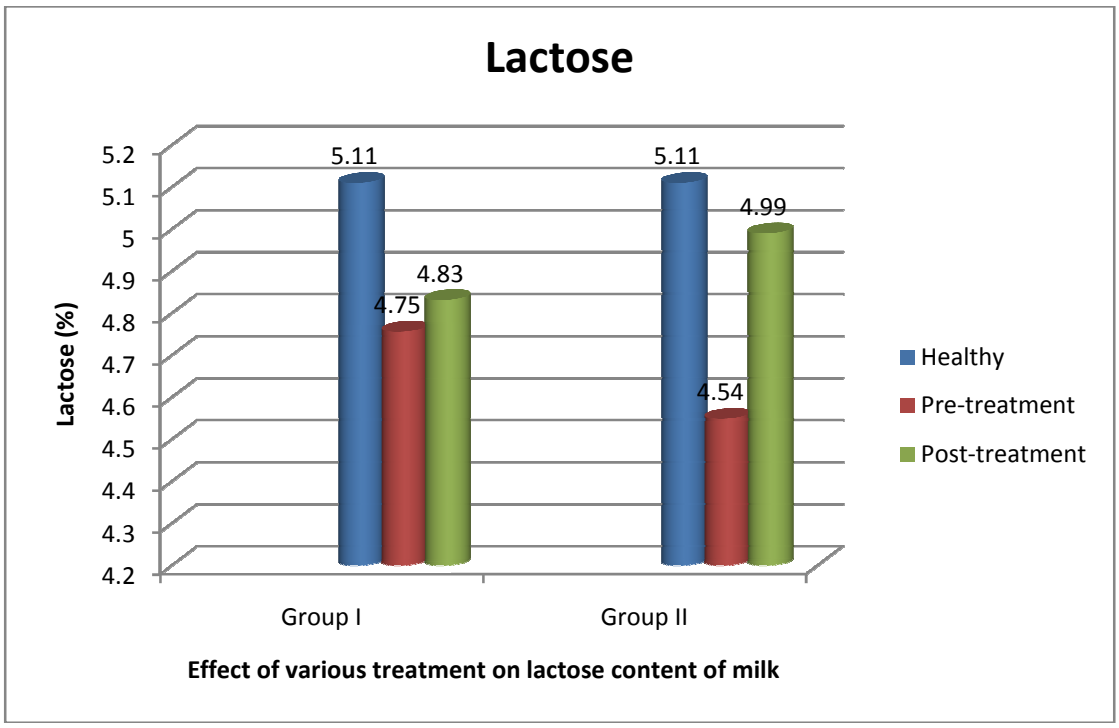

Figure 23: Pre- and post treatment mean ± SE values of lactose content of control group, Group I and Group II goats.

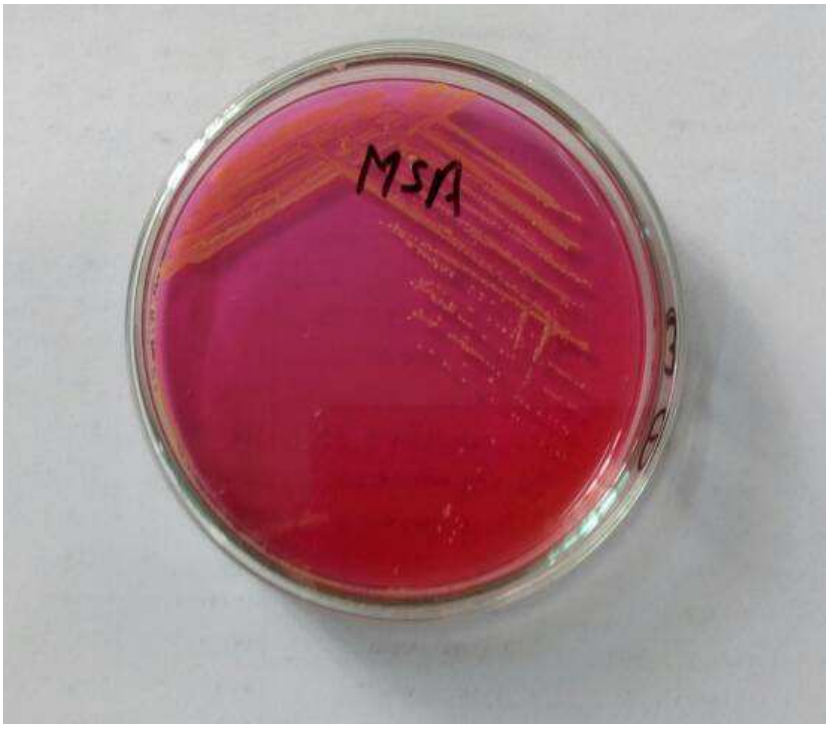

Figure 12: *Staphylocoocus* colonies on Mannitol Salt Agar (MSA) plate.

