EFFECT OF COW URINE DISTILLATE FORTIFIED WITH *Curcuma amada* EXTRACT THERAPY ON SUBCLINICAL MASTITIS IN COWS

T H E S I S

Submitted

In partial fulfillment of requirements for the degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY BIOCHEMISTRY

BY

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Enrolment No: V/16/338

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled "**EFFECT OF COW URINE DISTILLATE FORTIFIED WITH *Curcuma amada* EXTRACT THERAPY ON SUBCLINICAL MASTITIS IN COWS"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigate on have been duly acknowledged.

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Miss .**SHINDE KAJAL VIJAY** has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, "**EFFECT OF COW URINE DISTILLATE FORTIFIED WITH *Curcuma amada* EXTRACT THERAPY ON SUBCLINICAL MASTITIS IN COWS"** submitted by her is the result of research work is sufficient to warrant its presentation to the examination in the subject of **VETERINARY BIOCHEMISTRY** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by her for a degree of any other University.

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CERTIFICATE

This is to certify that the thesis entitled, "**EFFECT OF COW URINE DISTILLATE FORTIFIED WITH *Curcuma amada* EXTRACT THERAPY ON SUBCLINICAL MASTITIS IN COWS"** submitted by **SHINDE KAJAL VIJAY** to the Maharashtra Animal and Fishery Sciences University, Nagpur in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY BIOCHEMISTRY** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Place: Akola **(Shinde Kajal Vijay)**

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

Abbreviations/symbols		Full form
%	-	Percent
<	-	Less than
>	-	More than
ANOVA	-	Analysis of Variance
Avg	-	Average
C	-	Celsius
Cm	-	Centimeter
CMT	-	California Mastitis Test
Cp	-	Centipoise
CUD	-	Cow urine distillate
d	-	Day
DAHD	-	Department of Animal Husbandry and Dairy
DLC	-	Differential Leucocyte Count
DW	-	Distilled Water
EDTA	-	Ethelenediamine tetraacetic acid
et al.	-	Et alia (and other)
etc.	-	Etcetera
Fig.	-	Figure
g	-	gram
g	-	Gram
g/dl	-	Gram per deciliter
Hb	-	Haemoglobin
HF	-	Holestein Fresian
hr	-	Hour (s)
i.e.	-	That is
IDF	-	International Dairy Federation
IMI	-	Intra Mammary Infection
IU/l	-	International Unit per litre

Abbreviations/symbols		Full form
Kg	-	Kilogram
LDH	-	Lactate Dehydrogenase
LF	-	Left-fore
LH	-	Left-hind
Mg	-	milligram
mg	-	Milligram
min	-	Minute (s)
ml	-	Milliliter
PCV	-	Packed Cell Volume
PMN	-	Polymorphonuclear
RBC	-	Red Blood Cells
RF	-	Right-fore
RH	-	Right Hind
Rpm	-	Revolutions per minute
SC	-	Somatic Cell (s)
SCC	-	Somatic Cell Count
SCM	-	Sub Clinical Mastitis
SCM	-	Subclinical Mastitis
SE	-	Standard Error
SNF	-	Solid Not Fat
Sq.	-	Square
TEC	-	Total Erythrocytes Count
TLC	-	Total Leucocytes Count
WBC	-	White Blood Cells
WF	-	Working Factor
WST	-	White Side Test

CHAPTER – I

INTRODUCTION

In 2019, India's total cattle population was 192.49 million. (20th livestock census 2019). Over the preceding Livestock Census (2012), the total number of cattle increased by 0.8%. Over the previous census, the population of female cattle increased by 18.0%, while the population of male cattle declined by 30.2%. Cattle contributed about 36% of the total livestock.

With huge cattle population, India continues to be with world's top producer of milk. The government has begun several kinds of programmes that have increased livestock productivity, resulting to a significant increase in milk production. The total amount of milk produced in 2021–2022, exhibited annual growth of 5.29%, compared to 209.96 million tonnes in 2020-21 (DAHD, Annual Report 2022-23).

Mastitis is one of the most severe and deadly illnesses that affect lactating animals. It is a parenchymal mammary gland inflammation, characterized by pathological abnormalities in glandular tissues as well as physical, chemical, and usually microbial changes in milk. It causes substantial economic losses to farmers and changes in the glandular tissues quantity. It adversely affects animal health, milk quality, quantity and the economics of milk production (Sharma *et al.* , 2007).

"Mastitis," in simple terms, refers to udder inflammation. The majority of dairy farmers associate mastitis with an inflamed udder or quarter, which affects the appearance of the milk. This alteration is the result of the inflammation in response to the infection. When there are no apparent external changes to the udder despite the infection, mastitis may appear in a subclinical form (Sharma *et al.* , 2012).

Early diagnosis of subclinical mastitis is crucial for prompt treatment which improves recovery prospects and reduce medical costs involved in treatment of clinical mastitis (Sharma *et al.* , 2012). Subclinical mastitis is more difficult to detect because the milk appears normal but often has an increased

somatic cell count. Because changes in the udder tissue occur more quickly than they become apparent, early identification of mastitis is necessary. For the diagnosis of subclinical mastitis, a variety of techniques based on physical and chemical changes in milk are used. However, following the International Dairy Federation's (IDF) standards, the diagnosis of mastitis is based on the quarter's somatic cell count (SCC) (Sharma *et al. ,* 2009).

In simple terms, sub-clinical mastitis is a type of mastitis that can be distinguished by the absence of gross symptoms and the absence of visible changes in the milk. Subclinical mastitis if ignored, leads to clinical form resulting in a harder, larger gland and a decrease in milk production (Sharma *et al. ,* 2012).

Wani *et al. ,* (2022) reported three times more production losses are caused by subclinical mastitis than by clinical mastitis, which accounts for 60–70% of the overall economic losses caused by all mastitis forms (De Vliegher *et al. ,* 2012 ; Sinha *et al. ,* 2014; Sharun *et al. ,* 2019).

Currently, mastitis is mostly treated with allopathic medicines, but allopathic treatments are accompanied with residual effects, which poses a great health risk to the human population. Panchagavya therapy, or cowpathy, has been proposed as an alternative prophylactic and therapeutic approach to the health of livestock, which protects human health too (Dhama *et al. ,* 2013).

Cow urine, in general, has antibacterial properties (Raad *et al. ,* 2013) In addition, traditional medicines viz plant extracts can be utilized in therapy.

Cow urine possesses both antimicrobial and disinfectant properties. (Chauhan and Dhama, 2010) These activities are attributed to the presence of various components such as urea, phenol, carboxylic acids, calcium, manganese, along with specific amino acids and urinary peptides, as highlighted by Gulhane *et al. ,* (2017).

‘Ark' has been identified as a potent bioenhancer of commonly used antibiotics, antifungal, and anticancer drugs, thereby reducing the required dosage and treatment duration. When administered alongside antibiotics, it also hinders the development of microbial resistance to these medications (Bhadauria, 2002).

In the United States, a patent has been filed specifically for a distillate of cow urine known to enhance the efficacy of antifungal, antimicrobial, and anticancer agents (Khanuja, 2002; Saxena *et al. ,* 2004). Cow urine, containing quinolones and flavoquinolones, exhibits notable fungicidal properties.

Zingiberaceae includes a number of significant genera, including *Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria, and Costus.* Linnaeus coined the genus *Curcuma* in his 1753 Species *Plantarum.* The term most likely comes from the Arabic word "kurkum," which means yellow. Mango ginger, or *Curcuma amada* Roxb. (Family: Zingiberaceae), is a perennial rhizomatic aromatic herb that grows from November to April. *Curcuma amada roxb.,* sometimes known as mango ginger, is a distinctive spice that resembles ginger in morphology but tastes like raw mango. The genus has a wide distribution in the tropical regions of Asia, Africa, and Australia, having its origins in the Indo-Malayan region. Many of these naturally occurring medicinal plants are used as well in medicine (Samant, 2012).

Curcuma amada roxb has wide conventional applications in folks medicine. Its antimicrobial, insecticidal, antifungal, hypotriglyceridemic, hypoglycemic or anti-hyperglycemic, anthelmintic, antiallergic, anti-inflammatory activity and antioxidant properties have been proved by several investigations (Jatoi *et al. ,* 2007 ; Samant, 2012).

Numerous herbal extracts present in cow urine distillates have demonstrated effectiveness as antimicrobials. Rajapandiyan *et al. ,* (2011) explored the use of cow urine extract from *Azadirachta indica* as a novel control agent against clinically significant antimicrobials. Poornima *et al. ,* (2012) utilized cow urine extracts from *Polyathalia longifolia* to assess their antimicrobial activity.

Cow urine distillate can be fortified with *Curcuma amada* to enhance its medicinal properties, particularly antibacterial activity, which could be beneficial in treating diseases like mastitis. Therefore, the present study was planned to evaluate the therapeutic activity of *C.amada*-fortified cow urine distillate in

subclinical mastitis, with the objective **to study effect of cow urine distillate fortified with *Curcuma amada* extract therapy on subclinical mastitis in cows.**

CHAPTER – II

REVIEW OF LITERATURE

Study material related to mastitis and effect of *Curcuma amada* fortified with cow urine distillate is reviewed here under:

2.1. Prevalence of SCM in cattles

Bangar *et al.* (2015) studied provided the pooled estimate of the prevalence of subclinical mastitis in dairy cows in India under a random effects model, and it was concluded that there was a high prevalence of the disease. A significant variation in the prevalence of the disease across various regions of India was observed in the meta-analysis. This high prevalence of subclinical mastitis might have been the main cause of low productivity in dairy cows in India over the years and needed to be controlled through scientific, managerial, and therapeutic measures. Dairy farmers could have reduced the incidence and economic losses associated with subclinical mastitis with the guidance of field veterinarians.

Swami *et al.* (2017) studied that the prevalence of subclinical mastitis in cows was 35.00 percent, and in buffaloes, it was 28.33 percent. This indicated that the incidence of subclinical mastitis was higher in cows compared to buffaloes. The incidence per animal (6.67 percent) and per quarter (3.67 percent) was found to be higher in cows compared to buffaloes. It was observed that the prevalence of subclinical mastitis was higher in the hind quarter of buffaloes and in the fore quarter of cows. Additionally, the hind quarters were more affected than the fore quarters in both species. The pH of milk affected by subclinical mastitis increased, while fat, protein, lactose, total solids, and solids not fat decreased significantly, adversely affecting milk quality. Age, lactation number, stage of lactation, method of milking, and housing were identified as major risk factors precipitating the occurrence of subclinical mastitis.

Mourya *et al.* (2020) studied that prevalence of subclinical mastitis was discovered to be 31.55 percent in cows on an animal basis and 20.18 percent on a

quarter basis, in and around Jabalpur, Madhya Pradesh. Based on SCC, MCMT, and milk pH, the highest prevalence was recorded in the 5-7 years of age group (38.50%), 4th parity (44.12%), early lactation stage (56.25%), and in crossbred (35.06%) animals.

2.2. Losses due to SCM

According to Khan and Khan (2006), subclinical mastitis resulted in significant changes to milk casein, the primary milk protein with the highest nutritional value, which decreases, as lower quality whey proteins increase, adversely impacting the quality of dairy products like cheese.

In dairy cows who developed subclinical mastitis, Halasa *et al.* (2009) found a considerable decrease in milk, fat, and protein production; predictions of production losses were also more accurate.

Two blocks in the Pune district, Baramati and Daund, in central India, were selected for the collection of data by Sinha *et al.* (2014) because of the large amount of crossbred 187 cows within these blocks. Economic losses resulting from subclinical mastitis have been assessed in terms of decreased milk production, costs associated with medicine and veterinary care, and additional resources utilized. The total estimated losses per lactation were INR1390, with the decrease in milk production resulting in approximately 49% of the total economic loss, and veterinary expenses responsible for 37%.

Rathod *et al.* (2017) investigated the incidence and economic losses caused by subclinical mastitis in buffaloes, crossbred cows, and zebu/desi cows at 10 project villages in Karnataka state's Bidar district. The economic consequences of sub-clinical mastitis includes decreased milk production, increased daily prevention and treatment costs per animal, and a decrease in the adult animal's market value. Depending on the animal's condition, the overall loss for one lactation period could range from INR 21,677 to INR 88,340.

Das *et al.* (2018) conducted a survey on clinical and subclinical cases of mastitis in cattle and buffaloes throughout ten districts in Odisha. They observed

that a substantial amount of buffaloes' economic losses are caused by subclinical mastitis. Treatment contributes to the greatest amount of economic loss—roughly 60.46 percent of overall losses. In sub-clinical mastitis, the average daily loss of milk supply is 2.58 liters. The total monthly cost of subclinical mastitis to cows is $2222/-.

Kumari *et al.* (2018) revealed that subclinical mastitis (SCM) is a more severe form of the disease that costs the dairy sector substantially more. Losses from SCM are more than three times higher than those from clinical mastitis. While the milk in this case of mastitis looks normal and there are no obvious abnormalities—such as udder enlargement, hardness in the affected quarter, pain, or watery milk—physical and chemical changes occur in the milk, which aids in the diagnosis of SCM using a variety of methods of diagnosis.

Singh *et al.* (2021) investigated the economic losses resulting from sub-clinical mastitis in cattle by using data obtained from the Uttar Pradesh Animal Husbandry Gadget. The loss associated with subclinical mastitis is INR 832.517 crores. Veterinary expenses accounted for 37% of the overall loss, whereas the reduction in milk production by itself produced an overall loss to the extent of INR1390 for each lactation, or nearly 49%. Sub-clinical mastitis may result in losses due to ineffective treatment, lack of various disease causes, and its inability of preventive measures.

2.3. California Mastitis Test (CMT)

This test is quick, simple, and affordable. Also, it indicates the infection status of each quarter, which four-quarter composite sampling could fail to recognize (Schalm and Noorlander, 1957).

Dingwell *et al.* (2003) evaluated the California mastitis test for usefulness. CMT is a simple screening test for subclinical mastitis that is often performed from the cow side. It is a qualitative measurement of the somatic cell count in milk. They also examined the CMT's predictive values, specificity, and sensitivity. The CMT's capacity to identify an IMI's existence is known as its sensitivity. The CMT's predictive values indicate how the test results might be

interpreted in the field, and its specificity is its capacity to identify quarters lacking an IMI.

Iqbal *et al.* (2006) noticed that CMT was more effective than the other four tests: White Side Test (WST), White Side + Dye Test (WSTD), Surf Test, and Surf + Dye Test at the threshold between negative and trace, when udder inflammation was at the subclinical stage.

Salvador *et al.* (2014) concluded that the main advantages of CMT are that it serves as an "animal side test," quick, inexpensive, and simple. Based on CMT data, the estimated incidence of SCM was 30.29%. However, according to the SCC, the average incidence of SCM was 23.46%.

Badiuzzaman *et al.* (2015) investigated a total of 444 quarters of milk samples from 111 crossbred dairy cows in Bangladesh between 2010 and 2011. They assessed the efficacy of the California Mastitis Test (CMT) and somatic cell count (SCC) for diagnosing SCM. Positive samples by CMT accounted for 80.08%, with a specificity of 69.40%. Positive samples by SCC were 86.60%, with a specificity of 97.81%.

Maheshwari *et al.* (2016) found that milk samples from 212 quarters tested positive for SCM using CMT. They noted CMT scores of 1+, 2++, and 3+++ in 57.55% (122 out of 212 quarters), 31.60% (67 out of 212 quarters), and 10.85% (23 out of 212 quarters) of SCM-affected cows, respectively.

Ferronatto *et al.* (2018) revealed that even at the times of maximum cellularity, as in the colostrum and transition milk, the CMT score had been considered to be the optimum cut-off value for detecting mastitis at all times and, consequently, when selecting animals for further microbiological analysis based on their milk.

Ahmed and Fahim (2018) noted that the California Mastitis Test (CMT) was commonly used for diagnosing subclinical mastitis (SCM), identifying 63.50% of samples as positive.

Geleta *et al.* (2019) screened 1536 milk samples from 384 cows using CMT. They reported that 20.51% were CMT positive, 15.49% were CMT 1+, 11.52% were CMT 2++, and the remaining 45.32% were CMT negative.

Karabasanavar *et al.* (2019) observed that CMT was increasingly utilized as a reliable, easy-to-perform, and rapid diagnostic test for SCM.

Maramulla *et al.* (2019) diagnosed SCM using CMT in 492 quarter milk samples from 123 lactating animals around Hyderabad. They found 366 quarter milk samples negative by CMT. Among the positive samples, 8.13% showed CMT 1+ reaction, 10.98% showed CMT 2++, and 6.50% showed CMT 3+++.

Tresamol *et al.* (2019) analyzed 261 milk samples from organized dairy farms for SCM detection. They found 49.50% of samples positive by CMT, with 9.96% as CMT 1+, 7.67% as CMT 2++, and 31.80% as CMT 3+++.

Mourya *et al.* (2020) examined 412 lactating cattle for SCM and observed a prevalence of 31.35% on an animal basis and 20.18% on a quarter basis using CMT. CMT scores were distributed as follows: 58.18% (192/330) for CMT 1, 29.09% (96/330) for CMT 2++, and 12.73% (42/330) for CMT 3+++.

Shabaz *et al.* (2020) examined 823 milk samples using the California Mastitis Test (CMT) reagent. They found that 53.2% (497) were negative, followed by 17.01% (140) as CMT score 3+++, 14.09% (116) as CMT score 2++, and 8.50% (70) as CMT score 1+.

Amritha Priya *et al.* (2021) collected 420 quarter milk samples from 104 healthy dairy cows from University Livestock Farm, Mannuthy, and Livestock Research Station, Thiruvazhamkunnu. Among these, they found 55 samples graded as 3+++, 108 as 2+, 62 as 1, and 195 as 0 or negative by CMT.

Chouhan *et al.* (2021) examined 57 milk samples from presumably healthy udders of crossbred cows using CMT. They found that 31.57% were negative or trace, 28.07% were weakly positive (+), 21.05% were positive (+++), and 19.29% were strongly positive.

Getahun (2021) conducted a cross-sectional study on subclinical mastitis in Ejersa Lefo District, Oromia region, Ethiopia. Out of 184 milking cows, 111 were affected by subclinical mastitis. The grading of CMT results indicated 6.13% were weakly positive, 15.30% were distinctly positive, and 21.60% were strongly positive for SCM infection.

2.4. Somatic cell count (SCC)

Berning and Shook (1992) noted that somatic cell count (SCC) could detect latent infections even in the absence of visible udder inflammation, aiding in distinguishing between affected and unaffected individuals.

Sharma *et al.* (2011) revealed thatthe somatic cell count (SCC), which includes leucocytes (75%)—that is, neutrophils, macrophages, lymphocytes, erythrocytes, and epithelial cells—is a helpful indicator of intramammary infection (IMI). Stress, injury to tissues, and bacterial infection can cause an increase in leucocytes. Animal bodies are protected from infectious agents by somatic cells. Raw milk quality is negatively impacted by an increased SCC in the milk. insufficient milk supply, changes in milk consistency (density), less chance of proper milk processing, insufficient protein, and a significant risk of poor milk hygiene due to potential pathogenic organism presence are all associated with subclinical mastitis

Sarvesha *et al.* (2016) found significantly increased milk leukocytes (8.59 ± 0.43) and neutrophils (43.59 ± 0.19), along with a decrease in macrophages (27.88 ± 0.17) and lymphocytes (17.64 ± 0.18) in SCM-affected animals.

Souza *et al.* (2016) determined a threshold SCC value of >200,000 cells/ml in quarter milk samples for diagnosing SCM. They observed a decrease in SCC from 529,834 cells/ml to 29,637 cells/ml over three weeks post-sampling and recovery from intramammary infections.

Sri Balaji *et al.* (2016) examined 480 milk samples from 120 healthy dairy cows in Salem District, Tamil Nadu. They found SCC values of 4.25 ± 0.11 x 10^6 cells/ml and 437 ± 0.20 x 10^3 cells/ml in subclinical mastitis-affected

Jersey crossbreeds and Holstein Friesian crossbreeds, respectively, compared to healthy cows with SCC values of 1.78 ± 0.53 x 10^3 cells/ml and 1.95 ± 0.11 x 10^3 cells/ml.

Bobbo *et al.* (2017) reported that 58% of healthy cows had SCC scores <100,000 cells/ml in milk samples. They found SCC scores >100,000 cells/ml indicative of intramammary infections in composite milk samples, with microbial presence at 1,000 CFU/ml.

Petzer *et al.* (2017) found that an increase in SCC to >200,000 cells/ml correlated with increased daily milk production loss in subclinical mastitis cases.

Sumon *et al.* (2017) conducted a cross-sectional study on 13 dairy farms in Bangladesh, observing a significant increase in mean SCC in the rear quarter (341.25 x 10^3 cells/ml) compared to the front quarters (226.54 x 10^3 cells/ml).

Ahmed and Fahim (2018) noted that milk SCC ranged from 263 x 10^3 cells/ml to 2 x 10^6 cells/ml in positive CMT quarter milk samples, with a mean count of 61554.12 cells/ml.

Alhussien and Dang (2018) concluded that SCC served as an index for udder health status and was helpful in assessing milk quality in dairy animals.

Milk metabolites in Subclinical Mastitis

2.5. Milk Colour and Consistency in Subclinical Mastitis

Milk's colour is a combination of the distinct effects provided by: (1) the colloidal casein particles and the scattered fat globules, both of which scatter light, and (i) the carotene (to a certain extent xanthophyll), which gives a yellowish tinge, Milk appears in a variety of colours, including creamy white (buffalo milk) and yellowish-creamy white (cow milk). The amount of yellow in cow milk is influenced by a number of factors, including breed diets, the size of fat globules, the milk's fat content, etc. The milk from some cow breeds has a more intense yellow colour than milk from others. The colour of cow milk gets more

intense yellow the more green feed is consumed. The intensity of the yellow colour increases with the size of the fat globules and the fat content (De Sukumar, 1980).

Casein micelles and fat globules scatter visible light, giving milk its white colour. Because smaller, homogenized fat globules scatter light more readily, homogenized milk produces a whiter product. Whey's unique colour is due to riboflavin, which gives milk a greenish serum phase. Dairy products like butter and cheese get their colour from fat-soluble pigments called carotenoids, derived from plants and not from animal synthesis. As a result, feed greatly influences the colour of milk fat. Individuals and breeds of cattle distinct in their ability to convert carotenes into Vitamin A through metabolism.

Reddy *et al.* (2001) discovered that there were no apparent indications of subclinical mastitis (SCM) in crossbred cows across various dairy farms near Tirupati.

Chakrabarti (2004) observed that SCM-affected cows did not show any abnormalities in their udders, only experiencing a reduction in milk production.

Saravanan *et al.* (2009) documented that all positive cases of SCM had milk samples exhibiting normal colour, consistency, and odour.

Radostits *et al.* (2010) noted that subclinical mastitis is characterized by an increased somatic cell count in affected milk samples, with no visible abnormalities observed in either the milk or the udder.

Suresh *et al.* (2010) observed that cows aged between 4-6 years affected by SCM did not exhibit any clinical signs except for reduced milk production. Additionally, twenty-four quarter milk samples showed normal colour, consistency, and odour.

Maheshwari *et al.* (2016) stated that cows affected by SCM displayed no apparent symptoms other than a decrease in milk production, with milk maintaining normal color, odor, and consistency.

Mourya *et al.* (2020) conducted examinations of milk samples from all confirmed SCM cases, revealing no abnormalities in terms of color, texture, or smell. Furthermore, the udder and teats returned to normal upon manual palpation.

2.6. Milk Fat % in SCM

Bobade (2004) found that the average milk pH of the normal group was 6.48 ± 0.01, while for the clinical group, it was 7.04 ± 0.02. The average milk pH values were significantly higher in clinical mastitis compared to subclinical mastitis and lower in normal milk. However, no significant difference was observed between normal and subclinical groups.

Hange (2004) observed milk pH ranging from 6.53 ± 0.04 to 7.36 ± 0.10. The values were significantly higher in clinical mastitis milk compared to subclinical mastitis, while they were lower for normal milk.

Leitner *et al.* (2004) noted that the concentration of milk components in infected halves was significantly lower than those in uninfected halves. Specifically, fat (61.7 ± 0.26 vs. 64.9 ± 0.21 g/l), protein (53.5 ± 0.11 vs. 58.5 ± 0.07 g/l), lactose (33.5 ± 0.16 vs. 44.7 ± 0.08 g/l), and casein (40.5 ± 1.59 vs. 45.9 ± 1.36 g/l) were lower in infected halves. However, there was no difference in the concentration of Ca2+.

Bhoyar (2006) examined 36 crossbred cows for subclinical mastitis, finding that 22 cows (73.33%) and 46 quarters (34.07%) tested positive in the Modified California Mastitis Test (MCMT). They observed significant decreases in the average values of acidity, specific gravity, fat, total solids (TS), solid-not-fat (SNF), lactose, calcium, magnesium, phosphorous, and potassium in subclinical mastitis milk. Conversely, the average values of pH, total protein, chloride, and sodium increased significantly in subclinical mastitis milk.

Sonea *et al.* (2009) reported that the fat percentage of normal milk (3.9%) was higher than that of subclinical mastitis (SCM) milk (3.5%).

Bagri *et al.* (2018) In the past study of fat percentage, it was observed that the average percentage of fat significantly decreased in subclinical mastitis milk samples

Sharma *et al.* (2020) The study showed a decrease in fat, solid-not-fat, total solids, protein, and lactose contents with increased somatic cell count, suggesting that subclinical mastitis affected milk quality and may have decreased milk production. The average fat percentage in normal quarter milk and subclinical mastitis milk was observed to be 3.96 and 3.37 percent, respectively. The fat percentage of normal milk ranged between 1.70 to 8.31 percent, while in subclinical mastitis milk it ranged between 1.04 to 5.62 percent. In this study, a decrease of 14.89 percent in fat percentage was observed in subclinical mastitis milk samples. Similarly, Rajiv *et al.* (1998) observed a decrease in fat percent of subclinical mastitic milk, and this decrease in fat content in milk might have been due to impaired synthesis and secretory activity of the udder epithelial cell.

Harjanti *et al.* (2020) reported that the average milk production was 12.08 liters per day. Milk composition results were as follows: 3.36% lactose, 3.45% fat, and 2.97% protein. It was concluded that when the mammary infection in cows increased, milk production and milk components decreased.

2.7. Milk density in SCM

Specific gravity is the ratio of a substance's density to the density of water, whereas density is a substance's mass (weight) per unit volume. At 60°F (15.6°C), the specific gravity of milk is typically expressed. It is possible to calculate the weight of a known volume or the volume of a known weight in order to determine the density or specific gravity of milk. A pycnometer or a hydrostatic balance can be used to determine the weight of a known volume, whereas lactometers are used to determine the volume of a known weight. Water is lighter than milk. At 60°F, the average specific gravity of cow milk ranges from 1.028 to 1.030, whereas it varies for buffalo milk from 1.030 to 1.032 (De Sukumar, 1980).

In numerous studies investigating milk composition, specific gravity data have been consistently documented. MacMahon *et al.* (1932) analyzed milk

samples from 83 cows and reported an average specific gravity of 1.0302. Similarly, studies conducted in Bangalore (Annu. Rep. I.D.R.I., 1948-50) observed that the average specific gravity of herd milk from various cow breeds ranged from 1.0290 to 1.0298. It was noted that, except for approximately 8% of individual milk samples, none fell below the minimum standard of 1.028 specific gravity. The specific gravity of milk was observed to exhibit a slight decline during the initial months of lactation, followed by a period of relative stability up to the sixth month, after which a gradual decrease or irregular behavior was noted. Additionally, the authors reported a slight negative correlation between specific gravity and the fat percentage of milk.

With a lactometer, the specific density of milk was measured. At 15 degrees Celsius, the normal density of milk ranged from 1.028 to 1.033 g/ml, while water had a density of 1.0 g/ml. Therefore, by reading the lactometer, it was possible to determine whether water had been added to the milk. It was advisable to combine the lactometer reading with the fat test: if the fat test results were low and the density was high (e.g. 1.035), then the milk might have been skimmed. If the fat test results were low and the density was low (e.g. 1.027), then water might have been added to the milk. The lactometer reading could be used along with the fat percentage to estimate the Solids Non Fat (SNF) content of the milk (Draaijer, J. (2002).

The average specific gravity of normal cow milk ranged from 1.028 to 1.032, indicating that it was slightly denser than water due to its constituents, except for fat, which were more abundant than in water. Skim milk had a specific gravity ranging from 1.034 to 1.036, while cream typically had a specific gravity around 0.93. Buffalo milk exhibited a specific gravity ranging from 1.030 to 1.034. It was generally more economical to purchase milk by volume when the unit price remained the same (price per kilogram or liter (Mann *et al.* , 2013)

2.8. Milk viscocity in SCM

The Ostwald viscometer was used to determine the viscosities of several aqueous solutions without attempting to determine how accurately the instrument reproduced the viscosities.

Cox *et al.* (1956) conducted a study on 13 milk samples from individual cows, observing viscosity at 20°C ranging from 1.97 to 2.48 centipoise, with an average of 2.063. They attributed changes in viscosity to the presence of soluble non-fat solids.

Pejic *et al.* (1956) found the average viscosity of milk from indigenous Simmental cows to be 1.815 centipoise, with a specific gravity of 1.0325.

Kulkarni and Dole (1958) determined the viscosity of milk at 20°C, observing an average viscosity of 1.444 centipoise. They noted a decrease in viscosity upon dilution with water.

Satprakash (1963) investigated milk samples from Indian cows, finding that fresh milk had a viscosity of 13.20 millipoise at 20°C, with casein contributing significantly to viscosity.

Patel (1965) analyzed 192 milk samples from Kankrej cows for viscosity, finding considerable variation among individual animals, with viscosity ranging from 2.0553 to 1.4770 centipoise, and an average of 1.7422 at 20°C.

Blahova and Janal (1973) tested cow milk for temperature-dependent viscosity concerning holding time at low temperatures, finding minimal effect on viscosity at 20°C.

Sontakke (1979) reported mean viscosity values for individual and herd milk samples of Deoni cows to be 1.884 ± 0.013 and 1.872 ± 0.110 centipoise, respectively.

Kulkarni and Dole (1956) conducted research on estimating the viscosity of several samples of cow milk and established relationships involving the Viscosity Constant V, which represents the ratio of the time of outflow of the

sample through an opening to that of water. They investigated these relationships concerning both total solids and non-fat solids.For Sindhi cows (individual milk), the average viscosity recorded was 1.283 centipoises at 30°C. The average K value, which denotes the ratio of viscosity constant to total solids (TS), was found to be 0.1060, while the average ratio of viscosity constant to solids-not-fat (SNF) was 0.1623. In the case of herd milk, the viscosity measured in centipoises was 1.236.

2.9. Milk pH in SCM

Tale *et al.* (2002) found that the pH of normal cow milk ranged from 6.44 ± 0.02 to 6.48 ± 0.01, whereas in subclinical mastitis milk, it ranged from 6.69 ± 0.03 to 6.73 ± 0.02. Bansal and Randhawa (2003) indicated that mastitis led to a rise in milk pH due to the leakage of blood bicarbonates into the milk following damage to mammary epithelium, consequently decreasing the activity of milk-clotting enzymes.

Gokhare *et al.* (2003) provided the average milk pH values with standard errors, ranges, and regression in different health statuses. They observed that the average pH of milk was lowest (6.42 ± 0.02) in the normal group and highest (7.12 ± 0.05) in the clinical group.

Kuchitik and Sedlockva (2003) analyzed changes in milk composition and properties during the third lactation based on milk samples from 9 goats. They found that titrable acidity and pH remained relatively stable throughout lactation, except for a recorded milk pH value of 6.91 on the 135th day of lactation.

Bobade (2004) reported that the average milk pH of the normal group was 6.48 ± 0.01, while for the clinical group, it was 7.04 ± 0.02. Milk pH values were significantly higher in clinical mastitis compared to subclinical mastitis, and lower in normal milk. No significant difference was observed between normal and subclinical groups.

Hange (2004) observed that the pH of milk ranged from 6.53 ± 0.04 to 7.36 ± 0.10. These values were significantly higher in clinical mastitis milk compared to subclinical mastitis, while they were lower for normal milk.

Batavani *et al.* (2007) In the past, the pH of SCM (subclinical mastitis) milk was found to be higher than that of normal milk, aligning with findings from earlier studies (Kitchen, 1981; Sena and Sahmani, 2001; Wielgosz-Groth and Groth, 2003). Indirect pH testing could be considered a reliable method for detecting subclinical mastitis as it is cost-effective, simple, and rapid. Such testing can be conducted in the field at the time of milk collection.

Kumar (2007) noted that the mean milk pH value for apparently healthy cows and buffaloes was 6.48 ± 0.40 and 6.50 ± 0.52, respectively, with a significantly higher mean value observed in cases of subclinical mastitis. Guha and Gera (2012) found that the pH of normal milk and milk from quarters affected by subclinical mastitis was 6.85 ± 0.26 and 6.92 ± 0.17, respectively, with an insignificant increase in pH observed in subclinical mastitis cases.

Sood *et al.* (2008) observed that somatic cell count (SCC) and pH levels were notably higher in both subclinical and clinical mastitis samples, whereas Electrical Conductivity (EC) in mastitic milk from dairy animals showed a non-significant increase.

Sunder *et al.* (2013) recorded the average pH of healthy cow milk as 6.60 ± 0.12, while mastitis-affected milk had an average pH of 7.2 ± 0.17. Waghmare *et al.* (2013) reported a higher average pH of milk from subclinically affected cows (6.79 ± 0.016) compared to normal cows (6.55 ± 0.009).

Langer *et al.* (2014) reported that the sensitivity of milk pH in detecting subclinical mastitis was 56.84%, with a specificity of 61.1%. Balaji *et al.* (2016) analyzed 480 milk samples from 120 dairy cows and found that apparently healthy, non-infected cows had a mean ± SE milk pH of 6.93 ± 0.05, while samples suspected for subclinical mastitis had a pH of 7.59 ± 0.027.

Johri (2016) reported that the normal milk pH of Rathi cattle ranged between 6.02 and 6.79, while pH in subclinically affected quarters ranged between 7.01 and 7.5. Gogoi *et al.* (2017) analyzed 368 quarter milk samples and found their pH within acceptable limits regardless of their CMT status.

Swami *et al.* (2017) evaluated the effect of subclinical mastitis on the physicochemical properties of milk and observed a significant increase in the average pH value of subclinical mastitis-affected milk compared to normal milk. Specifically, the pH of cow milk increased from 6.57 ± 0.021 in normal milk to 6.88 ± 0.015 in subclinical mastitis-affected milk, while in buffaloes, it increased from 6.68 ± 0.016 in normal milk to 6.91 ± 0.021 in subclinical mastitis-affected milk.

Bagri *et al.* (2018) In the past study, on average, pH values of subclinical mastitis milk were found to be higher than normal milk pH values, but the difference was not statistically significant.

2.10. Total whey Protein and Albumin

Wheelock *et al.* (1966) observed higher concentrations of non-casein proteins in milk from udders with bacterial infections compared to normal milk, possibly due to increased permeability of udder tissues to blood components like serum proteins during mammary infection.

Ashworth *et al.* (1967) reported a significant increase in milk serum protein concentration (+0.21%) with increasing CMT score, indicating a higher total protein concentration in mastitic milk likely due to serum proteins seeping from the bloodstream through damaged udder tissues.

Haenlein *et al.* (1973) found an increase in total whey protein in mastitic milk (13.1 mg/ml) compared to normal milk (8.2 mg/ml) in Holstein and Guernsey breed cows.

Randolph *et al.* (1974) studied mastitis's influence on milk properties in bovines and found higher concentrations of bovine serum albumin, immunoglobulin, protease, and peptone in mastitic milk than in normal milk.

Singh and Ganguli (1975) observed an increasing trend in average milk whey protein values for normal (8.7 mg/ml) and mastitis positive clinical and subclinical (19.8 mg/ml) milk samples of Tharparkar cows.

Kitchen (1981) recorded elevated total milk whey protein in mastitic milk (19.8 mg/ml) compared to normal milk (8.7 mg/ml) in cows.

Guidry *et al.* (1983) studied changes in milk components during mastitis in cows and found increased albumin concentration in mastitic milk (4.49 mg/ml) compared to normal milk (0.38 mg/ml), with peak values reaching 9.37 mg/ml after 4 hours on average post-infection.

Rao (1990) found increased non-casein protein, especially blood proteins, in milk from mastitic quarters in cows due to increased capillary permeability during inflammation.

Raniya and Kumarson (1996) found increased total proteins in subclinical mastitic affected quarters in cows (3.49 gm/dl) compared to normal milk (2.97 gm/dl).

Singh *et al.* (1995) reported elevated milk protein values (3.12 ± 0.55 gm/dl) from milk of subclinical mastitic cows compared to healthy cows (2.97 ± 0.55 gm/dl).

Kolte *et al.* (1999) reported elevated total protein concentration in milk from subclinical mastitic cows (3.90 ± 0.17 gm/100 ml) compared to milk from healthy cows (2.5-3.5 gm/100 ml).

Hoeben *et al.* (2000) recorded elevated bovine serum albumin levels in milk samples from mastitic cows (5.5 ± 13 gm/l) compared to non-mastitic cows (3.1 ± 0.6 g/l).

Vijaylakshim *et al.* (2001) reported elevated bovine serum albumin (BSA) in mastitic cow milk compared to normal healthy cow milk. The mean BSA values for mastitic milk and normal milk from healthy cows were 4588 ± 0.33 mg/ml and 0.76 ± 0.15 mg/ml, respectively.

Tale *et al.* (2002) observed that the total milk whey protein content increased in subclinical mastitic cow milk compared to milk from healthy cows.

Waghmare *et al.* (2002) noted an increase in total whey protein in milk from subclinical mastitic cows (2.90 ± 0.01 gm/dl) compared to milk from healthy cows (2.88 ± 0.08 gm/dl).

Charjan *et al.* (2003) reported elevated total proteins in milk whey obtained from subclinical mastitic cows compared to normal milk whey (3.05 ± 0.02 gm/100 ml).

Batavani *et al.* (2007) The albumin content of milk in subclinical mastitis was significantly increased compared to the healthy samples. The increase in albumin content during mastitis has been reported in cows (Urech *et al. ,* 1999; Vijayalakshmi *et al. ,* 2001; Coulon *et al. ,* 2002), sheep (Leitner *et al. ,* 2004a), and goats (Leitner *et al. ,* 2004b). Although it was generally thought that the liver is the main site of albumin synthesis and that albumin enters the milk by leaking through the epithelial tight junctions from the bloodstream (De Wit, 1998), extrahepatic synthesis of albumin has been demonstrated in mammary gland epithelial cells, albeit to a lesser extent than in the liver (Shamay *et al. ,* 2005). The significant increases in albumin in mastitic animals suggest that the gland itself is a major source of the increase in albumin content in milk under inflammatory conditions.

Rathaur *et al.* (2020) revealed that there was an increase in Whey Protein, Na, pH, Electric Conductivity in mastitis milk compared with normal milk and a significant decrease in Specific gravity, SNF, Fat, and Protein value in mastitis milk. These results were also found in cows as reported by Badran *et al. ,* (1986) and Haggag *et al. ,* (1991). Decrease in the specific gravity in mastitis-affected cows was attributed to the increase in chloride and decrease in lactose contents.

The study also reported that there was a positive correlation with the severity along with pH of the milk in mastitis. The decreasing trend in the specific gravity in mastitis milk was owed to the reduced lactose contents and increased chlorides.

2.11. LDH enzyme activity in SCM

There was a noted partial correlation between lactate dehydrogenase (LDH) levels and the pH of milk collected from inflamed glands. LDH activity increased progressively as milk pH rose from 6.9 to 7.1. However, LDH levels in mastitic milk at pH 7.2 and 7.3 were lower than those observed at pH 7.1. In normal milk, no correlation was found between milk pH and LDH activity. A CMT score of 3 and 4 was associated with a higher mean LDH activity (Bogin and Ziv., 1973).

Bogin *et al.* (1977) investigated the distribution pattern of LDH isoenzymes in normal and inflamed bovine udders. Results showed that the characteristic proportion of LDH in normal udder milk was 2.29 ± 1.28%, whereas in acute inflamed udder tissue and leukocytes, it was 11.03 ± 1.13%.

Kato *et al.* (1989) reported that LDH activity in normal milk ranges from 50 to 100 U/ml (78.3 ± 10.5), whereas mastitic milk had higher LDH activity, i.e., 19133 U/ml. They found that the increased LDH activity shifted from the fast-moving isoenzyme (LDH,) to the slow-moving isoenzyme (LDH) present in the anaerobic muscle of the mammary gland.

Mert *et al.* (1992) reported that the mean values of LDH activity in 5 healthy cows and 15 sub-clinically mastitic cows were 45.20 U/lit and 38.1 U/lit, respectively.

Klinkon *et al.* (2003) reported that LDH activity in normal milk ranged from 7 to 173 U/ml of milk, and if the somatic cell count in milk exceeded 40,000 cells/ml, the enzyme activity also exceeded 100 U/ml of milk.

Battrawy *et al.* (2002) reported a significant elevation of LDH enzyme in sub-clinically mastitic milk.

The mean activity of LDH was higher in milk from sub-clinically mastitic udders than in milk from healthy udders ($P < 0.01$). The incidence of increment in LDH activity in udder milk showed the presence of tissue damage provoked by sub-clinical mastitis (Batavani *et al.* , 2003).

Batavani *et al.* (2007) investigations have shown that udder tissue abnormalities in subclinical mastitis are associated by a substantial increase in LDH activity in the secretions, although this has no apparent impact on the enzyme levels in blood serum. Previous studies on cows and sheep has higher LDH activity in milk serum of inflamed udders. It is evident that blood serum was not the only source of this enzyme in mastitic milk and that it was probably also released from disintegrated leukocytes and the parenchymal cells of the udder is shown by the higher level of LDH in mastitic milks than blood serum LDH activity.

Similarly, Hussain *et al.* (2012) observed Enzymes like lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, and sodium were found to be significantly higher in mastitic cattle compared to healthy ones. Additionally, the levels of potassium, phosphorus, calcium, magnesium, zinc, and iron were significantly elevated in the milk of mastitic animals.

2.12. Importance and medicinal properties of cow urine distillate

2.12.1.Cow Urine Distillate's Medicinal Properties:

Khanuja (2002) investigated the properties of cow urine distillate. Cow urine distillate contains bioactive molecules that are activity enhancers and availability facilitators, including anti-infective and anti-cancer medicines. "Bioactive molecules" refers to molecules that exhibit any activity in the form of either increasing or decreasing a biological function, such as medicines, nutraceuticals, cardiovascular, hepatoprotective, neurotonics, etc. These studies directly affect the dosage of antibiotics, medications, and anti-cancer agents while significantly improving the effectiveness of bioactive molecule absorption. In this investigation, the goal is to develop an innovative technique using cow urine

distillate in various formulations to improve the activity and bioavailability of antibiotics, drugs, and other compounds.

Jarald *et al.* (2008) studied that by employing bacteria including Escherichia coli, Staphylococcos aureus, Staphylococcos epidermitis, Bacillus subtilis, Klebsiella pneumoniae, and Proteus, studied the antimicrobial activity of cow urine and its distillate.

Gururaja *et al.* (2009) revealed the effect of cow urine distillate on liver function was studied in vivo in rats intoxicated with carbon tetrachloride (CCl4). the in vivo effects of cow urine distillate on liver function. Six groups (n = 6) were randomly assigned to the animals. The last three groups were given a single dose of CCl4/olive oil (1:1, 5 mL/kg, i.p.) on the seventh day after receiving cow urine distillate orally once a day for seven days at doses of 2.7, 5.4, and 10.8 ml per kg body weight. In a dose-dependent manner, the distillate of cow urine decreased the levels of SGOT, SGPT, ALP, GGT, and total bilirubin. The potential useful effect of distillate from cow urine on liver function could be associated to the antioxidants that are present in the urine.

Sathasivam *et al. ,* (2010) conducted an examination of the antimicrobial properties of cow urine distillate in vitro against clinical pathogenic microorganisms utilizing the agar well diffusion test. Similarly, Goswami and Kaushik (2017) effectively employed the disc diffusion assay to compare cow urine distillate with antibiotics.

Wate *et al.* (2012) studied the rat-tail immersion method, the analgesic effects of cow urine and its distillate have been investigated in this study. The activity of a conventional Diclofenac sodium solution administered to a different group of animals was compared to the activity observed within the experiment's animals. The analgesic activity of cow urine and its distillate is comparatively significant.