Figure 13: *Klebsiella* colonies on Mac conkey Agar media plate

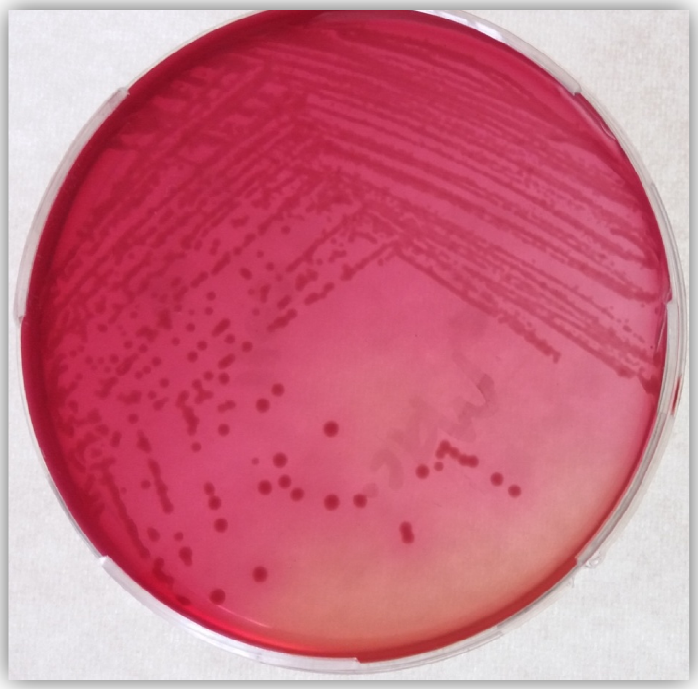

Figure 14: Colonies of *E.coli* on Mac conkey agar media plate

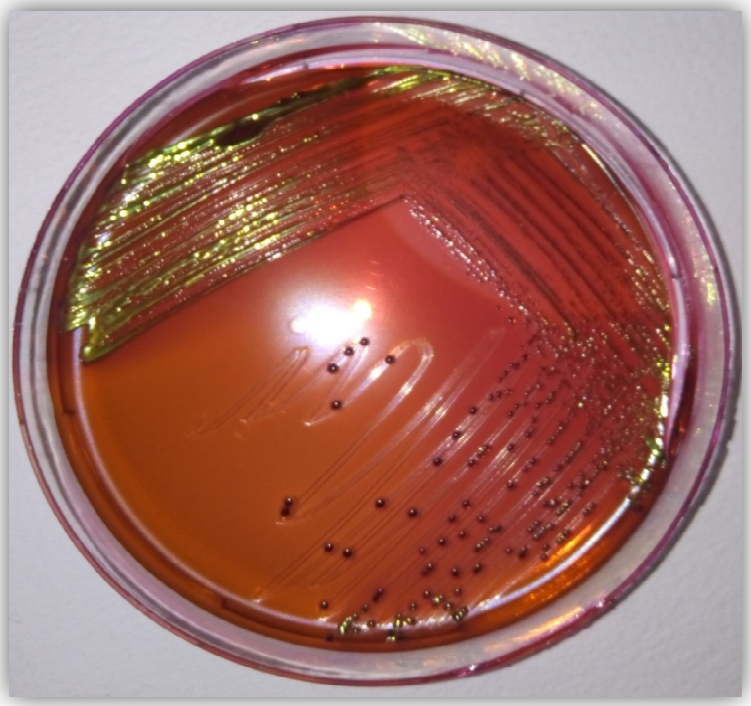

Figure 15: colonies of *E. coli* on Eosin Methylene Blue (EMB) agar media plate.