Sachdev *et al.* (2012) studied the impact of Gomutra ark (GoA) on rats with experimentally induced diabetes due to alloxan was investigated. Measured biochemical markers included malondialdehyde release, vitamin C, and blood

sugar. Acute and chronic toxicity studies were used to assess the safety profile of GoA. GoA considerably decreases blood glucose in diabetic rats, however it has a lesser apparent effect than glibenclamide. In diabetic rats, it drastically decreases vitamin C and malondialdehyde levels. This study supports up the traditional usage of Gomutra ark, which has a high therapeutic index and is safe to use for diabetes.

Shukla *et al.* (2013) investigated that the anti-urolithiatic effects of for medicinal purposes distilled cow urine, or cow urine ark, in regard to renal calculi caused by ethylene glycol (EG).Serum creatinine, blood urea level, urine oxalate, kidney weight, and CaOx deposits were all markedly elevated by EG. The availability of cow urine ark resulting in significantly lower levels of blood urea, urine oxalate, serum creatinine and CaOx depositions. Significantly, kidney weight was also increased. Calcium phosphate crystallisation has been avoided by cow urine ark by 40% and 35%, respectively. For Wistar rats, cow urine ark can be beneficial in both treating and preventing EG-induced urolithiasis.

Sharma *et al.* (2017) evaluated the ability of distillate cow urine to prevent obesity in Wistar rats and compare it with fresh cow urine in the presence of high-fat diets. After 60 days of daily oral treatment against high-fat diet-induced obesity, a qualitative analysis of fresh cow urine and its distillate was conducted and used to evaluate anti-obesity parameters such as BMI, abdominal circumference, obesity index, atherogenic index, lipid profile analysis, and histopathological evaluation. The BMI, abdominal circumference, obesity index, atherogenic index, total cholesterol, triglycerides, LDL-C, and VLDL-C all appeared to be reduced in the treatment groups given fresh cow urine and its distillate. HDL-C levels were considerably higher in the treated group than in the control group.This ultimately results in the conclusion that both fresh cow urine and its distillation have considerable anti-obesity activity against high fat diet induced obesity in Wistar rats.

Meena *et al.* (2019) considered that cow urine is a really multidimensional drug. As it is an anti-cancerous, anti-microbial, anti-diabetic,

anti-urolithiatic and anti-psychotic drug and also enhances the immunity of animals and humans.

According to the experiment's findings, broilers supplementing with cow urine distillate from the Ongole and Sahiwal breeds of cows was more effective than using a crossbreed cow, a Holstein Friesian, in reducing oxidative stress and providing a higher antioxidant effect (Sushma *et al. ,* 2021).

2.13. Importance and medicinal properties of *Curcuma amada*

2.13.1. *Curcuma amada :*

A plant or any part of a plant is used to produce medicine to aid in the healing process during illness and disease, and this practise is known as herbal medicine, botanical medicine, or phytomedicine (Samant, 2012).

Natural compounds found in medicinal plants are acceptable for humans and animals. These substances cannot be created in a lab. Several phytoconstituents coming from the categories of flavonoids, alkaloids, glycosides, and many others are present in *Curcuma amada* roxb (Samant, 2012).

Mango ginger, also known as *Curcuma amada* roxb. (Family: Zingiberaceae), is a perennial rhizomatic aromatic herb that is available from November to April. The rare spice mango ginger (*Curcuma amada* roxb.) morphologically resembles ginger but tastes like raw mango. The genus is extensively distributed throughout the tropics of Asia, Africa, and Australia, having its origins in the Indo-Malayan region (Samant, 2012).

Samant, (2012) also concluded that Curcumin, demethoxy curcumin, and bis-demethoxy curcumin are the key elements in *C. amada's* acetone extract. A-pinene, a-and b-curcumene, camphor, cuminyl alcohol, myristic acid, and turmerone are all present in the essential oil. The rhizome's distinctive mango odour is a result of the contributions of car-3-ene and cis-ocimene. Rhizomes produce 1% essential oil, which is composed of 18.% d-a-pinene, 47.2% ocimene, 11.2% linalool, 9.1% linalyl acetate, and 9.3% safrole. The free acids in mango

ginger are as follows: gallic (10%, 75 mg/g), cinnamic (7%, 52.5 mg/g), protocatechuic (7%, 52.5 mg/g), gentisic (24%, 180 mg/g), and ferulic (20%, 150 mg/g). Syringic (4%, 30 mg/g) and p-coumaric acids (2%, 15 mg/g) are also present in tiny levels *C.amada Roxb*. The rhizome contains 45% starch, and reports indicate it additionally possesses beneficial properties.

Tumbare *et al.* (2021) conducted a study on the "Efficacy of Curcumin Against Subclinical Mastitis in Goats" in the vicinity of Parbhani. The research aimed to document the prevalence of subclinical mastitis and assess the effectiveness of curcumin in treating this condition in goats.In the study, three groups, each comprising 10 animals, were administered different treatments: Group 1 received oral curcumin at a dosage of 2 mg/kg/body weight once daily (OD) for 7 days; Group 2 was treated with a herbal paste containing curcumin (100 mg) and gum acacia applied topically OD for 7 days; Group 3 received a combination treatment of a herbal paste containing curcumin and gum acacia applied topically OD, along with oral curcumin at a dosage of 2 mg/kg/body weight OD for 7 days.The comparative efficacy of the treatments revealed that Group 1 achieved an efficacy of 80%, Group 2 achieved 50%, and Group 3 achieved 90%. Notably, Group 3, which received the combination treatment of oral curcumin and topical application of the herbal paste containing curcumin and gum acacia, exhibited the highest efficacy among the three treatment groups.

2.13.2. *Curcuma amada* Medicinal Properties

Mujumdar *et al.* (2000) revealed anti-inflammatory activity of *Curcuma amada* roxb. in albino rats. The *Curcuma amada* extract has anti-inflammatory properties have been examined in albino rats using the use of the chronic granuloma pouch model and acute carrageenan paw oedema model. The extract demonstrated dose-dependent anti-inflammatory action in both acute and chronic model, as well as being composed of chemical compounds with hydroxyl, ester, carbonyl, and olefin functionalities. After both acute and chronic administration to albino rats, the rhizome extract of *Curcuma amada* indicated anti-inflammatory properties.

Mujumdar *et al.* (2004) *Curcuma amada* rhizome ethanol extract was shown to have anti-inflammatory properties. Based on these results, pharmacological studies were carried out using the following techniques on albino mice: tail-flick test, barbiturate sleeping period, acetic acid-induced writhing, and exploratory activity. This percentage demonstrated CNS depressing effects by reducing exploratory activity and increasing barbiturate sleeping time. Additionally, it demonstrated a decrease in the tail-flick response, writhings caused by acetic acid, and inflammation induced by carrageenan, suggesting possible antinociceptive and antiphlogistic activity, respectively.

Policegoudra *et al.* (2007) concluded that gram-negative and gram-positive bacteria were highly susceptible to the antibacterial effects of mango-ginger preparations and extracted difurocumenonol.

Policegoudra *et al.* (2010) revealed that, with potential bactericidal action against a number of bacteria, *Curcuma amada* shows a broad spectrum of antibacterial activity. In addition, the purified substance exhibited cytotoxicity, antioxidant activity, and platelet aggregation inhibitory activity.

Policegoudra *et al.* (2011) studied that mango ginger extracts also shown possible cytotoxicity and inhibitory effects on platelet aggregation. Mango ginger extracts' significant levels of antioxidant activity, cytotoxicity, and platelet aggregation inhibitory effect are associated with their phenolic content and other bioactive compounds. Mango ginger extracts can be utilised as a natural source of phenolic and terpenoid components because these bioactive compounds are well-known for their health-promoting qualities. Mango ginger extracts have the potential to be used in the production of culinary and nutraceutical products because they have antioxidant and platelet aggregation inhibitory properties.

Hypotriglyceridemic, hypoglycemic, and anti-hyperglycemic, antioxidant, anthelmintic, antiallergic, anti-inflammatory, platelet aggregation inhibitory and cytotoxic these are the characteristics of *Curcuma amada* (Samant, 2012)

Upadhyay *et al.* (2013) evaluated antitumor activity of *Curcuma amada* Roxb rhizome. *Curcuma amada* roxb. rhizome extracts, both aqueous and

alcoholic, have been examined for their antitumor properties in Swiss albino mice.The brine shrimp bioassay method was used to validate in-vitro cytotoxicity investigations. After being treated with both extracts in the Ehrlich Ascites Carcinoma (EAC) model, the tumor-induced mice showed a marked increase in life span and a decrease in the number of cancer cells and weight of the tumour. The ethanolic and aqueous extracts in the tumour improved the haematological parameters.It was discovered that *Curcuma amada* roxb. had a very strong alcoholic extract in an in vivo model of cancer. The tumor-induced alterations in the measured parameter were significantly reversed by the plant extract. These findings point to the potential antitumor effects of alcoholic and watery extracts of *Curcuma amada* rhizomes in ehrlich ascites carcinoma.

Hossain *et al.* (2015) revealed that *Curcuma amada* has analgesic properties and is used in Ayurvedic, Unani, and folk medicines for the treatment of rheumatic disorders, as well as having pharmacological action.

Sharma *et al.* (2015) *Curcuma amada* roxb.(Rhizomes) promotes wound healing process. Indian traditional medicine uses *Curcuma amada* roxb, also known as mango ginger (Zingiberaceae), a rhizomatous aromatic herb, for healing wounds. This study provides a scientific assessment of the various *Curcuma amada* rhizome extracts' capacity to heal wounds. In an excision wound model, topical administration of *Curcuma amada* rhizome ethanolic extract ointment (10%) decreased the epithelialization time and histological alterations while increasing the percentage of wound contraction (99%) compared to the control group (90.%). *Curcuma amada* rhizomes 10% ethanolic extract greatly assisted in wound healing, suggesting the plant's traditional therapeutic uses.

Pandve, E. R. (2015) conducted clinical investigation and it can be deduced that the 2.5% ethanol leaves extract of Bryophyllum pinnatum proves to be a more efficacious formulation for topical application in wound healing compared to both the 2.5% ethanol rhizome extract of Curcuma amada and the control group. Moreover, the Bryophyllum pinnatum extract demonstrates a range of beneficial properties, including antiseptic, antibacterial, anti-inflammatory,

astringent, hematenic, and immunomodulatory effects when incorporated into 2.5% ethanolic extract ointments.

Palanisamy and Samiappan (2017) evaluated that the chemotherapeutic and antioxidant properties of *Curcuma amada* rhizome extract on Sprague Dawley rats that were induced to develop cervical cancer using benzo(a)pyrene (BaP). Treatment with the standard the medicine cisplatin and the rhizome extract of *C.amada* restored the tissue enzymes, serum indicators, and antioxidants. Concluded that the oxidative stress caused by BaP in rats' cervical carcinogenicity was reduced by the *C. amada* rhizome extract.

Raj *et al.* (2018) conducted a experimental study on anxiolytic activity and locomotor behavior of *Curcuma amada* rhizomes using wistar Albino rats.Mango ginger, also known as *Curcuma amada* Roxb. (CA), is a rhizomatous aromatic herb which is used in this country for its culinary and medicinal uses. Three groups of wistar albino rats were used: the control group (distilled water with 0.1% CMC), the standard group (Diazepam, 1 mg/kg), and the test group (Ethanolic Extract of *Curcuma amada* Rhizome, 250 mg/kg). They had given medicines orally for ten days, during which time their anxiolytic action was monitored.*Curcuma amada* (250 mg/kg) exhibited statistically significant reductions in locomotor behaviour and an increase in time spent in light arena.*Curcuma amada* has significant CNS depressive and anxiolytic properties.

Mitra *et al.* (2020) revealed the *Curcuma amada* rhizome's most effective fraction for its aqueous-methanol extract's antidiabetic and antioxidant properties was the major purpose of this experiment. For four weeks, the streptozotocin-induced diabetes groups administered fractions of the aqueous-methanol extract (n-hexane, chloroform, ethyl-acetate, or n-butanol) at a dose of 10 mg/100 g body weight/day. Following the fraction treatment, the ethyl-acetate fraction revealed the most significant recovery in the majority of sensors, including the glycemic, enzymatic, genomic, and β cell populations, as well as the pancreatic islet diameter and toxicity level. The ethyl-acetate fraction of *C.amada* was the most powerful solvent in these investigation, it can be concluded.

Maria *et al.* (2023) revealed that ethanolic extract of *Curcuma amada* possesses significant antimicrobial activity against Streptococcus mutans, it can be concluded.

2.14. Medicinal Plant Extract combination with Cow Urine

Jarald *et al.* (2008) studies were conducted on alloxan-induced diabetic rats at two dose levels (200 and 400 mg/kg) to determine the antidiabetic efficacy of cow urine and herbal preparations. The investigation was conducted over a 21-day period. Insulin (1 unit/kg i.p.) and control were used as reference standards against which the activity was measured. In a dose-dependent manner, herbal treatments substantially lowered the blood sugar level of diabetic rats whereas the mixture made with cow urine showed greater activity than the one prepared with water. The potential of cow urine to extract more active ingredients from herbal preparations and hence enhance antidiabetic activity makes it an excellent extraction solvent.

Rajapandiyan *et al.* (2011) studied the antibacterial activity of *Azadirachta indica* cow urine extract was assessed against MDR clinical isolates. The findings showed that *A. indica* cow urine extract has more antibacterial activity against MDR E. coli and Klebseilla pneumonia than its organic fraction. According to the results of the phytochemical test, the constituents for each of the 20 days tested positive for quinine, coumarin, tannin, saponin, flavonoids, and alkaloids. The chemical components of *Azadirachta indica* cow urine extract and organic extracts were associated with antimicrobial activity. Neem-cow urine extract has antibacterial properties because it contains a variety of complex components. Thus, the key elements of the *Azadirachta indica* cow urine extract's antibacterial action include its diversity of main and minor ingredients as well as its synergistic effects.

Killari *et al.* (2019) revealed that distilled cow urine appears to possess a capacity to enhance the bioavailability of pharmaceuticals. Thus, the purpose of this study is to assess the antioxidant and anti-inflammatory properties of wheat

grass powder enhanced with distillate of cow urine. Wistar rats with paw edoema caused by carrageenan were used to test the anti-inflammatory effects of wheat grass powder supplemented with cow urine distillate at doses of 100, 200, and 400 mg/kg. The ferric thiocyanate method was employed to assess the antioxidant potential of the wheat grass powder. The results of this study indicate that including cow urine distillate to wheat grass powder increased its antioxidant and anti-inflammatory properties.

Virshette (2020) conducted a study to evaluate the in vitro antibacterial efficacy of *Azadirachta indica* leaf extracts in various solvents derived from cow urine against Escherichia coli and Pasturella multocida, employing the agar well diffusion method. The study also included a comparison with ciprofloxacin (30mcg). Twelve Deoni cows were divided into two groups: pregnant and non-pregnant, each consisting of six cows. The results indicated that the extracts of A. indica dissolved in different cow urine solvents exhibited superior antibacterial activity against E. coli compared to P. multocida. Particularly, the extraction process involving cow urine distillate and photoactivated urine demonstrated enhanced antibacterial activity against both E. coli and P. multocida compared to cow urine distillate and photoactivated urine alone.

2.15. Complete blood count (CBC)

Singh *et al.* (2014) observed that subclinical mastitis affected cattle exhibited a mean PCV value of 25.67 ± 0.63, TLC of 9.20 ± 0.16, and monocyte count of 1.33 ± 0.19, whereas healthy cattle showed values of 28.12 ± 0.48, 6.87 ± 0.12, and 1.40 ± 0.02, respectively.

Siddiqe *et al.* (2015) discovered a decrease in Hb levels in subclinical mastitis cattle (8.30 ± 0.4864) compared to healthy cattle (8.92 ± 0.687). The difference in basophils between healthy and subclinical mastitis-affected cattle was not significant (0.2143 ± 0.425 and 0.4286 ± 0.534, respectively).

Krishnappa *et al.* (2016) reported that subclinical mastitis-affected animals displayed mean values of Hb, TEC, TLC, neutrophils, and lymphocytes

as 8.96 ± 0.15, 6.87 ± 0.05, 10.61 ± 0.15, 65.60 ± 0.26, and 30.56 ± 0.29, respectively, while healthy animals exhibited values of 10.45 ± 0.30, 7.22 ± 0.17, 6.82 ± 0.23, 35.89 ± 0.78, and 60.43 ± 0.65, respectively.

Das *et al.* (2018) found that cows with subclinical mastitis exhibited a significant ($P<0.05$) decrease in Hb (9.46 ± 0.13), TEC (6.69 ± 0.08), PCV (26.53 ± 0.68), and lymphocyte count (33.78 ± 0.81) compared to healthy cows (Hb: 10.89 ± 0.21, TEC: 7.16 ± 0.12, PCV: 33.40 ± 0.84). Additionally, the mean values of TLC and neutrophils were significantly ($P<0.05$) higher in subclinical mastitis cases (TLC: 11.28 ± 0.28, neutrophils: 63.02 ± 0.82) compared to healthy animals (TLC: 6.84 ± 0.41, neutrophils: 36.6 ± 0.84). The mean value of monocytes did not show a significant difference between healthy and subclinical mastitis-affected animals (3.20 ± 0.28 and 3.28 ± 0.16, respectively).

Tripathy *et al.* (2018) revealed a significant ($P<0.05$) decrease in Hb (8.8 ± 0.11), TEC (4.66 ± 0.06), PCV (27.30 ± 0.36), and lymphocyte count (44.31 ± 0.66), along with significant ($P<0.05$) increases in TLC (10670 ± 153.6), neutrophils (50.96 ± 0.59), and eosinophils (3.20 ± 0.24) in mastitis-affected cows compared to healthy ones (Hb: 10.65 ± 0.27, TEC: 5.53 ± 0.12, PCV: 32.60 ± 0.84, lymphocytes: 61.70 ± 1.52, TLC: 6960 ± 177.8, neutrophils: 35.10 ± 1.47, eosinophils: 1.70 ± 0.21). Additionally, the mean value of MCHC did not show a significant difference between healthy and affected cattle (32.68 ± 0.05 and 32.35 ± 0.08, respectively).

Choudhary *et al.* (2019) was conducted a study to determine the effect of subclinical mastitis (SCM) on hematological parameters and serum mineral profile of indigenous cattle. Hematology revealed significantly higher average values of TLC in subclinically (9.20 x 10^3/µl) infected animals than healthy animals (6.87 x 10^3/µl). Differential leukocyte counts revealed neutrophilia and lymphopenia. The changes in hematological and mineral constituents were important indicators of the physiological or pathological state of the animal infected with subclinical mastitis.

Rathaur *et al.* (2020) reported that the parameters of hematological in mastitis-suffering cows were compared with healthy cows. There was a significant increase ($p<0.01$) in TLC in mastitis-suffering cows compared with healthy cows and a significant decrease ($p<0.01$) in HB, TEC, PCV, and MCV. Different types of leukocyte count (DLC) such as Neutrophil, Eosinophil, and Monocyte significantly ($p<0.01$) increased in mastitis-suffering cows compared with healthy cows.

Dhakal and Pokhrel (2022) observed a significant ($P<0.05$) increase in total leukocyte count in subclinical mastitis-affected animals compared to healthy ones, with values of 15.74 ± 1.08 and 11.23 ± 1.66, respectively. The mean value of neutrophils also increased significantly ($P<0.05$) in subclinical mastitis (53.11 ± 4.56) compared to healthy animals (47.22 ± 2.34).

Yadav *et al.* (2022) revealed that blood samples of cows were taken for hematological studies. There was a 16.29% animal-wise prevalence of subclinical mastitis in dairy cattle of Bikaner. A significant increase ($p<0.05$) in neutrophils and a significant decrease ($p<0.05$) in hemoglobin, total erythrocyte count, and packed cell volume were observed. No significant differences were observed in monocyte, lymphocyte, and total leukocyte count in cows with mastitis groups.

Lakshmi *et al.* (2024) Cows affected by subclinical mastitis showed lower mean values for haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), and higher total leukocyte count (TLC) compared to the control group. These findings were also in accordance with earlier reports where significant reductions in RBC, Hb, and PCV values leading to anaemia in animals affected with mastitis were reported (Zaki *et al. ,* 2010; Das *et al. ,* 2018; Saleem *et al. ,* 2021).

CHAPTER-III

MATERIAL AND METHODS

The present study entitled "Effect of cow urine distillate fortified with *Curcuma amada* extract therapy on subclinical mastitis in cows" was undertaken to evaluate the physicochemical, biochemical, haematological and Enzymatic parameters in cows positive for subclinical mastitis. The present work was carried out at the Department of Veterinary Biochemistry, PGIVAS Akola and Adarsh Goseva Evam Anusandhan Prakalp Mhaispur, Dist.Akola and Dr.Panjabrao Deshmukh Krishi Vidyapeeth Dairy Farm, Akola during 2023-24.

This chapter deals with the description of methods and procedures used to observe the effect of *Curcuma amada* fortified with cow urine distillate.

The present research program was carried out under the following subheads

Location of study

The research was conducted in the Amravati division of Maharashtra state, specifically in Akola, which is administered by the Akola Municipal Corporation. Akola shares borders with Amravati district to the north and east, Buldhana district to the west, and Washim district to the south. The city is situated along the watershed of the Purna River, which includes the Shahanoor River passing through the upper north part of Akola. Akola is located at a latitude of 20.7°N and a longitude of 77.09°E. The city's elevation varies between 925 feet (287 meters) and 1036 feet (316 meters) above sea level.

Geography and Climate

Akola's geographic coordinates are latitude 20.7°N and longitude 77.09°E, with elevations ranging from 925 feet (287 meters) to 1036 feet (316 meters) above sea level. The climate of Akola is characterized as tropical savanna, with a humid subtropical climate in its vicinity. The city's local weather station serves as

the national weather station. Annual temperatures in Akola fluctuate between a high of 47.6°C (117.68°F) and a low of 2.2°C (35.96°F). Due to its proximity to the Tropic of Cancer, Akola experiences intense summer heatwaves, particularly in May. The city receives an average of 800 millimeters (31 inches) of rainfall annually, with the majority occurring during the monsoon season from June to September, although some precipitation

Approval of IEC-VCR

Before the start of the experiment, the experimental protocol was prior approved by the Institutional Ethics Committee for Veterinary Clinical Research (IEC-VCR) of Post Graduate Institute of Veterinary & Animal Sciences, Akola-vide IEC-VC Resolution number **IEC-VCR (2) 2024 (08).**

Procurement of Materials

3.1 Material

3.1.1. *Curcuma amada*

Curcuma amada were procured from the local market and was identified by botanists. Prof. Dr.S.P. Rothe, Ex-principal, MB Science and Commerce college Akola.

3.1.2. Cow urine Distillate: Cow urine was procured from Adarsh Goseva Evam Anusandhan Prakalp, Mhaispur, Dist. Akola. The Cow urine distillate was prepared by using distillation unit available in the Department of Veterinary Biochemistry, PGIVAS Akola.

3.1.3. Procurement of biochemical reagents

CMT reagent, and CMT Paddle were procured from Akansha chemicals, Akola.

3.1.4. Glassware and plasticware

Sterile 50 ml milk sample bottles, icepacks, , CMT paddle, hand gloves, teat dips, clean towels, syringe and needles, EDTA vials, cotton swabs, container, beaker, conical flasks, spatula, pipette (10, 100 and 1000 microlitre) , clean glass

slides, cover slips, diamond marker and permanent marker pen oil immersion, microscope,

3.1.5. Chemicals and Reagents

CMT reagent, ethanol, glacial acetic acid, acetone, methylene blue, basic fuschin stain

3.1.6. Selection of Cows

After screening for CMT and having SCC between 2.5 to 5.0×10^5 cells/ml from cattle farm, total 30 cattles (mid lactating cattle and were aged between 4-7 years and body weight about 300-400 kgs approx.) in the mid-lactation period were selected randomly for experiment.

3.2 Methods

3.2.1 Preparation of *Curcuma amada* aquous extract

The rhizome part of the *Curcuma amada plant was* procured from the local market and had been identified by botanists. The aquous extract of *Curcuma amada* was prepared as per the method of Upadhyay *et al.,* (2013) with some modifications.

For aquous extraction, a dried coarse powder was transferred to a round bottom flask, which was then filled with 1.5 to 2 litres of distilled water and soaked for 24 hours. Then it was boiled for 4-5 hours. Filter it by clean cloth so aquous extract is allow for drying resulting in dried course powder formed.

3.2.2 Preparation of *C.amada* fortified cow urine distillate:

The *Curcuma amada* aquous extract obtained dried extract was mixed with cow urine distillate 3-4 times as per the method of Killari *et al. ,* (2019).

3.2.3. Dose calculation of Cow Urine distillate

Animal equivalent Dose = km x mg/kg (Nair & Jacob 2016)

Cow Equivalent Dose = Rat dose x km (rat) ÷ km (cattle)

CED = Cow Equivalent Dose

BSA = Body Surface Area

BW = Body Weight

$BSA = 0.09 \times W^{0.67}$ (Berman, 2003)

Therefore for body weight (W) 350 kg of dairy cattle,

$BSA = 0.09 \times 350^{0.67}$

$= 4.5\ m^2$

Km factor for Cattle calculation are as follows:

Km = Body weight of cattle ÷ Body Surface Area of Cattle

$= 350\ kg \div 4.5\ m^2$

$=77.77$

$= 78$

Km factor for cattle is 78

Dose calculation of Cow Urine distillate (CUD) by the method of Nair and Jacob, 2016)

For, Cow urine distillate,

CUD dose in rat is 2ml (Wate *et al.* , (2012)

Rat km factor = 6 (Nair and Jacob, 2016)

The average body weight of mid-lactating cattle is **350kg.**

Cow Equivalent Dose of Cow Urine Distillate = Rat dose x km (rat) ÷ km (cattle)

= 2 x 6 ÷ 78

(CUD) = 0.15 ml/kg Bwt.

Therefore, Cow Urine Distillate (CUD) dose in cattle is 0.15ml/kg Bwt.

3.2.4. Dose calculation of *Curcuma amada*

The dose of *Curcuma amada* was extrapolated from rats to animals (Cattle) and the evaluated dose was used in the study **(Jarald *et al.* , 2008; Raj *et al.* , 2018).**

Dose calculation of *Curcuma amada* (Jarald *et al.* , 2008; Raj *et al.* , 2018)

For *Curcuma amada* ,

C.amada dose in rat is 250mg/kg

So, **Cow Equivalent Dose of *C.amada* = Rat dose** x **km (rat) km (cattle)**

= 250 x 6 ÷ 78

= 19.2mg/kg

= 20mg/kg Bwt.

Therefore, *C.amada* dose in cattle is 20mg/kg Bwt.

Zederone is the active principle for analgesic activity of *Curcuma amada.* The median lethal dose (LD50) of zederone at 24 hours is approximately 223 mg/kg body weight in mice (Hossain et al., 2015).

3.2.5. Testing of cows for subclinical mastitis

DIAGNOSIS OF SUBCLINICAL MASATITIS

a) Califonia Mastitis Test (CMT) CMT Reagent and CMT Paddle were procured from the Aakansha Chemicals,Akola.

- The CMT was conducted in the milking shed at the start of each cattle on 0^{th} 3^{rd},7^{th}, 14^{th} and 28^{th} days of the experiment.
- The test was conducted as per the standard procedures by Schalm and Noorlander (1957).

The description is represented in the table 3.1

Table 3.1 Description of the visible reaction interpretation during the modified California mastitis Test

Sr. No.	Precipitation/gel formation	Colour	Interpretation
1	No thickening of the mixture	Grey	Normal milk
2	Slight thickening of the mixture. Thickening was disappeared after rotating the paddle for 10 seconds.	Light Purple	Weak Positive
3	Distinct thickening of mixture. Thickening was disappeared after rotating the paddle for 20 seconds.	Purple	Distinct Positive
4	Gel is formed and the surface of the mixture becomes elevated. Central peaks remain projected even after the CMT paddle rotation was stop	Dark Purple	Strong Positive

Plate 3.1. Collection of milk sample in CMT paddle by hand milking method

Plate 3.2. Screening of cows for subclinical mastitis through CMT

b) Milk pH

The milk pH of each milk sample was recorded with a digital pH meter on days 0^{th} 3^{rd},7^{th}, 14^{th} and 28^{th} days.

c) Somatic Cell Count (SCC)

Preparation of stain

The stain was prepared with the method given by Duttschaever and Legggatt, (1965) given as follows:

1. 0.6 g of methylene blue (Bacto-Methylene Blue certified) was mixed in 52 ml of 95% ethanol and 44 ml of tetrachloroethane.
2. It was allowed to stand in the water bath at 45°C with occasional agitation until the methylene blue was completely dissolved.
3. It was cooled.
4. 4 ml of glacial acetic acid was added and filtered.
5. 2 ml of saturated alcoholic solution of basic fuchsin (1 g in 15 ml of 95% ethanol) was added.

Technique

The SCC was determined as described by Duttschaever and Legggatt, (1965) and Dhakal, (2006). A square of 1 cm^2 was prepared on a glass slide with the diamond marker. Milk was mixed thoroughly before testing.

Preparation of milk smears

The milk sample was thoroughly mixed, to obtain uniform distribution of cells. Ten micro liters of milk (0.01ml) from each sample bottle was spread over a 1 cm^2 marked area on a grease-free glass slide with the help of a platinum loop. The smears on the slides were left undisturbed at room temperature, air-dried, and preserved till staining was done.

Staining

- The slide was immersed in acetone for 2 minutes; for reconstituted milk, it was immersed for one minute.
- Acetone was allowed to evaporate to dryness
- The stain was applied for 2 minutes, and the stained smears were dried at 35-37°C within the same timeframe.
- It was important not to dry for too long; otherwise, smears might crack, and excess methylene blue could not be removed.
- Washing was done with gentle agitation in warm water until excess methylene blue was removed.
- The samples were dried and examined.

Somatic cells were counted under 100 magnification using oil immersion. Somatic cells were purple with blue back ground and were counted as follows:

Cell counting

Cell counting was made by examining the smears under oil immersion objective as per the procedure used by Sukumar *et al,* (2019). The cells were counted in 25 fields. The total number of cells counted was multiplied with the working factor to obtain the number of cells per ml of milk and calculated as follows:

The SCC/ml = Total no. of cells counted in 25 fields × WF

Calculation of the working factor (WF) of the microscope

A binocular microscope was used with 10x oculars and a 1.8 mm oil immersion objective. The diameter of the field was measured with the help of a stage micrometer.

Diameter of microscopic field = 0.16 mm or 0.016 cm

Area of the field = πr^2

$= 3.14 \times (0.008)^2$

$= 0.0002$ sq. cm

Plate 3.3. Milk sample examined for colour and consistency parameters

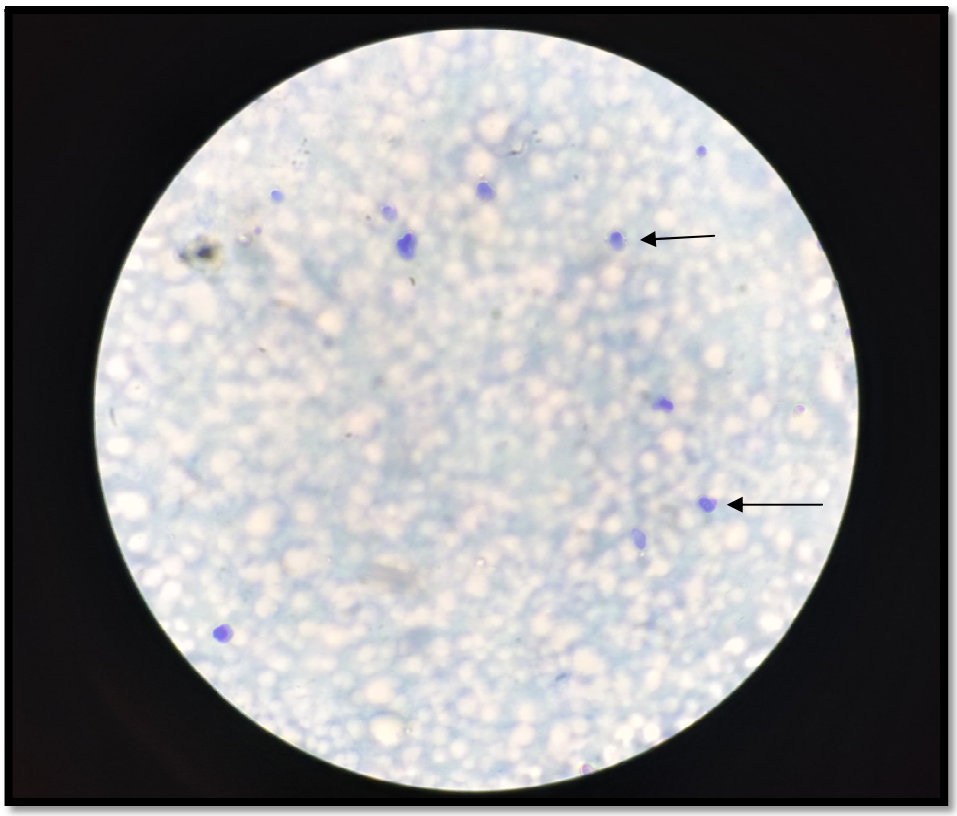


Plate 3.4 Somatic cells observed under microscope

Since 0.01 ml of milk was spared in 1.0 cm^2, the possible number of fields counted in 1 sq. cm is 5000.

Milk volume represented by each field = 1/5000 × 1/100

= 1/500000 ml.

Hence, microscopic factor (MF) = 5, 00,000

Working factor (WF) = MF/ No. of fields counted

= 500000/25

Working factor = 20000

3.2.6. Design of Experiment

Details of Experimental treatment

The mid lactating cows were selected randomly and divided into five treatment groups of 6 cows in each group as represented in table 3.2. Group T_0 served as a control group of healthy animals. In Group T_1 animals were received cow urine distillate @0.15ml/kg b.w.t diluted in water by oral administration (post-milking), Group T_2 animals were received *Curcuma amada* aquous extract @20mg/kg b.w.t diluted in water by oral administration (post-milking), while in Group T_3 received Fortified or combined with *Curcuma amada* aquous extract (@20mg/kg b.w.t), cow urine distillate (@0.15ml/kg b.w.t) diluted in water by oral administration (post-milking) and Group T_4 received standard treatment of morbofloxacin @8mg/kg BW intramuscular single dose (post-milking).

Table 3.2 Details of experimental treatment

Sr. No.	Group	Treatment provided to Cows	Number of Cows
1	**T_0**	Normal or healthy cows kept as **Control**	**6**
2	**T_1**	treated with Cow urine distillate @0.15ml/kg b.w.t diluted in water by oral administration (post-milking)	**6**
3	**T_2**	treated with *Curcuma amada* aquous extract @20mg/kg b.w.t diluted in water by oral administration (post-milking)	**6**
4	**T_3**	treated with fortified or combined with *Curcuma amada* aquous extract (@20mg/kg b.w.t) **combined** with cow urine distillate (@0.15ml/kg b.w.t) diluted in water by oral administration (post-milking)	**6**
5	**T_4**	treated with morbofloxacin @8mg/kg BW IM single dose (post-milking).	**6**
Total number of cows			**30**

Collection of milk samples

The mid-lactating cows were selected and milk samples were collected from each of the quarters, all of the selected cows by hand milking method in the morning from each animal of the control and treated group on days 0^{th}, 3^{rd},7^{th}, 14^{th} and 28^{th} respectively at the time of milking. The first 3-4 streams of milk were discarded. Milk samples were collected in sterilized, dry plastic bottles with a volume of 50 ml to study somatic cell count, total viable count, and

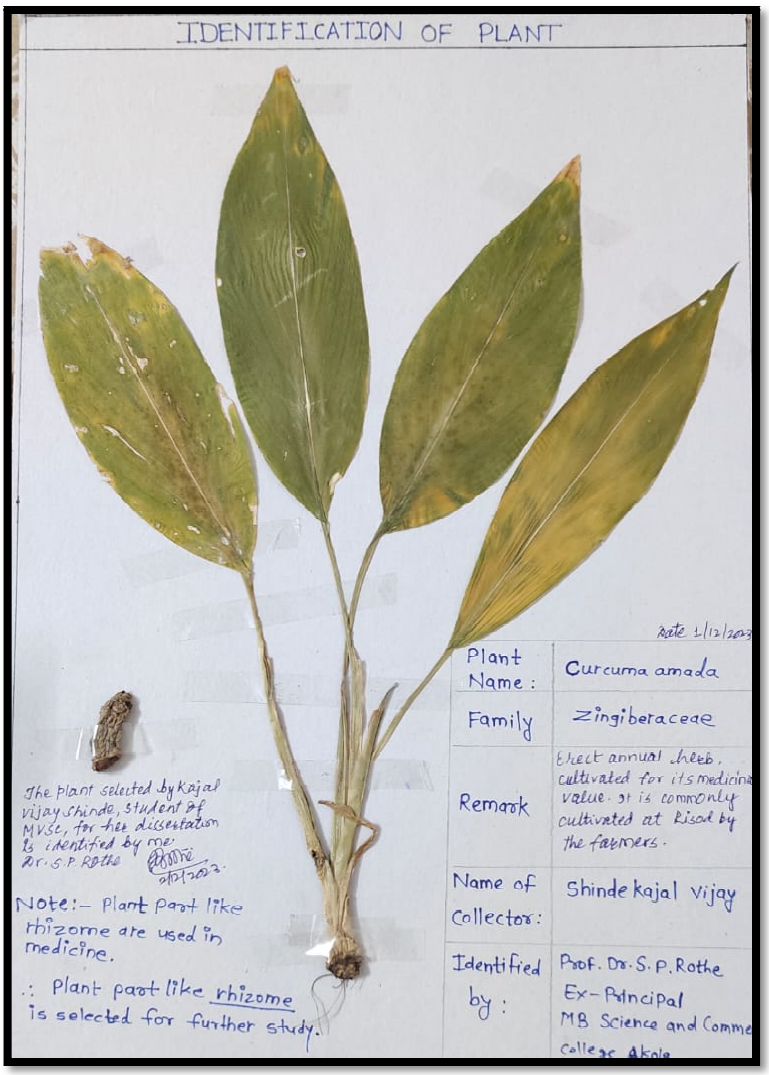


Plate 3.5 Herbarium sheet of *Curcuma amada* plant identified by botanist.

Plate 3.6. *Curcuma amada* aquous extract in dried form

milk composition. The name or number of cows from which the sample was collected was labelled on bottle. All milk samples were brought to the laboratory on ice packs for further analysis.

3.2.7. Physico-chemical parameters

Milk samples collected on days 0^{th}, 3^{rd}, 7^{th}, 14^{th} and 28^{th} were subjected to analysis of milk physico-chemical parameters such as colour, consistency, viscosity, fat and density.

3.2.7.1. Colour

Colour of milk recorded by Visual Examination

The colour of the milk was observed as white or light yellow, and it was recorded whether the colour of the milk was normal or abnormal.

3.2.7.2. Consistency

Consistency of milk recorded by white tile method.

The consistency of the milk was recorded as normal, watery, thick, ropy, or slimy.

3.2.7.3. Viscosity (Centipoise)

Viscosity of milk is determined by method (Mann *et al.,* 2013)

Requirements: Ostwald's viscometer, measuring pipette, suction bulb ,milk sample.

Procedure:

1. The viscometer was thoroughly cleaned by washing successively with dilute alkali solution, tap water or chromic acid, tap water, and distilled water.

2. It was then rinsed with acetone (or ethanol or a combination) and dried by suction, cooling, and blowing with dust-free air.

3. A known quantity of milk was transferred into the wider limb of the viscometer using a pipette.

4. The viscometer with milk was placed in a thermostatically controlled water bath maintained within ± 1°C.

5. After allowing for temperature equilibrium (for about 30 minutes), the milk was drawn by applying suction to the capillary limb, attaching a rubber tube to the tip of the limb above the uppermost mark.

6. The milk was allowed to flow down under its own pressure.

7. The flow time was measured by recording the time of flow between the two marks of the capillary limb using a stopwatch with an accuracy to read to 0.1 second.

8. The procedure for measuring the flow time was repeated 5-6 times while the viscometer was dipped in the water bath at the fixed temperature, and the average flow time was recorded.

9. The viscometer was emptied, and the milk residue was washed away by forcing tap water through it. Then, it was cleaned and dried as described in steps (1) and (2) above.

10. The same volume of freshly boiled and cooled distilled water as that of milk under step (3) was transferred into the viscometer.

11. The average flow time for water was determined after temperature equilibrium, as in the case of milk.

12. The density of milk relative to the distilled water used, both tempered to the experimental temperature, was measured.

Calculations: Average time of flow for the milk = t1 seconds Average time of flow for water = t2 seconds Weight of empty pycnometer = a grams Weight of pycnometer + water = b grams Weight of pycnometer + milk = c grams Mass of distilled water having volume V of the pycnometer = (b - a) grams Mass of milk having volume V of the pycnometer = (c - a) grams Relative viscosity of the milk = S Absolute viscosity of milk = Relative viscosity of milk × viscosity of water at experimental temperature.

3.2.7.4. Fat (%)

Milk fat determined by Gerber's method described by (Kleyn *et al. ,* 2001)

10.75 ml Milk is weighed into Gerber butyrometer containing 10ml $H_2SO_4$and 1ml Isoamyl alcohol is added and butyrometer contents are vigorously mixed to dissolve curd and release fat. Fat is isolated by centrifuged at 1100 rpm for 4mins and quantified in the graduated portion of the Gerber butyrometer.

3.2.7.5. Density (g/ml)

Milk density was determined by Lactometer method by (Mann *et al. ,* 2013).

Using a lactometer, the density or specific gravity of milk could also be determined. The lactometer was a specialized hydrometer calibrated within the range of 1.020 to 1.035 (or 20 to 35 as lactometer reading). After obtaining the lactometer reading at the calibrated temperature or the corrected lactometer reading at the milk temperature after applying the correction factor, the specific gravity of milk could be calculated using the formula: Specific gravity = 1 + CLR/1000.

The measurement of specific gravity or density of milk by a lactometer was based on the Archimedes principle, wherein a floating object sinks until it has

displaced a weight of fluid equal to its own weight. The greater the volume of displaced fluid, the lower the density of the fluid, resulting in a lower lactometer reading.

3.2.8. Milk Biochemical Parameters

3.2.8.1. Preparation of Whey

Whey was prepared by adding 100mg citric acid to 10ml of milk sample and centrifuged at 3000 rpm for 10 min. The whey was collected without disturbing uppermost fat layer and whey samples were preserved in the refrigerator at 4 $^{\circ}$C for further biochemical estimations.

3.2.8.2. Estimation of Total Protein (gm/dl)

The total protein in milk whey estimated by Biuret method described by Johnson and Swanson (1952). On days 0^{th}, 3^{rd},7^{th}, 14^{th} and 28^{th}

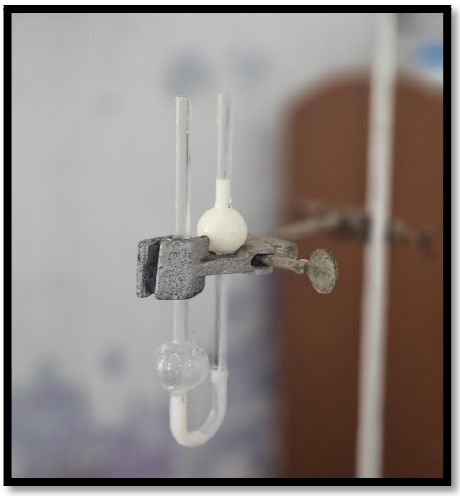
Total protein Method: Biuret

Priciple : The peptide bonds of protein react with copper ions in alkaline solution to form a blue-violet colored complex, each copper ion complexing with 5 or 6 peptide bonds. Tartarate is added as a stabaliser whilst iodide is used to prevent autoreduction of the alkaline copper complex. The color formed is proportional to the protein concentration and is measured at 546nm (520-560).

Reagent composition in test :

Active ingredients	Concentration
Copper Sulphate	19mmol/l
Potassium Sodium Tartarate	43mmol/l
Potassium Iodide	30mmol/l
Sodium Hydroxide	600mmol/l

Plate 3.7: (A) Cow urine distillate, (B) *Curcuma amada* with distilled water and (C) Cow urine distillate fortified with *C.amada* used for treatment of cows under experiment trial



3.8.Viscosity of milk determined by Ostwald viscometer

Pate 3.9. Analysis of biochemical Parameters (Total protein, Albumin and LDH enzyme) by using semi-automatic Biochemical analyzer

Reagent preparation : Reagents supplied were ready to use.

Calculations: The results were calculated automatically by the AGD semi-automatic biochemical analyzer instrument.