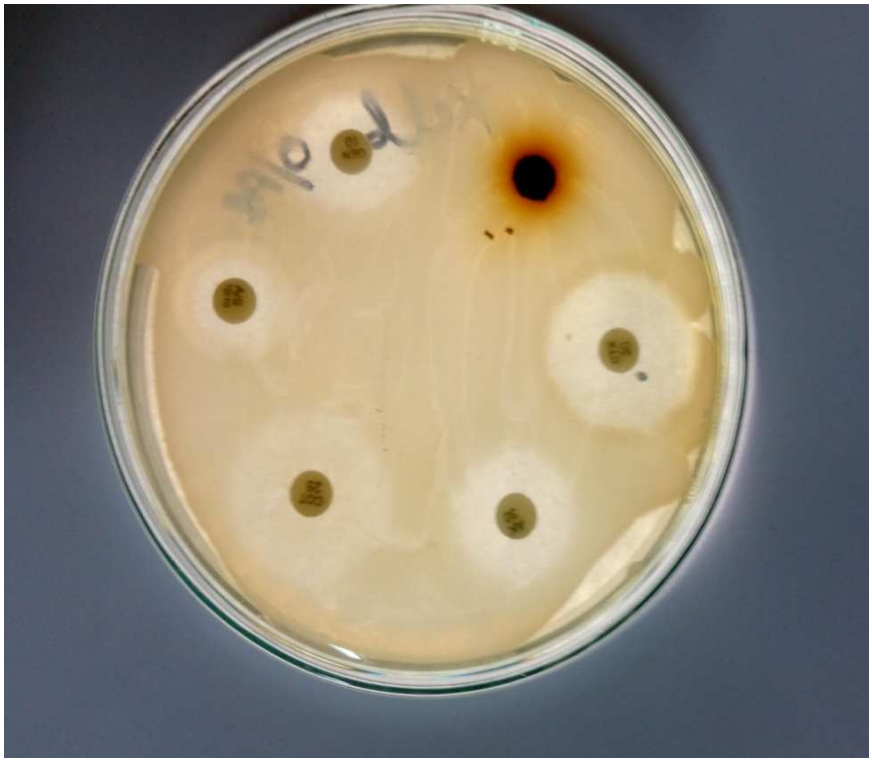

Figure 16: Photograph of aqueous Tulsi extract showing antibacterial activity @ 150 mg/kg

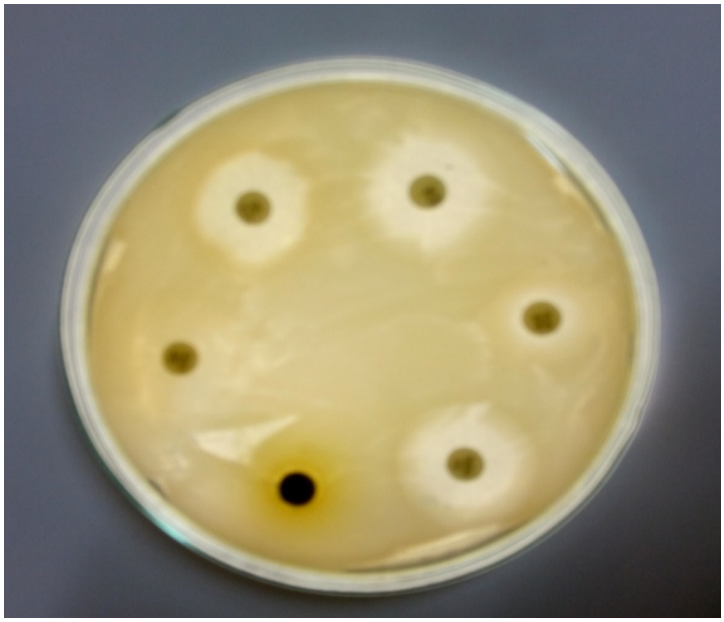

Figure 17: Photograph of alcoholic Tulsi extract showing antibacterial activity @ 125 mg/kg.

Figure 4: Photograph showing Extraction process by soxhlet method. (Soxhlet extraction assembly)

Figure 1: Gel formation is clearly visible on CMT examination of subclinical mastitic milk of goat.