3.2.8.3. Estimation of Albumin (gm/dl)

The albumin content in milk whey was estimated by the BCG method described by Lieske *et al. ,* (2005). On days 0^{th} 3^{rd},7^{th}, 14^{th} and 28^{th} Albumin Method: BCG (Bromocresol green method)

Principle: Albumin binds with bromocresol green (BCG) at pH 4.2 causing a causing a shift in absorbance of the yellow BCG dye. The blue-green color formed is 28 proportional to the concentration of albumin, when measured photometrically between 580-630nm with max. absorbance at 625nm.

Reagent composition in test :

Active ingredients	Concentration
Bromocresol green	0.08 mmol/l
Succinate buffer	50 mmol/l
Sodium azide	1 gm/l

Reagent preparation : Reagents supplied were ready to use.

Calculations : The results were calculated automatically by the AGD semi-automatic biochemical analyzer instrument.

3.2.8.4..Estimation of LDH Activity (U/ml)

The determination of lactate dehydrogenase (LDH) activity in milk whey as described by Larsen (2005). On days 0^{th}, 3^{rd},7^{th}, 14^{th} and 28^{th}.

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Method

PRINCIPLE

LDH catalyses the transformation of pyruvate is Lactate in Tris buffer with NaCl in the NADH coenzyme. The transformation of NADH to NAD is accompanied by a decrease absorbance at 340 nm. The change in absorbance correlates with the LDH activity in the serum

Pyruvate + NADH + H^+ LDH Lactate + NAD^+

REAGENT COMPOSITION

Reagent 1

Tris buffer pH 7.5	100 mmol/L
Sodium Pyruvate Stabilizer excipients and surface active agents.	>1.2 mmol/L

Reagent 2

The buffer pH > 9.0	20 mmol/L
NADH Stabilizer, excipients and surface active agents.	>1. mmol/L

Reagent preparation: Reagents supplied were ready to use.

Calculations: The results were calculated automatically by the AGD semi-automatic biochemical analyzer instrument.

3.2.9. Complete blood count (CBC)

The CBC was evaluated or described by Jones and Allison (2007) on the 0th, 3rd, 7th, 14th, and 28th days.

Blood samples were collected from the mid lactating selected cows from the jugular vein after taking all aseptic precautions, 2ml blood were collected using a 16-gauge hypodermic sterile needle from subclinical mastitis positive cows as well as from healthy cows in a sterile ethylene diamine tetra-acetic acid (EDTA) tube. Haematological tests were also performed on the 0^{th}, 3^{rd},7^{th}, 14^{th} and 28^{th} days after treatment from 30 randomly divided cows in different treatment groups with subclinical mastitis. All collected samples were subjected to haematological investigation using an auto haematoanalyzer for the estimation of the following parameters.

Total RBC Count (TEC; $\times 10^6$ µl)

- Total WBC Count (TLC; $\times 10^3$ µl)
- Haemoglobin (Hb; g/dl)
- Packed Cell Volume (PCV; %)
- Granulocytes ($\times 10^3$ µl)
- Lymphocytes ($\times 10^3$ µl)
- Monocytes ($\times 10^3$ µl)

3.2.10. Statistical analysis

The statistical analysis of the data obtained for different parameters like SCC, CBC, milk physiochemical parameters such as (colour, consistency, viscosity, fat and density) and Milk biochemical parameters (Milk pH, Total Protein, Albumin, and LDH activity of milk) was done using one-way ANOVA with interaction described by Snedecor and Cochran (1980). The means were tested for significant differences using Completely randomized design experiment. The data were analysed statistically by Wasp 2.0 software.

CHAPTER - IV

RESULT AND DISCUSSION

The present study entitled “Effect of cow urine distillate fortified with *Curcuma amada* extract therapy on subclinical mastitis in cows” was undertaken to study the effect of cow urine distillate fortified with *Curcuma amada* extract on physiochemical, biochemical and hematological parameters in subclinical mastitis of cows. The study was conducted on a total of thirty non descript SCM cows of Adarsh Gosewa Evam Anusandhan Prakalp, Mhaispur, Akola and on cows at livestock farm of Dr. PDKV, Akola. The observations recorded during the study were tabulated and analyzed using standard statistical procedures, and the results obtained are interpreted and discussed in this chapter.

California mastitis test (CMT)

The present study utilized the California Mastitis Test (CMT) as a primary diagnostic tool for identifying subclinical mastitis. The CMT was conducted on total 52 cows out of which 24 animals were found positive for SCM and 6 were selected as healthy cows. The SCM prevalence was 46 percent of the total cows screened with CMT. For the screening of SCM California Mastitis Test has been widely used due to its simplicity, affordability, rapidity, and reliability, making it particularly suitable for field applications compared to alternative methods. These findings align with prior investigations by Geleta *et al.* (2019), Tresamol *et al.* (2019), Shabaz *et al.* (2020), and Getahun (2021), which also affirmed the high percent of SCM positivity (range 42-54%) by CMT test. Consequently, among available indirect tests, CMT is recognized as the most immediate and efficient field-based method for detecting subclinical mastitis. The CMT operates by detecting leukocytes in milk, with a positive reaction indicated by the formation of a gel or viscous mass upon contact with sodium laurel sulfate. This surfactant disrupts leukocytes on the milk's surface, causing the release of DNA from their nuclei, which then reacts with the reagent, resulting in precipitation or gel formation. This reaction is most effective at an alkaline pH, explaining why CMT

exhibits a specific positive response in cases of subclinical mastitis, where mastitic milk tends to be alkaline and contains elevated leukocyte levels (Patel, 2023).

Colour

The colour of the milk was observed as white or light yellow, and it was recorded at 0^{th}, 3^{rd}, 7^{th}, 14^{th} and 28^{th} days interval but there non- significant improvement were shown.

whether the colour of the milk was normal or abnormal.

Colour	**Subclinical mastitis positive sample**	**Subclinical mastitis Negative sample**
Yellowish white/ Normal	17	6
White	5	-
Slightly cloudy	2	-
Total	24	6

Factor M= Subclinical mastitis positive samples

Factor N= Subclinical mastitis Negative samples

Factor M Means

M 1	11.500
M 2	2.500
M 3	1.000

Factor N Means

N 1	8.000
N 2	2.000

Chi Square Statistic is not significant

Consistency

The consistency of the milk was recorded as normal, watery, thick, ropy, or slimy.

Factor M= Subclinical mastitis positive samples

Factor N= Subclinical mastitis Negative samples

The consistency of the milk was recorded as normal, watery, thick, ropy, or slimy and it was recorded at 0^{th}, 3^{rd}, 7^{th}, 14^{th} and 28^{th} days interval but there non-significant improvement were shown.

Consistency	**Subclinical mastitis positive sample**	**Subclinical mastitis Negative sample**
Normal	**-**	**6**
Watery	17	
Slightly thick	4	-
Thick	3	-
Total	24	6

Factor M Means

M 1	11.500
M 2	2.000
M 3	1.500

Factor N Means

N 1	8.000
N 2	2.000

Test Results

Chi-Square Statistic	2.283
Chi-Square Table (0.05)	5.99
Chi-Square Table (0.01)	9.21

Chi Square Statistic is not significant

4.1 Milk pH

The pH values of various treatments groups measured at different intervals are summarized in the Table 4.1. The pH was recorded using digital pH meter in healthy as well as subclinical mastitis cows at different time intervals. The mean pH recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 6.54±0.02, 6.82±0.06, 6.85±0.07 6.77±0.03 and 6.63±0.06, respectively. The study revealed that the overall pH value of treatment groups T1, T2 and T3 increased significantly ($p<0.05$) as compared to control, however non significant improvement in milk pH was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk pH was observed in T4 group which received standard drug morbifloxacin when compared to T1 group. Similar trend of pH values was observed at different intervals but non significant changes were observed between control and different treatment groups at 28th day. At different time intervals in all treatment groups the pH values found increased initially on day 0 which was decreased progressively from day 3rd to 28th i.e. towards the end of experiment upon treatment with CUD, *C. amada* or CUD fortified urine indicating non significant but positive response of CUD, *C. amada* or CUD fortified urine .

The results of the present study are in agreement with the results of earlier studies conducted by Sunder *et al.,* (2013), Swami *et al.,* (2017) in which milk pH found to be increased initially and later found to decreased gradually toward normal in most of the cases in SCM. No earlier reports are available about milk pH post oral administration of Cow urine distillate, *Curcuma amada* or fortified cow urine distillate. Harmon (1994) conducted research on mastitis physiology

and factors influencing somatic cell count (SCC). It was observed that the pH values of milk could elevate from 6.6 to 6.9 or beyond, potentially due to heightened enzyme activity and the presence of blood with altered pH from affected tissue, such as mastitis. The pH of SCM milk was higher than that of normal milk, which was consistent with the results of previous reports (Kitchen, 1981; Sena and Sahmani, 2001; Wielgosz-Groth and Groth, 2003). Bagri *et al.,* (2018) examined an elevated pH value in SCM milk (6.7 to 6.9) compared to normal milk (6.4 to 6.8). Additionally, pH values were found to be increased in cases of subclinical mastitis. The rise in pH of subclinical mastitis milk in the study might be attributed to the heightened permeability of gland tissue to blood components, resulting in higher values in the milk. Gharban,(2021) reported that the milk pH in the mastitis group showed a higher value compared to the healthy group. These studies observed a positive correlation between milk pH and the severity of inflammatory processes. The milk pH testing could serve as a cost-effective and practical on-farm diagnostic tool for subclinical mastitis (SCM). However, subsequent findings concluded that milk pH lacked clinical utility in diagnosing SCM in dairy cattle.

Table 4.1. Mean ± SE values for pH of the cows under different treatment groups

Treat-ment /Interval	**Day 0th**	**Day 3rd**	**Day 7th**	**Day 14th**	**Day 28th**	**Pooled mean**
T_0	6.58^{b}±0.04	6.54^{b}±0.03	6.53^{b}±0.03	6.52^{b}±0.04	6.53±0.03	**6.54±0.02^{c}**
T_1	6.92^{a}±0.07	6.90^{a}±0.06	6.90^{a}±0.07	6.78^{a}±0.06	6.61±0.05	**6.82±0.06 ^{a}**
T_2	6.97^{a}±0.07	6.95^{a}±0.07	6.91^{a}±0.06	6.78^{a}±0.05	6.63±0.06	**6.85±0.07 ^{a}**
T_3	6.85^{a}±0.05	6.82^{a}±0.06	6.78^{a}±0.05	6.75^{a}±0.06	6.67±0.06	**6.77±0.03 ^{ab}**
T_4	6.88^{a}±0.05	6.60^{b}±0.06	6.58^{b}±0.06	6.56^{b}±0.06	6.53±0.05	**6.63±0.06 ^{bc}**
Significant/ NS CD 5%=0.148	*	*	*	*	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant, (n=30)

4.2 Total Protein (g/dl)

The total protein (TP) values of control and various treatments groups measured at different intervals are presented in the Table 4.2. The TP in milk whey was analyzed using semi-automatic biochemical analyzer by Biuret method in healthy as well as subclinical mastitis cows at different time intervals. The mean TP recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 2.837±0.03, 3.827±0.07, 3.836±0.06, 3.702±0.07 and 3.558±0.14, respectively. The study revealed that the overall TP value of treatment groups T1, T2 and T3 increased significantly ($p<0.05$) as compared to control. Non significant improvement in milk TP was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk TP was observed in T4 group which received standard drug morbifloxacin when compared to T1 group. Similar trend of significant ($p<0.05$) increased TP values in different treatment groups was observed at different intervals except on day 28th when compared to control. In the initial stage on day 0 the levels of milk TP were increased and as time progressed TP values in groups T4, T3, T2, and T1 were decreased and found nonsignificant to normal control on day 28th.

Table 4.2. Mean ± SE values for TP (g/dl) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	2.89^{b}±0.04	2.83^{b}±0.04	2.80^{b}±0.05	2.82^{b}±0.03	2.85±0.02	**2.837^{c}±0.03**
T_1	3.94^{a}±0.07	3.94^{a}±0.05	3.94^{a}±0.04	3.71^{a}±0.04	3.62±0.03	**3.827^{a}±0.07**
T_2	3.95^{a}±0.18	3.93^{a}±0.18	3.90^{a}±0.19	3.75^{a}±0.20	3.65±0.19	**3.836^{a}±0.06**
T_3	3.82^{a}±0.38	3.81^{a}±0.38	3.78^{a}±0.39	3.62^{a}±0.42	3.50±0.41	**3.702^{ab}±0.07**
T_4	3.67^{a}±0.05	3.76^{a}±0.05	3.77^{a}±0.05	3.47^{a}±0.10	3.20±0.10	**3.558^{b}±0.14**
Significant/ NS CD 5%=0.204	*	*	*	*	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

This increase in milk total protein level in subclinical mastitis is consistent with findings reported by Singh *et al.* (1998), Kolte *et al.* (1999), Tale *et al.* (2002), and Charjan *et al.* (2003). The results of the present study are also in agreement with the earlier findings of Daimi (2004) conducted a research in that the average milk total protein in the normal healthy group ranged from 2.89±0.03 to 2.92±0.03 gm/dl, with an overall average of 2.91±0.01 gm/dl. In the subclinical mastitic group, the milk total protein concentration before treatment initiation ranged from 3.77±0.04 to 3.99±0.05 gm/dl. which was significantly higher compared to control. The elevation in milk total protein in subclinical mastitis may be attributed to the leakage of serum proteins from the bloodstream following damage to udder tissues due to subclinical mastitis (Wheelock *et al.,* 1966). The increase in milk protein during subclinical mastitis may be attributed to pathological changes resulting from mammary epithelium infection, leading to increased capillary permeability during mastitic infection (Kitchen, 1981; Rao, 1990).

4.3. ALBUMIN (g/dl)

The mean values of albumin (ALB) of control and various treatments groups measured at different intervals are presented in the Table 4.3. The Albumin in milk whey was analyzed using semi-automatic biochemical analyzer by BCG Method in healthy as well as subclinical mastitis cows at different time intervals. The mean ALB recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 1.08±0.02, , 3.89±0.04, 3.39±0.04, 3.32±0.05 and 2.82±0.04 g/dl, respectively. The study revealed that the overall ALB value of treatment groups T1, T2 and T3 increased significantly ($p<0.05$) as compared to control. Significant ($p<0.05$) improvement in milk ALB was observed in T2 and T3 group received *C. amada* extract and cow urine distillate fortified with *C. amada,* respectively. The significant improvement in milk ALB was observed in T4 group which received standard drug morbifloxacin when compared to T1 group. Similar trend of ALB values was observed at different intervals in which ALB values increased initially and decreased progressively towards the end experiment.

This increase in milk albumin content in subclinical mastitis in present study align with findings by Randolph *et al.* (1974), Quidry *et al.* (1983), and Vijayalaximi *et al.* (2001). Our findings also found consistent with Daimi (2004) conducted an experiment in that the average milk albumin content in the normal healthy group ranged from 1.05 ± 0.03 to 1.08 ± 0.03 g/dl, with an overall mean of 1.06 ± 0.01 gm/dl. In subclinical mastitic groups, the milk albumin content before treatment initiation ranged from 2.56 ± 0.29 to 3.46 ± 0.10 g/dl, which was significantly higher compared to the normal healthy group. The observed elevation in milk albumin in subclinical mastitis may be due to increased capillary permeability associated with inflammation of the udder, leading to the breakdown of the blood-milk barrier and resulting in the transudation of serum proteins into the lacteal secretion (Vijayalaximi *et al.,* 2001).

Table 4.3. Mean ± SE values for Albumin(g/dl) of the cows under different treatment groups

Treatment /Interval	**Day 0th**	**Day 3rd**	**Day 7th**	**Day 14th**	**Day 28th**	**Pooled mean**
T_0	1.08^{c}±0.10	1.10^{c}±0.10	1.08^{c}±0.09	1.08^{c}±0.10	1.08^{c}±0.10	**1.08^{d}±0.02**
T_1	3.98^{a}±0.28	3.95^{a}±0.28	3.91^{a}±0.28	3.84^{ab}±0.28	3.76^{a}±0.27	**3.89^{a}±0.04**
T_2	3.45^{ab}±0.19	3.44^{ab}±0.19	3.42^{ab}±0.18	3.36^{ab}±0.18	3.27^{ab}±0.18	**3.39^{b}±0.04**
T_3	3.37^{ab}±0.38	3.35^{ab}±0.38	3.32^{ab}±0.38	3.32^{ab}±0.41	3.27^{ab}±0.41	**3.32^{b}±0.05**
T_4	2.90+^{b}±0.32	2.88^{b}±0.32	2.84^{b}±0.30	2.78^{b}±0.30	2.69^{b}±0.31	**2.82^{c}±0.04**
Significant/NS **CD Values -** 1%=0.120, 5%=0.088	*	*	*	*	*	*

The mean bearing different superscript ^{a,b,c,d} within the column differ significantly

* ($p<0.05$), NS –Non Significant

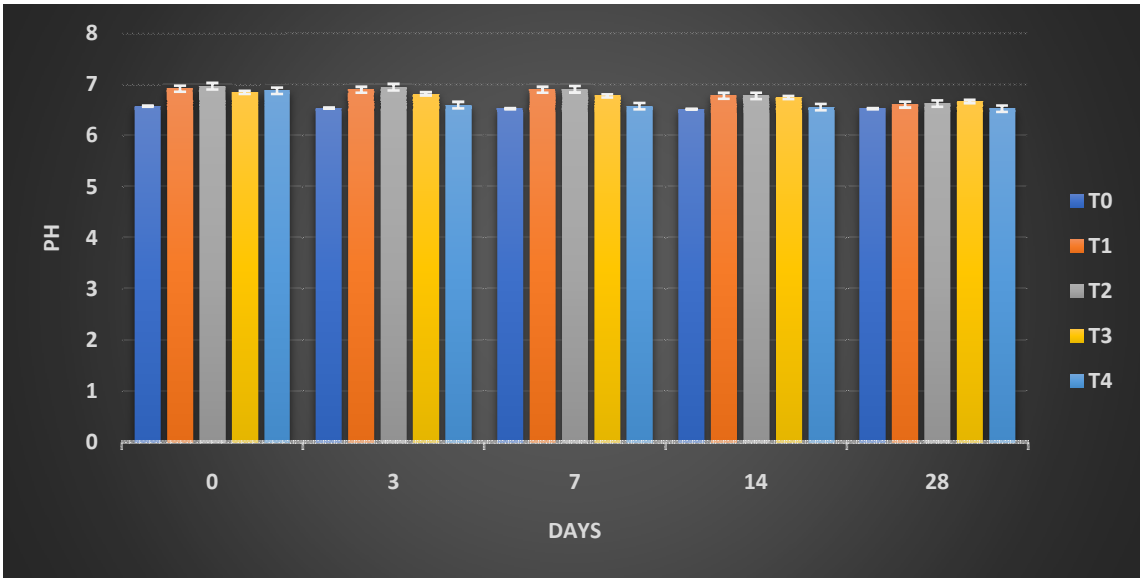


Fig.4.1 pH of the Cows under different treatment groups.

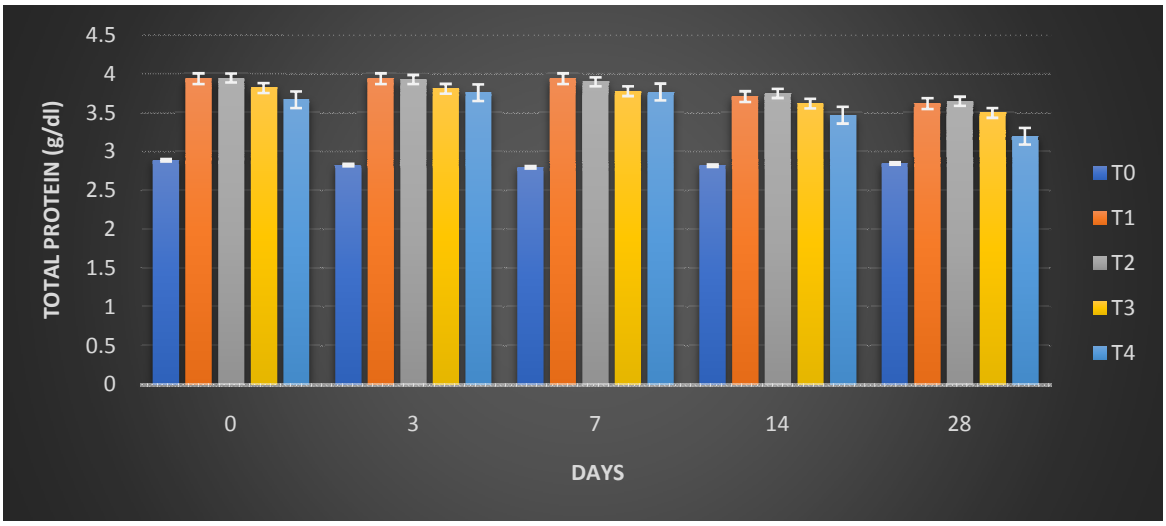


Fig.4.2 TP (g/dl) of the Cows under different treatment groups.

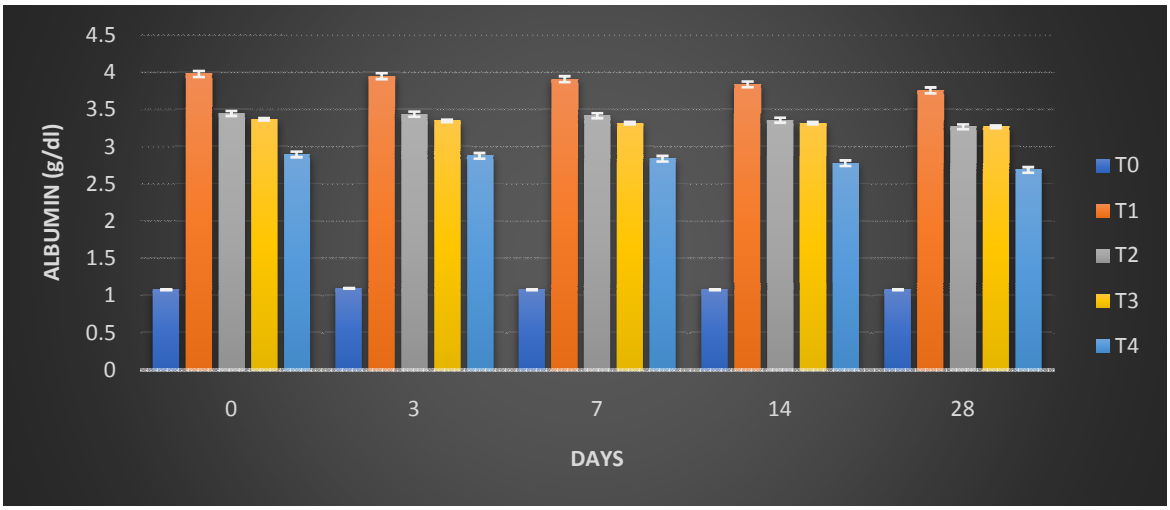


Fig.4.3 Albumin (g/dl) of the Cows under different treatment groups.

4.4. LDH Enzyme (U/ml)

Average values of milk lactate dehydrogenase in control and different treatment groups of cows at different time intervals are presented in the Table 4.4. The Lactate dehydrogenase enzyme in milk whey was analyzed using semi-automatic biochemical analyzer by IFCC Method in healthy as well as subclinical mastitis cows at different time intervals. The mean LDH recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 414.38±3.24, 1126.78±22.09, 1095.84±13.44, 1049.26±3.55 and 925.62±64.21 U/ml, respectively. The study revealed that the overall LDH value of treatment groups T1, T2 and T3 increased significantly ($p<0.05$) as compared to control, however non significant improvement in milk LDH was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk LDH was observed in T4 group which received standard drug morbifloxacin when compared to T1 group. Similar trend of LDH values was observed at different intervals in which significant changes were observed between control and different treatment groups at different intervals during the study period. The treatment of cow urine distillate fortified with *Curcuma amada* in subclinical mastitic cows showed non significant improvement at different intervals as compared to group T1 and T2.

The increase in LDH activity in subclinically and clinically mastitic milk was attributed to the release of parenchymal cells from the udder and leukocytes from disintegrating cells or other sources like serum (Bogin and Ziv, 1973). Worthington (1972) had previously noted that this increase could not solely be from serum, as enzymes are large molecules that cannot passively penetrate from blood into milk, except through active selective transfer mechanisms (Folley, 1956). Kitchen *et al.* (1970) similarly reported that mastitis damages the muscle tissue of the mammary gland, releasing enzymes into body fluids.

Table 4.4. Mean ± SE values for LDH enzyme (U/ml) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	413.67[b] ±18.15	413.10[c] ±20.62	414.82[c] ±18.57	415.28[c] ±16.29	415.03[b] ±19.80	**414.38[c] ±3.24**
T_1	1163.37[a] ±131.5	1156.75[a] ±132.78	1145.73[a] ±128.15	1120.65[a] ±123.32	1047.40[a] ±119.86	**1126.78[a] ±22.09**
T_2	1121.12[a] ±115.65	1119.98[ab] ±113.82	1099.28[ab] ±107.86	1089.38[a] ±107.97	1049.43[a] ±97.16	**1095.84[a] ±13.44**
T3	1054.05[a] ±40.0	1054.35[ab] ±41.71	1051.13[ab] ±41.41	1048.97[ab] ±39.18	1037.82[a] ±43.70	**1049.26[a] ±3.55**
T_4	1175.72[a] ±115.47	890.47[b] ±72.06	868.42[b] ±73.5	849.43[b] ±64.27	844.08[a] ±70.27	**925.62[b] ±64.21**
Significant/NS	*	*	*	*	*	*

The mean bearing different superscript [a,b,c] within the column differ significantly * ($p<0.05$), NS –Non Significant

Previous studies have suggested that elevated LDH activity in subclinically and clinically mastitic milk indicates tissue damage from bacterial infection (Batavani *et al.,* 2003). LDH activity in individual milk samples has been recognized as a significant indicator of bovine mastitis (Larson, 2005), consistent with the findings of their study.

4.5. Somatic Cell Count (SCC x10^5 cells/ml**)**

The average somatic cell count in different groups of cows at various intervals is presented in the Table 4.5. The mean somatic cell count recorded

(pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups were 2.55±0.04, 6.51±0.10, 6.25±0.10, 6.15±0.10 and 5.25±0.29 x10^5 cells/ml, respectively. The study revealed that the overall somatic cell count value of treatment groups T1, T2 and T3 increased significantly ($p<0.05$) as compared to control, however non significant improvement in milk somatic cell count was observed in T3 group received cow urine distillate fortified with *C. amada* as compared to CUD or *C. amada* treated groups. The significant improvement in milk somatic cell count was observed in T4 group which received standard drug morbifloxacin when compared to other treatment groups. As compared to pooled mean values similar trend of somatic cell count values was observed between control and different treatment groups at different intervals.

Table 4.5. Mean ± SE values for Somatic Cell Count (SCC x10^5 cells/ml**) of the cows under different treatment groups**

Treatment /Interval	**Day 0th**	**Day 3rd**	**Day 7th**	**Day 14th**	**Day 28th**	**Pooled mean**
T_0	2.54^{b}±0.22	2.54^{b}±0.21	2.55^{c}±0.20	2.55^{c}±0.19	2.56^{b}±0.20	**2.55^{c}±0.04**
T_1	6.73^{a}±0.63	6.65^{a}±0.63	6.57^{a}±0.63	6.40^{a}±0.65	6.18^{a}±0.66	**6.51^{a}±0.10**
T_2	6.40^{a}±0.54	6.33^{a}±0.54	6.23^{ab}±0.50	6.19^{ab}±0.49	6.09^{a}±0.51	**6.25^{a}±0.10**
T_3	6.33^{a}±0.66	6.31^{a}±0.65	6.27^{ab}±0.66	5.99^{ab}±0.59	5.84^{a}±0.56	**6.15^{a}±0.10**
T_4	6.38^{a}±0.54	5.17^{a}±0.53	4.92^{b}±0.50	4.88^{b}±0.51	4.87^{a}±0.52	**5.25^{b}±0.29**
Significant/ NS CD 5%=0.429	*	*	*	*	*	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

The results of the present study also found similar with the results of earlier studies by Petzer *et al.* (2017), Kandeel *et al.* (2019) and Sumon *et al.* (2020) stated that the increase in SCC suggested the inflammatory reaction that may be caused by a transfer of leukocytes to the udder after infection entry in the mammary gland. The rise in somatic cells in milk represents the animal's defense against the pathogen in terms of fighting sickness (Schalm, 1968).The findings of Berning and Shook (1992) stated that the somatic cell count was proved to be a more effective means of distinguish between affected and unaffected individuals and support our observation that the SCC was able to discriminate the latent infections in the absence of udder inflammation indications.

4.6. Milk Fat (%)

The milk fat % was determined by using Gerber's method in healthy as well as subclinical mastitis cows at different time intervals. Average values of milk fat in different groups of cows before and at 3^{rd},7^{th}, 14^{th} and 28^{th} day after treatment are presented in the Table 4.6.

The mean milk fat recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 4.12±0.07, 3.41±0.08, 3.39±0.06, 3.43±0.06 and 3.60±0.08 %, respectively. The study revealed that the overall milk fat value of treatment groups T1, T2, T3 and T4 was decreased significantly ($p<0.05$) as compared to control, however non significant improvement in milk fat was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk fat was observed in T4 group which received standard drug morbifloxacin when compared to other treatment groups. Like pooled mean values similar trend of fat values was observed at different intervals wherein milk fat values differ significantly between control and treatment groups. Improvement in fat values of different treatment groups found non significant at different time intervals.

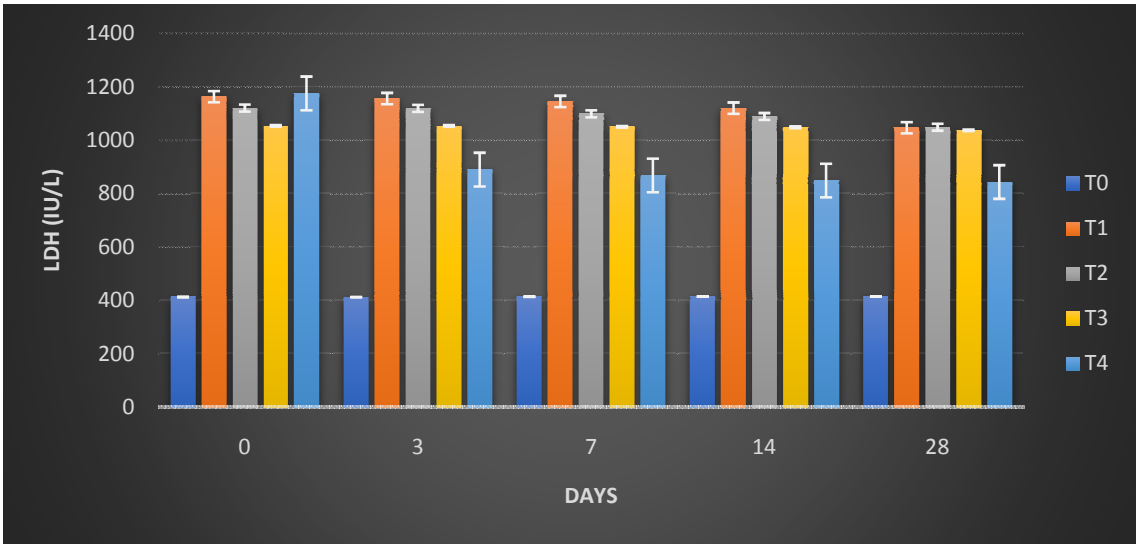


Fig.4.4 LDH of the Cows under different treatment groups.

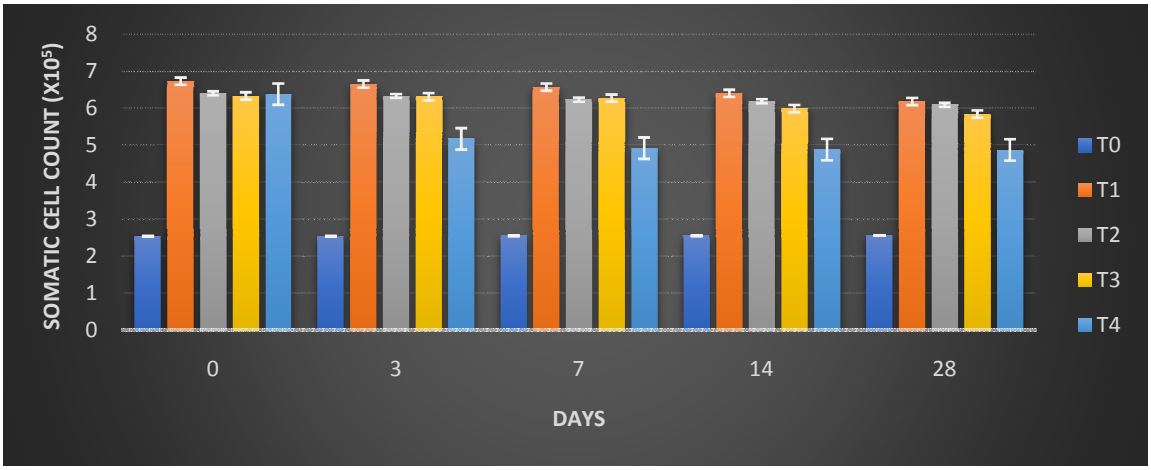


Fig.4.5 Somatic cell count (×10^5 cells/ml) of the Cows under different treatment groups.

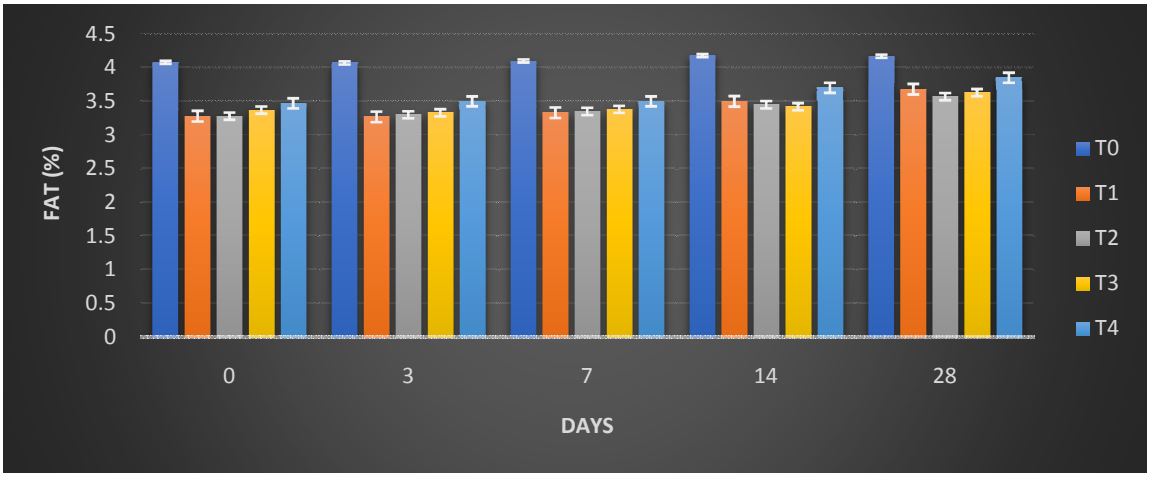


Fig.4.6 Fat (%) of the Cows under different treatment groups

Table 4.6. Mean ± SE values for milk fat (%) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	4.08^{a}±0.06	4.07^{a}±0.07	4.10^{a}±0.17	4.18^{a}±0.10	4.17^{a}±0.10	**4.12^{a}±0.07**
T_1	3.28^{b}±0.13	3.27^{b}±0.13	3.33^{b}±0.11	3.50^{b}±0.12	3.68^{b}±0.10	**3.41^{c}±0.08**
T_2	3.28^{b}±0.15	3.30^{b}±0.15	3.35^{b}±0.14	3.45^{b}±0.14	3.57^{b}±0.14	**3.39^{c}±0.06**
T_3	3.37^{b}±0.13	3.33^{b}±0.14	3.38^{b}±0.13	3.42^{b}±0.11	3.63^{b}±0.07	**3.43^{c}±0.06**
T_4	3.47^{b}±0.11	3.50^{b}±0.11	3.50^{b}±0.11	3.70^{b}±0.10	3.85^{b}±0.08	**3.60^{b}±0.08**
Significant/ NS CD 5%=0.176	*	*	*	*	*	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

The results of the present study are in align with the results of earlier studies conducted by Singh *et al.* (1998) observed a reduction in fat percentage in milk affected by subclinical mastitis (SCM). Additionally, this decrease in fat content in SCM can be attributed to the impaired synthesis and secretion activity of udder epithelial cells (Schultz, 1977). Pingle (1980) documented that the mean fat percentage in normal Gaolao milk was 4.26%. Similarly, Sherkar (1973) reported that the average fat content of normal milk from the dairy farm at the College of Agriculture, Nagpur, was 4.86%. Similar decreases in fat content during mastitis have been reported by other researchers (Uallah *et al.,* 2005 ; Danchuk *et al.,* 2021). In the milk of diseased cows, fat levels were reduced by 42.5% in cows with streptococcal mastitis (SCM) and by nearly 60% in those with coliform mastitis (CM) compared to healthy animals. During mastitis, the degradation of fat in milk occurs due to the presence of various enzymes concentrated at the site of inflammation. Harjanti *et al.* (2020) demonstrated that lipolysis of milk globule membranes by leukocyte lipases or by plasmin, which

hydrolyzes lipoproteins, diminish the synthetic and secretary capacity of the mammary gland, leading to a decrease in fat concentration during mastitis.

4.7. Viscosity (Centipoise)

The viscosity was determined by using Ostwald viscometer in healthy as well as subclinical mastitis cows at different time intervals. Average values of milk viscosity in different groups of cows before and on 3^{rd},7^{th} 14^{th} and 28^{th} day after treatment are presented in Table 4.7.

The mean viscosity recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 1.65±0.04, 1.10±0.02, 1.13±0.03, 1.14±0.04 and 1.21±0.03, respectively. The study revealed that the overall viscosity value of treatment groups T1, T2 and T3 decreased significantly ($p<0.05$) as compared to control. The non significant improvement in milk viscosity was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk viscosity was observed in T4 group which received standard drug morbifloxacin when compared to T1 group. The milk viscosity values found decreased significantly in SCM cows in different groups on 0, 3^{rd},7^{th} 14^{th} and 28^{th} day. Like pooled mean values of different groups very similar trend of viscosity values was observed at different intervals in treatment groups. In the beginning of experiment viscosity values found decreased which were improved progressively from day 3^{rd} to 28^{th} i.e. towards the end of experiment.

The viscosities of various commercial milk types—skim milk, 1% fat milk, 2% fat milk, whole milk, half and half, and whipping cream—were measured with a coaxial viscometer across temperatures from 0 to 30°C. Viscosity exhibited an exponential relationship with temperature and a linear correlation with fat content. Within the tested temperature and fat content ranges, viscosity values spanned from 1 to 60 centipoise. Additionally, the densities of these milk products, determined using a vibrational densitometer, ranged from 990 to 1035 kg/m³ (Bakshi and Smith, 1984).

Table 4.7. Mean ± SE values for Viscosity (Cp) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	1.642^{a}±0.0523	1.610^{a}±0.0432	1.650^{a}±0.0563	1.685^{a}±0.0310	1.667^{a}±0.0615	**1.65^{a}±0.04**
T_1	1.078^{b}±0.0253	1.080^{b}±0.0263	1.082^{b}±0.0252	1.097^{b}±0.0257	1.180^{b}±0.0604	**1.10^{c}±0.02**
T_2	1.080^{b}±0.0575	1.092^{b}±0.0553	1.107^{b}±0.0638	1.124^{b}±0.0664	1.242^{b}±0.0865	**1.13^{c}±0.03**
T_3	1.057^{b}±0.0436	1.081^{b}±0.0361	1.095^{b}±0.0467	1.207^{b}±0.0540	1.245^{b}±0.0512	**1.14^{bc}±0.04**
T_4	1.113^{b}±0.0439	1.202^{b}±0.0706	1.233^{b}±0.0790	1.225^{b}±0.0739	1.265^{b}±0.0852	**1.21^{b}±0.03**
Significant/NS CD 5%=0.077	*	*	*	*	*	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

4.8. Density (g/ml)

The density of milk was determined by using lactometer reading in healthy as well as subclinical mastitis cows at different time intervals. Average values of milk density in different groups of cows at different time intervals are presented in the Table 4.8.

Table 4.8. Mean ± SE values for density (g/ml) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	1.030^{a} ±0.0008	1.031^{a} ±0.0008	1.033^{a} ±0.0012	1.032^{a} ±0.0009	1.031 ±0.0007	**1.032^{a} ±0.0007**
T_1	1.027^{b} ±0.0007	1.027^{c} ±0.0007	1.028^{b} ±0.0005	1.029^{ab} ±0.0004	1.030 ±0.0007	**1.028^{b} ±0.0008**
T_2	1.028^{ab} ±0.0010	1.028^{bc} ±0.0012	1.028^{b} ±0.0013	1.028^{b} ±0.0013	1.030 ±0.0014	**1.028^{b} ±0.0005**
T_3	1.027^{b} ±0.0007	1.027^{c} ±0.0007	1.028^{b} ±0.0005	1.029^{ab} ±0.0004	1.030 ±0.0007	**1.028^{b} ±0.0008**
T_4	1.030^{ab} ±0.0012	1.031^{ab} ±0.0008	1.032^{a} ±0.0013	1.032^{a} ±0.0009	1.032 ±0.0009	**1.031^{a} ±0.0009**
Significant/NS CD 5%=0.001	*	*	*	*	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$),

NS –Non Significant

The mean density recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 1.032±0.0007, 1.028±0.0008, 1.028±0.0005, 1.028±0.0008 and 1.031±0.0009, respectively. The study revealed that the overall density value of treatment groups T1, T2, T3 and T4 decreased significantly ($p<0.05$) at different time intervals as compared to control. O day 28^{th} mean density values recovered to normal in all treatment groups. The non significant improvement in milk density was observed in treatment group. Like pooled mean values, similar trend of density values was observed at different intervals in treatment groups.

Yesil *et al.* (2020) conducted a study involving the analysis of 110 milk samples obtained from healthy black and white cows and determined the density of the milk to be 1.032±0.0003 g/ml. The results of the present study coincides with the results of earlier studies conducted by Rathaur *et al.* (2020) observed a decrease in the specific gravity of milk from mastitis-affected cows, which they attributed to an increase in chloride levels and a decrease in lactose content. The decline in specific gravity in mastitis milk was attributed to the decreased lactose content and increased chlorides. Ueda (1999) observed seasonal fluctuations in milk density, which was strongly associated with changes in the quantities of fat, protein, lactose, and other solid components. The study suggested that estimating density using specific weight conversions for fat, protein, lactose, and other solid components closely approximates the values determined experimentally.

Complete Blood Count in Subclinical mastitis cows

4.9. Hb(g/dl)

Average values of hemoglobin in different groups of cows at 0, 3^{rd},7^{th} 14^{th} and 28^{th} day after treatment are presented in the Table 4.9.

The mean Hb recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 11.24±0.05, 10.69±0.51, 10.24±0.22, 11.08±0.28 and 10.16±0.19, respectively. The study revealed that the overall Hb value of treatment groups T1, T2 and T3 decreased non significantly as compared to

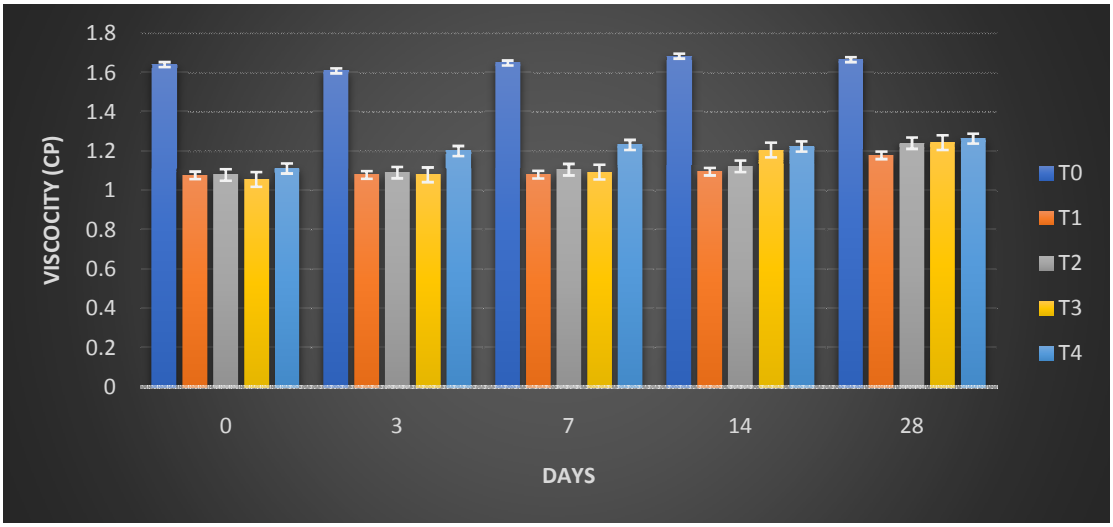


Fig.4.7. Viscosity (centipoise) of the Cows under different treatment groups.