Figure 2

Figure 3

Figure 2: Photograph of Pen type EC 035 (ATC) Conductivity meter; EC = 2.95 mS/cm at 20.6°C

Figure 3: Photograph of Pen type pH 035 (ATC) pH meter; showing pH = 6.56 at 22.8°C

5(a) Aqueous Extract of Tulsi

5(b) Alcoholic Extract of Tulsi

Figure 6: Photograph showing evaporation of alcohol in rotatory evaporator

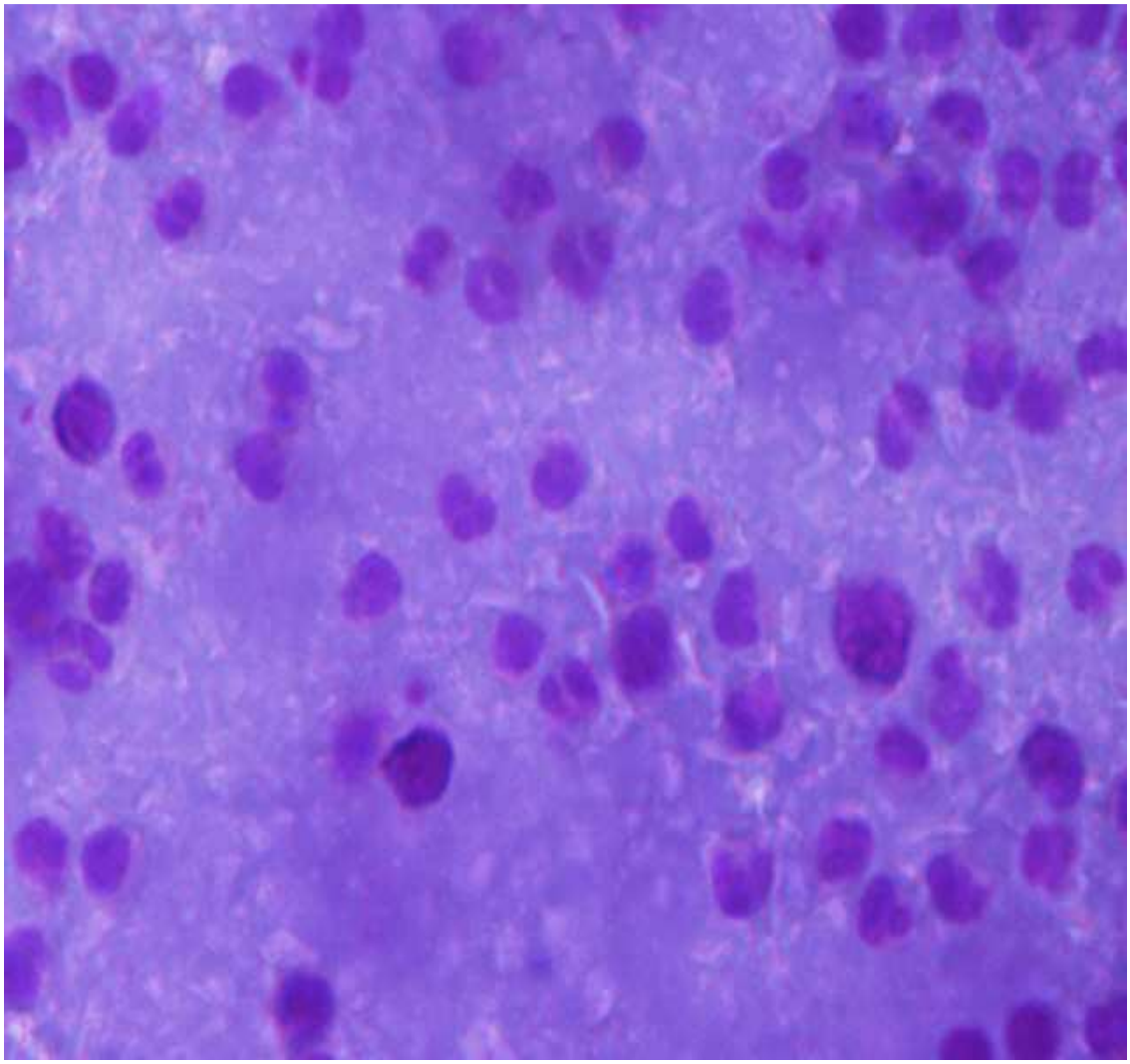

Figure 9: Photograph of Total Somatic Count (TSCC); Somatic cells are visible under 100X oil immersion microscope.