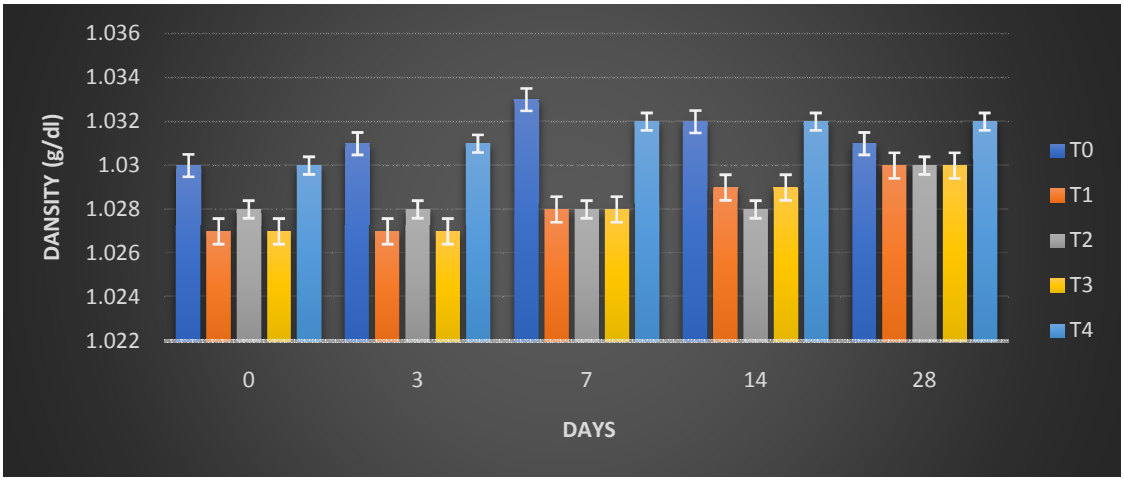


Fig.4.8. Density (g/ml) of the Cows under different treatment groups.

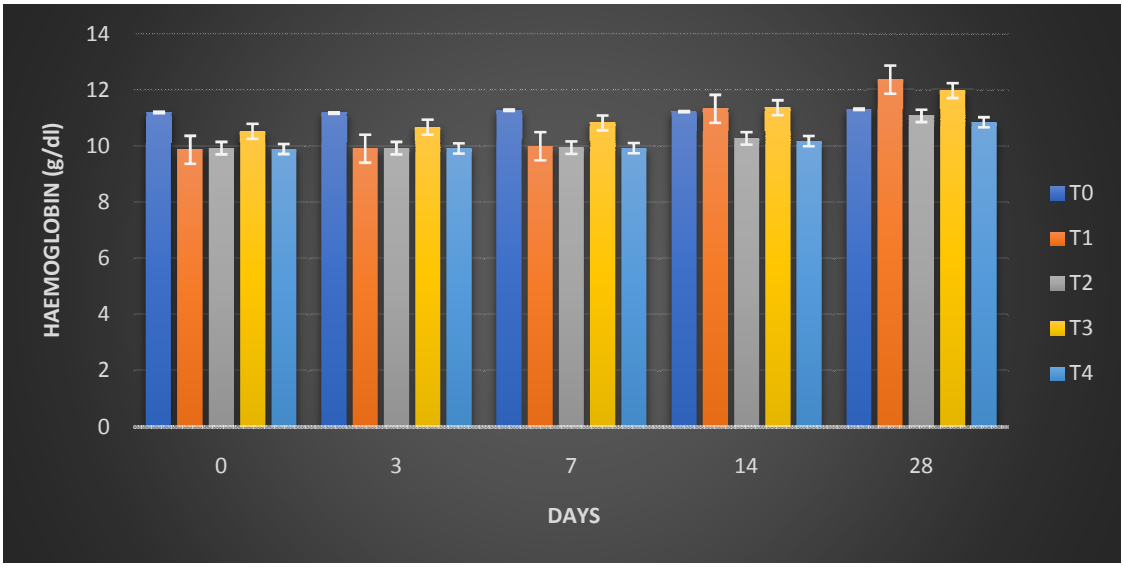


Fig.4.9.Hb (g/dl) of the Cows under different treatment groups.

control. Non significant decrease in Hb was observed at different time intervals in treatment groups. The Hb values were found to improve in cow urine and in CUD fortified group on 14th and 28th day and were found in the normal range.

Table 4.9. Mean ± SE hemoglobin (g/dl) values of cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	11.2±0.56	11.18±0.54	11.28±0.49	11.23±0.52	11.32±0.47	**11.24^{a}±0.05**
T_1	9.87±0.88	9.91±0.89	10.00±0.88	11.33±1.12	12.37±1.24	**10.69^{ab}±0.51**
T_2	9.93±0.76	9.93±0.76	9.95±0.75	10.28±0.74	11.08±0.82	**10.24^{b}±0.22**
T_3	10.53±0.68	10.68±0.64	10.83±0.64	11.37±0.73	11.98±0.86	**11.08^{a}±0.28**
T_4	9.90±0.44	9.92±0.45	9.93±0.46	10.18±0.43	10.85±0.49	**10.16^{b}±0.19**
Significant /NS CD 5%=0.837	NS	NS	NS	NS	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

This observation on Hb values aligns with the findings reported by Zaki*et al.* (2010), Padhya *et al.* (2014) and Siddiqe *et al.* (2015) in their studies on mastitis where decreased Hb values were observed. The results of the present study are also in agreement with the results of earlier studies conducted by Yadav *et al.* (2022) demonstrated in their study that the mean hemoglobin (Hb) level in both clinical and subclinical groups was significantly lower compared to the mean value in the control groups. Sarvesha *et al.* (2017) reported that no significant changes were observed in hemoglobin (Hb) and total erythrocyte count (TEC) levels in cows affected by clinical mastitis (CM) compared to healthy cows. Similarly, Sischo *et al.* (1997) did not observe significant trends in packed cell volume (PCV) and hemoglobin levels in cases of mastitis.

4.10. Total WBC (TLC; 10^3/µl)

The TLC values for various treatments measured at different intervals are summarized in the Table 4.10. The mean TLC recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 9.68±0.07, 11.70±0.21, 11.92±0.57, 11.32±0.40 and 10.92±0.55, respectively. The study revealed that the overall TLC value of treatment groups T1, T2 and T3 increased significantly ($p<0.05$) as compared to control, however non significant improvement in TLC was observed in T3 group received cow urine distillate fortified with *C. amada* as compared to group T1 and T2. The non significant improvement in TLC was also observed in T4 group which received standard drug morbifloxacin. The TLC values observed at different intervals in all treatment groups found non significant when compared among the groups. In the initial stage upon entry of infection TLC values found increased which were decreased progressively from day 3rd to 28th i.e. towards the end of experiment.

Table 4.10. Mean ± SE values for Total WBC (TLC; 10^3/µl) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	9.67±0.46	9.72±0.47	9.73±0.48	9.63±0.48	9.65±0.50	**9.68^{b}±0.07**
T_1	12.12±1.10	11.97±1.13	11.83±1.11	11.63±1.11	10.95±1.15	**11.70^{a}±0.21**
T_2	13.42±1.37	12.12±1.27	11.97±1.23	11.73±1.21	10.38±0.79	**11.92^{a}±0.57**
T_3	12.42±1.32	11.27±0.68	11.17±0.68	10.87±0.65	10.88±0.74	**11.32^{a}±0.40**
T_4	12.95±0.64	10.85±0.52	10.57±0.56	10.18±0.62	10.03±0.52	**10.92^{a}±0.55**
Significant /NS CD5%=1.054	NS	NS	NS	NS	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

The results of the present study are in agreement with the results of earlier studies conducted by Sadek *et al.* (2017) and Choudhary *et al.* (2019) reported significantly higher TLC with neutrophilia and lymphopenia conditions in SCM-affected dairy cows. Rathaur *et al.* (2020) investigated various haematological

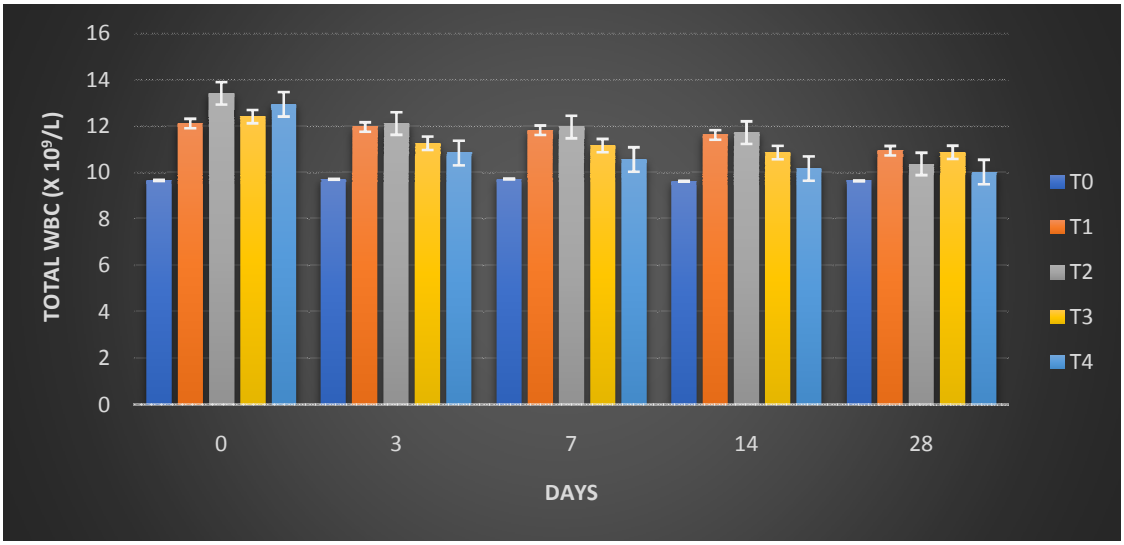


Fig.4.10 Total WBC (TLC; × 10^3µl) of the Cows under different treatment groups.

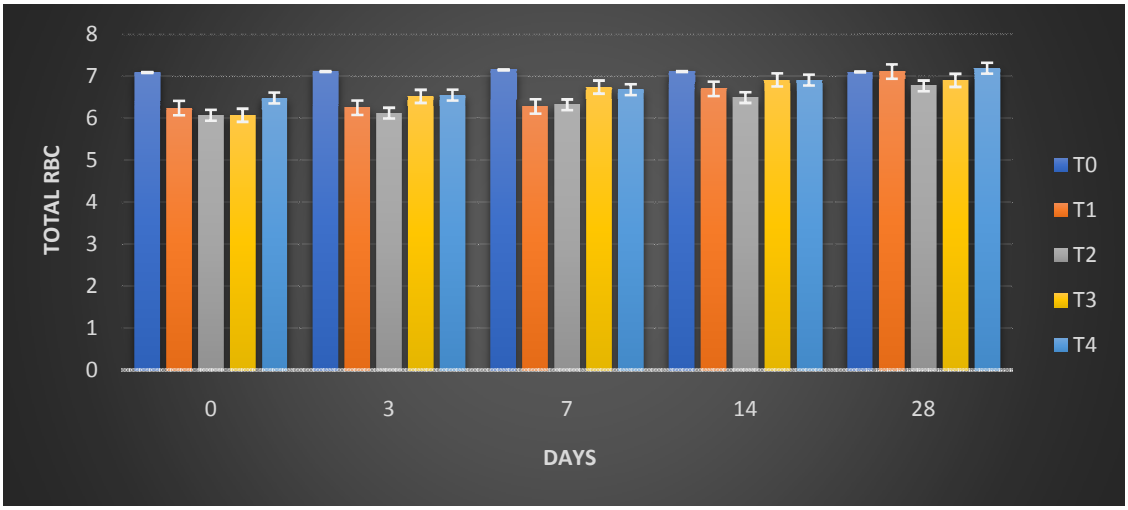


Fig.4.11.Total RBC (TEC; ×10^6µl) of the Cows under different treatment groups.

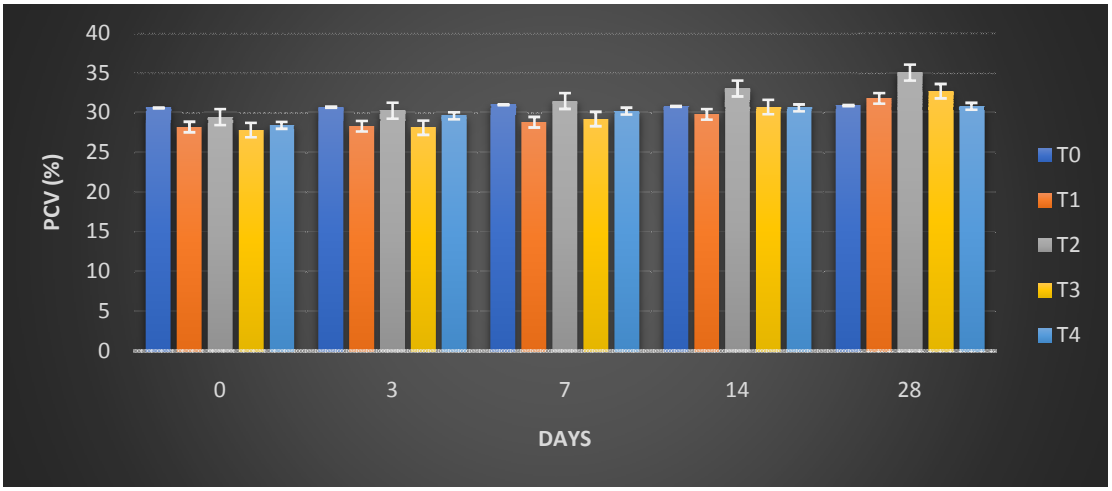


Fig.4.12. PCV % of the Cows under different treatment groups.

parameters in cows suffering from mastitis and compared them with those of healthy cows. They found a significant increase in total leukocyte count (TLC) in mastitis-affected cows compared to healthy ones. Zaki *et al.* (2008) and Khan *et al.* (1997) also reported an increase in total leukocyte count (TLC) with an increase in the absolute number of monocytes, eosinophils, and neutrophils in mastitis.

4.11. TOTAL RBC (TEC; 10^6/µl)

Average values of total RBC (TEC) in different groups of cows before and on 3^{rd},7^{th} 14^{th} and 28^{th} day of treatment are presented in the Table 4.11.

The mean TEC recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 7.11±0.04, 6.52±0.17, 6.35±0.13, 6.63±0.15 and 6.76±0.13, respectively. The study revealed that the overall TEC value of treatment groups T1, T2 and T3 decreased significantly ($p<0.05$) as compared to control, however non significant improvement in TEC was observed in T3 group received cow urine distillate fortified with *C. amada* as compared to other treatment groups. The significant improvement in TEC was observed in T4 group which received standard drug morbifloxacin when compared to other treatment groups. The TEC values of all treatment groups at different time intervals found non significant when compared among groups including control

Table 4.11. Mean ± SE values for Total RBC (TEC; 10^6/µl) of the cows under different treatment groups

Treatment /Interval	Day 0^{th}	Day 3^{rd}	Day 7^{th}	Day 14^{th}	Day 28^{th}	Pooled mean
T_0	7.09±0.38	7.11±0.36	7.15±0.32	7.11±0.33	7.10±0.34	**7.11^{a}±0.04**
T_1	6.24±0.20	6.25±0.21	6.28±0.20	6.70±0.21	7.11±0.20	**6.52^{bc}±0.17**
T_2	6.07±0.24	6.12±0.25	6.32±0.20	6.49±0.23	6.77±0.20	**6.35^{c}±0.13**
T_3	6.07±0.24	6.52±0.25	6.74±0.31	6.91±0.33	6.9± 0.33	**6.63^{bc}±0.15**
T_4	6.48±0.19	6.55±0.18	6.68±0.20	6.91±0.18	7.19±0.17	**6.76^{ab}±0.13**
Significant /NS CD 5%=0.390	NS	NS	NS	NS	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly
* ($p<0.05$), NS –Non Significant

The results of the present study are in agreement with the results of earlier studies conducted by Yadav *et al.* (2022) stated that the mean TEC values in both clinical and subclinical mastitis groups were significantly lower compared to the control group. This observation is also consistent with the findings reported by Zaki *et al.* (2010), Padhya *et al.* (2014), and Siddiqe *et al.* (2015) in their research on mastitis.

4.12. Packed Cell Volume (PCV %)

Average values of PCV in different groups of cows before and on 3rd, 7th, 14th and 28th day of treatment are presented in the Table 4.12 and represented graphically in fig. 4.12. The mean PCV recorded (pooled mean) at different intervals in

The mean PCV recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 30.82±0.07, 29.40±0.67, 31.87±1.00, 29.73±0.91and 29.95±0.43, respectively. The overall PCV value of control and different treatment groups at different time intervals differ non significantly. In all treatment groups the PCV values found decreased initially upon entry of infection which was improved progressively from day 3rd to 28th i.e. towards the end of experiment upon treatment with CUD, *C. amada* or CUD fortified urine.

Table 4.12. Mean ± SE values for PCV (%) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	30.62±1.09	30.72±1.09	31.02±1.09	30.82±1.09	30.92±1.09	**30.82±0.07**
T_1	28.22±2.42	28.32±2.42	28.82±2.42	29.82±2.42	31.82±2.42	**29.40±0.67**
T_2	29.47±1.74	30.27±1.74	31.50±2.80	33.07±2.80	35.07±1.74	**31.87±1.00**
T_3	27.83±1.62	28.13±1.62	29.23±1.62	30.73±1.62	32.73±1.62	**29.73±0.91**
T_4	28.43±1.02	29.63±1.02	30.23±1.02	30.63±1.02	30.83±1.02	**29.95±0.43**
Significant /NS	NS	NS	NS	NS	NS	NS

Significantly * ($p<0.05$), NS –Non Significant

The results of the present study are in agreement with the results of earlier studies conducted by Siddiqe *et al.* (2015) found that normal cows had higher packed cell volume (PCV) levels (27.42) compared to affected cows. Yadav *et al.* (2022) reported the mean values ± SE of packed cell volume (PCV) for subclinical mastitis was 25.9%-36.3%, and 26.7%-36.1% for the control group. The study found that the mean PCV values in both clinical and subclinical mastitis groups were significantly lower compared to the control group. This finding is consistent with the results reported by Padhya *et al.* (2014) in their studies on mastitis.

Differential Leukocyte Count (DLC)

4.13. Lymphocytes (%)

Average values of lymphocytes in different groups of cows before and on 3^{rd},7^{th}, 14^{th} and 28^{th} day of treatment are presented in the Table 4.13. The mean lymphocytes recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 52.38±0.57, 37.66±1.24, 38.82±0.82, 39.27±1.48 and 39.48±1.35, respectively. The study revealed that the overall lymphocytes value of treatment groups T1, T2 and T3 decreased significantly ($p<0.05$) as compared to control, however non significant improvement in lymphocytes was observed in different treatment groups. In all treatment groups the lymphocytes values found decreased initially upon entry of infection which were improved progressively from day 3^{rd} to 28^{th} i.e. at different time intervals upon treatment with CUD, *C. amada* or CUD fortified urine.

Table 4.13. Mean ± SE values for Lymphocytes (%) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	52.45[a]±3.98	52.57±4.01	52.43±4.09	52.25±4.22	52.22±4.27	**52.38[a]±0.57**
T_1	35.05[b]±5.87	35.68±5.93	36.75±6.15	39.08±6.22	41.72±5.82	**37.66[b]±1.24**
T_2	36.82[b]±4.62	37.65±4.61	38.58±4.71	39.60±4.62	41.43±4.64	**38.82[b]±0.82**
T_3	34.05[b]±2.04	38.77±2.36	40.02±2.33	41.72±2.15	41.82±2.26	**39.27[b]±1.48**
T_4	36.17[b]±4.26	37.98±4.15	38.97±4.55	40.72±4.10	43.55±3.87	**39.48[b]±1.35**
Significant /NS CD 5%=3.165	*	NS	NS	NS	NS	*

The mean bearing different superscript [a,b,c] within the column differ significantly * ($p<0.05$), NS –Non Significant

The results of the present study are in coincides with the results of previous study by conducted Sarvesha *et al.* (2017) discovered that the total milk leukocyte and granulocyte counts were notably higher, whereas lymphocyte and macrophage populations were significantly lower in animals infected with mastitis, indicating an infection in the mammary gland. Milk leukocyte count stands as one of the most reliable biomarkers for mastitis. The elevated level of milk leukocytes observed in this study suggests an immune response triggered by microorganisms in the mastitis-affected mammary gland (Djabri *et al.,* 2002; Gargouri *et al.,* 2008).

4.14. Monocytes (%)

The monocytes values for various treatments measured at different intervals are summaries in the Table 4.14. The mean monocytes recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 3.86±0.06, 4.21±0.49, 4.32±0.47, 4.16±0.48 and 4.11±0.50, respectively. The pooled mean monocytes values for various treatments differ non significantly as compared to control. The study also revealed that the overall monocytes value of treatment

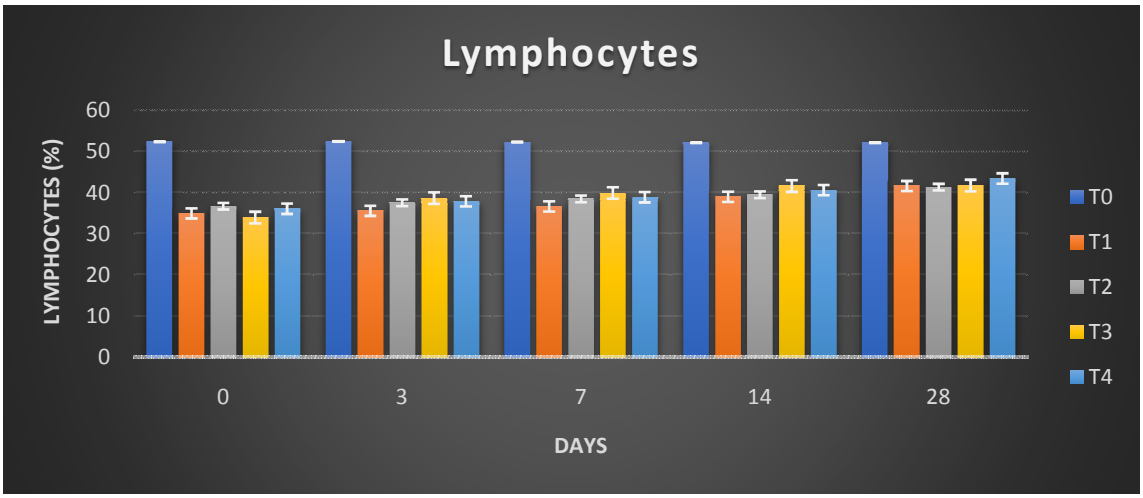


Fig.4.13. Lymphocytes ($\times 10^3$µl)of the Cows under different treatment groups.

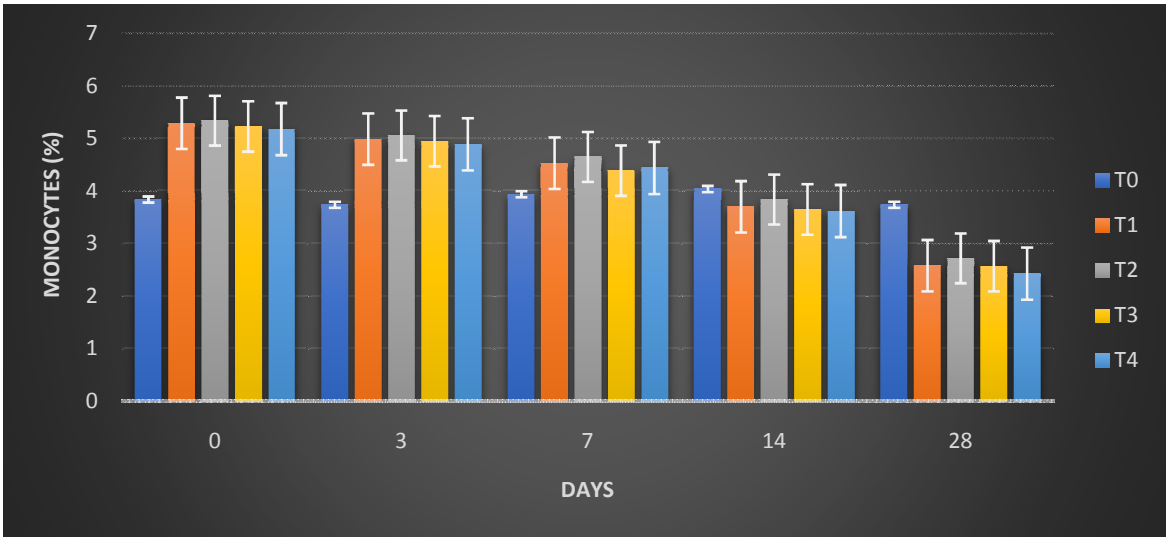


Fig.4.14. Monocytes ($\times 10^3$µl) of the Cows under different treatment groups.

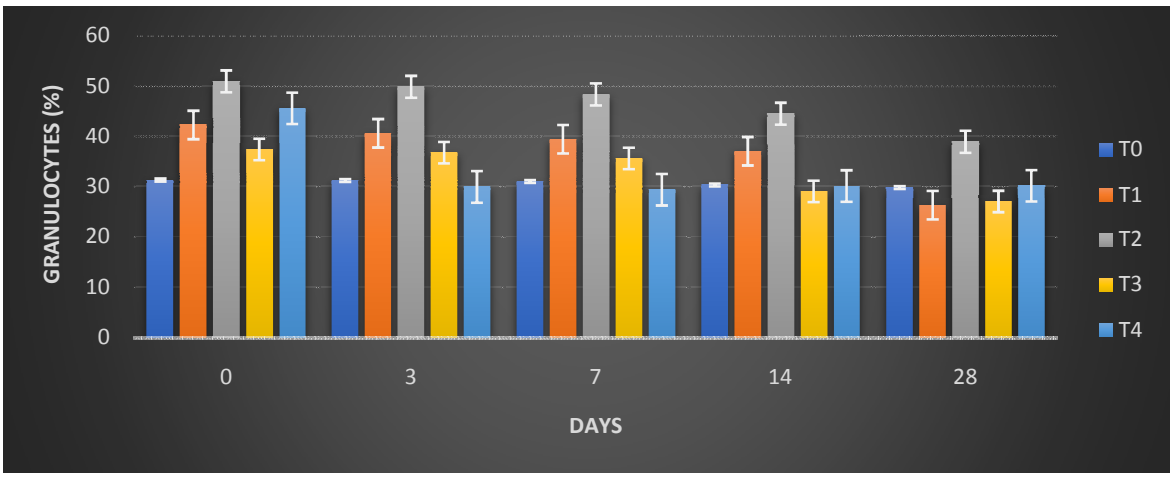


Fig.4.15. Granulocytes of the Cows under different treatment groups

groups at different time intervals differ non significantly as compared to control. In all treatment groups the monocytes values found increased initially upon entry of infection which were improved progressively from day 3rd to 28th i.e. at different time intervals upon treatment with CUD, *C. amada* or CUD fortified urine. Similar to findings of present study Zaki *et al.* (2008) and Khan *et al.* (1997) reported an increase in total leukocyte count (TLC) with an increase in the absolute number of monocytes, eosinophils, and neutrophils in mastitis.

Table 4.14. Mean ± SE values for monocytes (%) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	3.84±0.18	3.74±0.18	3.94±0.18	4.04±0.18	3.74±0.18	**3.86±0.06**
T_1	5.29±0.43	4.99±0.43	4.53±0.42	3.70±0.42	2.58±0.43	**4.21±0.49**
T_2	5.34±0.51	5.06±0.50	4.65±0.51	3.84±0.54	2.72±0.54	**4.32±0.47**
T_3	5.23±0.33	4.95±0.33	4.39±0.33	3.65±0.33	2.57±0.33	**4.16±0.48**
T_4	5.18±0.50	4.89±0.51	4.44±0.54	3.62±0.52	2.43±0.53	**4.11±0.50**
Significant/NS	NS	NS	NS	NS	NS	NS

NS- Nonsignificant

4.15. Granulocytes (%)

The granulocytes values for various treatments measured at different intervals are summaries in the Table 4.15. The mean granulocytes recorded (pooled mean) at different intervals in T0, T1, T2, T3 and T4 groups was 30.74±0.32, 37.14±2.85, 46.52±2.25, 33.18±2.30

and 33.04±3.14, respectively. The study revealed that the overall granulocytes value of treatment groups found to be increased. Granulocytes of T2 group increased significantly ($p<0.05$) as compared to control, however Granulocytes of in groups T1, T3, and T4 found comparable to the control group (T0) which indicate protective effect of CUD and CUD fortified extract. Improvement in

granulocytes was observed in T3 group received cow urine distillate fortified with *C. amada*. The non-significant improvement in granulocytes was observed in T4 group which received standard drug morbifloxacin when compared to T1 group. In all treatment groups the granulocytes values found increased initially upon entry of infection which were improved progressively from day 3rd to 28th i.e. at different time intervals upon treatment with CUD, or CUD fortified urine.

Table 4.15. Mean ± SE values for granulocytes (%) of the cows under different treatment groups

Treatment /Interval	Day 0th	Day 3rd	Day 7th	Day 14th	Day 28th	Pooled mean
T_0	31.32^{c}±1.60	31.22^{c}±1.60	31.02^{bc}±1.60	30.35^{b}±1.57	29.82±1.60	**30.74^{b}±0.32**
T_1	42.28^{ab}±0.33	40.62^{b}±0.33	39.43^{b}±0.33	37.05^{ab}±0.33	26.32±0.33	**37.14^{b}±2.85**
T_2	50.95^{a}±3.99	49.87^{a}±3.99	48.35^{a}±3.99	44.52^{a}±4.46	38.93±3.31	**46.52^{a}±2.25**
T_3	37.38^{bc}±2.76	36.77^{bc}±2.73	35.63^{bc}±2.67	29.05^{b}±1.99	27.05±3.17	**33.18^{b}±2.30**
T_4	45.58^{ab}±2.62	29.95^{c}±1.99	29.40^{c}±1.89	30.12^{b}±1.95	30.17±1.98	**33.04^{b}±3.14**
Significant/ NS CD 5%=6.895	*	*	*	*	NS	*

The mean bearing different superscript ^{a,b,c} within the column differ significantly * ($p<0.05$), NS –Non Significant

From the literature cited it is observed that various herbs including *Curcuma amada* (Ambe Haldi) have reported to be useful in the treatment of SCM, but no literature is available on CUD or CUD fortified *Curcuma amada* extract in the treatment of mastitis.

In related literature reviewed on use of ethnoveterinary practices and herbs in treatment of SCM Kolte *et al.* (2008) studied cows affected by subclinical mastitis (SCM) were treated with a herbal preparation containing a combination of roots of *Withania somnifera* (Ashwagandha), *Asparagus racemosus* (Shatavari), *Curcuma amada* (Ambe Haldi), and fresh leaves of *Ocimum sanctum* (Tulsi), which was found to be more effective than an alternative herbal preparation. **Balakrishnan *et al.* (2017)** reported the efficacy of a combination of Aloe vera leaves, Burm fruits, *Curcuma longa* leaves, and calcium hydroxide

in treating clinical mastitis, attributing the cure to its broad-spectrum antimicrobial, anti-inflammatory, and immune modulatory activities. The study conducted by **Mooventhan *et al*. (2016),** Group I animals afflicted with subclinical mastitis (SCM) were treated with a paste comprising 100g of *Moringa oleifera* leaves, 10g of turmeric powder, and 10g of common salt, which was applied externally over the udder twice daily for 7 days. On the other hand, Group II animals were administered an oral bolus consisting of 100g of Tulasi (*Ocimum sanctum*) dry powder, 3 teaspoons of honey, and the juice from one lemon, twice daily for 7 days. Among the 16 SCM-affected quarters from 10 animals in Group II, 90% of the animals and 93.75% of the quarters showed complete recovery post-treatment. **Mukherjee *et al.* (2005)** observed a reduction in bacterial count and an increase in neutrophil and lymphocyte count with enhanced phagocytic activity and phagocytic index following treatment of SCM-affected cows with aqueous leaf extract *of Ocimum sanctum.*

In conclusion based on various biochemical parameters present study demonstrates that CUD fortified *Curcuma amada* extract showed positive activity in the control of SCM in cows. The anti-mastitic activity of CUD fortified *Curcuma amada* extract was found better than CUD or *C. amada* alone treated groups. However, the group which received standard drug morbifloxacin showed better anti-mastitic activity in SCM affected cows.

CHAPTER - V

SUMMARY AND CONCLUSION

The present study entitled "Effect of cow urine distillate fortified with *Curcuma amada* extract therapy on subclinical mastitis in cows." was undertaken to study the effect of cow urine distillate, *C. amada* aqueous extract and cow urine distillate fortified with *C. amada* on milk physicochemical, biochemical and haematological parameters in subclinical mastitic cows. The study was conducted on 30 cows of which 24 were SCM positive from Adarsh Goseva Evam Anusandhan Prakalp Mhaispur, Dist. Akola and Dr. Panjabrao Deshmukh Krishi Vidyapeeth Dairy Farm, Akola. Thirty cows in the mid-lactation period (4-6 years and 300-400 kgs) were randomly selected and divided into five equal-treatment groups. The T1 group was given cow urine distillate @0.15ml/kg b.wt. diluted in water orally; T2 was given *Curcuma amada* aquous extract @20mg/kg b.wt orally,T3 was given CUD fortified *Curcuma amada* orally, Group T4 given standard treatment of morbofloxacin @8mg/kg b.wt. and group T0 was treated as healthy. The parameters, viz. Milk pH, total protein, albumin, lactate dehydrogenase enzyme, Somatic Cell Count (SCC), milk fat density and viscosity were recorded and haematogical parameters were analyzed at different intervals during the experimental period. The collected data were computerized and put into the statistical analysis using standard statistical methods using one-way ANOVA.

The CMT was conducted on total 52 cows out of which 24 animals were found positive for SCM. The SCM prevalence was 46 percent of the total cows screened with CMT.

The study revealed that the overall pH value of treatment groups T1, T2, T3 and T4 increased significantly ($p<0.05$) as compared to control. Similar trend of pH values was observed at different intervals but non significant changes were observed between control and different treatment groups at 28^{th} day. In treatment groups at different time intervals, the pH values found increased initially on day 0

and it was decreased progressively from day 3rd to 28th i.e. towards the end of experiment upon treatment with CUD, *C. amada* or CUD fortified urine.

The total protein (TP) values of control and various treatments groups measured at different intervals increased significantly ($p<0.05$) as compared to control. Non significant improvement in milk TP was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk TP was observed in T4 group which received standard drug morbifloxacin. The mean ALB recorded (pooled mean) at different intervals in T1, T2, T3 and T4 groups was increased significantly ($p<0.05$) as compared to control. Significant ($p<0.05$) improvement in milk ALB was observed in T2 and T3 group received *C. amada* extract and cow urine distillate fortified with *C. amada,* respectively.

Average values of milk lactate dehydrogenase in control and different treatment groups of cows at different time intervals increased significantly ($p<0.05$) as compared to control, however non significant improvement in milk LDH was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk LDH was observed in T4 group which received standard drug morbifloxacin. Similar trend of LDH values was observed at different intervals in which significant changes were observed between control and different treatment groups.

The Average somatic cell count in different groups of cows at various intervals increased significantly ($p<0.05$) as compared to control, however non significant improvement in milk somatic cell count was observed in T3 group received cow urine distillate fortified with *C. amada* as compared to CUD or *C. amada* treated groups. The significant improvement in milk somatic cell count was observed in T4 group which received standard drug morbifloxacin when compared to other treatment groups.

The mean milk fat recorded (pooled mean) at different intervals in T1, T2, T3 and T4 groups decreased significantly ($p<0.05$) as compared to control, however non significant improvement in milk fat was observed in T3 group

received cow urine distillate fortified with *C. amada*. The significant improvement in milk fat was observed in T4 group which received standard drug morbifloxacin when compared to other treatment groups. Like pooled mean values similar trend of fat values was observed at different intervals wherein milk fat values differ significantly between control and treatment groups.

The mean viscosity and density recorded (pooled mean) at different intervals in T1, T2, T3 and T4 groups were decreased significantly ($p<0.05$) as compared to control. The non significant improvement in milk viscosity and density were observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk viscosity and density were observed in T4 group which received standard drug morbifloxacin when compared to T1 group. The milk viscosity and density were values found decreased significantly in SCM cows in different groups on 0, 3^{rd},7^{th} 14^{th} and 28^{th} day.

The mean Hb recorded (pooled mean) at different intervals in T1, T2, T3 and T4 groups was decreased non significantly as compared to control. Non significant decrease in Hb was observed at different time intervals in treatment groups. The Hb values were found to improve non significantly in cow urine and in CUD fortified group on 14^{th} and 28^{th} day and were found in the normal range. The overall PCV value of control and different treatment groups at different time intervals differ non significantly. In all treatment groups the PCV values found decreased initially upon entry of infection which was improved progressively from day 3^{rd} to 28^{th} i.e. towards the end of experiment upon treatment with CUD, *C. amada* or CUD fortified urine.

The TLC values T1, T2, T3 and T4 groups increased significantly ($p<0.05$) as compared to control, however non significant improvement in TLC was observed in T3 group received cow urine distillate fortified with *C. amada* as compared to group T1 and T2. The TLC values observed at different intervals in all treatment groups found non significant when compared among the groups. In the initial stage upon entry of infection TLC values found increased which were decreased progressively from day 3^{rd} to 28^{th} i.e. towards the end of experiment.

The mean TEC recorded (pooled mean) at different intervals in T1, T2, T3 and T4 groups was decreased significantly ($p<0.05$) as compared to control, however non significant improvement in TEC was observed in T3 group received cow urine distillate fortified with *C. amada* as compared to other treatment groups. The significant improvement in TEC was observed in T4 group which received standard drug morbifloxacin when compared to other treatment groups.

Average values of lymphocytes in T1, T2, T3 and T4 groups was decreased significantly ($p<0.05$) as compared to control, however non significant improvement in lymphocytes was observed in different treatment groups. In all treatment groups the lymphocytes values found decreased initially upon entry of infection which were improved progressively from day 3rd to 28th i.e. at different time intervals upon treatment with CUD, *C. amada* or CUD fortified urine. The monocytes values for various treatments T1, T2, T3 and T4 groups was differ non significantly as compared to control. In all treatment groups the monocytes values found increased initially upon entry of infection which were improved progressively from day 3rd to 28th i.e. at different time intervals upon treatment with CUD, *C. amada* or CUD fortified urine. The study revealed that the overall granulocytes value of treatment groups found to be increased. Granulocytes of T2 group increased significantly ($p<0.05$) as compared to control, however granulocytes of in groups T1, T3, and T4 found comparable to the control group (T0) which indicate protective effect of CUD and CUD fortified extract. Improvement in granulocytes was observed in T3 group received cow urine distillate fortified with *C. amada*.

In conclusion subclinical mastitis leads to significant hematological abnormalities and alterations in SCC, Milk pH, total protein, albumin, LDH enzyme, density, viscosity in affected cows compared to healthy ones.

CUD fortified herbal therapy offers a favorable therapeutic outcome, particularly for mild to moderate infections like subclinical mastitis. Based on various biochemical parameters present study demonstrates that CUD fortified *Curcuma amada* extract showed positive activity in the treatment of SCM in cows.

The anti-mastitic activity of CUD fortified *Curcuma amada* extract was found better than CUD or *C. amada* alone treated groups. However, the group which received standard drug morbifloxacin showed better anti-mastitic activity in SCM affected cows.

The study demonstrates the efficacy of cow urine distillate plus *Curcuma amada* extract in treating subclinical mastitis, offering a promising solution to this prevalent issue in the dairy industry.

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VITAE

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THESIS ABSTRACT

a)	**Title of the thesis**	**:**	**EFFECT OF COW URINE DISTILLATE FORTIFIED WITH *Curcuma amada* EXTRACT THERAPY ON SUBCLINICAL MASTITIS IN COWS**
b)	**Full name of student**	**:**	**Shinde Kajal Vijay**
c)	**Name and address of Major advisor**	**:**	**Dr. P. M. Kapale** Assistant and Professor Head, Department of Veterinary Biochemistry, Post Graduate Institute of Veterinary and Animal Sciences, Akola
d)	**Degree to be awarded**	**:**	M.V.Sc. (Veterinary Biochemistry)
e)	**Year of award of degree**	**:**	2024
f)	**Major subject**	**:**	Veterinary Biochemistry
g)	**Total number of pages in the thesis**	**:**	83
h)	**Number of words in the abstract**	**:**	268
i)	**Signature of Student**	**:**	
j)	**Signature, Name and address of forwarding authority**	**:**	

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ABSTRACT

The present study was carried out to evaluate the effect of cow urine distillate fortified *Curcuma amada* extract therapy on subclinical mastitis in cows. The diagnostic tools such as California Mastitis Test (CMT) and somatic cell count were utilized for the diagnosing subclinical mastitis. The study was conducted on SCM cows at dairy farms. Thirty cows in the mid-lactation state were randomly selected and divided into five equal-treatment groups viz T0 was control, T1 group was given cow urine distillate, T2 was given *Curcuma amada*

aquous extract, T3 was given fortified with *Curcuma amada* aquous extract(@20mg/kg b.w.t), cow urine distillate (@0.15ml/kg b.w.t)diluted in water by orally, for 28 days. Group T4 given standard treatment of morbofloxacin. The CMT was conducted on total 52 cows out of which 24 animals were found positive for SCM. The SCM prevalence was 46 percent. The study revealed significant alterations in the physical properties of milk samples from subclinical mastitis cows, including increased pH, total protein, albumin, and lactate dehydrogenase enzyme levels, alongside decreased milk fat, density, and viscosity. Hematological parameters also showed significant changes, with increased total white blood cell count, monocytes, and granulocytes, and decreased hemoglobin, packed cell volume, total red blood cell count, and lymphocytes. The anti-mastitic activity of CUD fortified *Curcuma amada* extract was found better than CUD or *C. amada* alone treated groups. However, the group which received standard drug morbifloxacin showed better anti-mastitic activity in SCM affected cows. The study demonstrates the efficacy of cow urine distillate and *Curcuma amada* extract in treating subclinical mastitis, offering a promising solution to this prevalent issue in the dairy industry**.**

प्रबंध सारांश

१.	**प्रबंधाचे शिर्षक**	**: ''गायींमधील सुप्त अवस्थेतील स्तनदाह रोगावर गोमूत्र संकरित आंबेहळद अर्क उपचाराच्या परिणामाचे मूल्यांकन''**
२.	**विद्यार्थ्याचे पूर्ण नांव**	**: शिंदे काजल विजय**
३.	**मुख्य मार्गदर्शकाचे नांव व पत्ता**	**: डॉ. पी . एम. कपले** सहाय्यक प्राध्यापक व पशुजीवरसायनशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला
४.	**प्रदान केली जाणारी पदवी**	: एम. व्ही. एस. सी.
५.	**पदवी प्रदान करण्याचे वर्ष**	: २०२४
६.	**मुख्य विषय**	: पशुजीवरसायनशास्त्र विभाग
७.	**प्रबंधामधील एकुण पाने**	: ८३
८.	**प्रबंध सारांशामधील एकुण शब्द**	: २८२
९.	**विद्यार्थ्याची सही**	:
१०.	**प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता**	:

(डॉ. पी . एम. कपले)

सहाय्यक प्राध्यापक व पशुजीवरसायनशास्त्र विभाग प्रमुख,
स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला

सारांश

"गायींमधील सुप्त अवस्थेतील स्तनदाह रोगावर गोमूत्र संकरित आंबेहळद अर्क उपचाराच्या परिणामाचे मूल्यांकन" करण्यासाठी सध्याचा अभ्यास करण्यात आला. सुप्त अवस्थेतील स्तनदाह रोगाचे निदान करण्यासाठी कॅलिफोर्निया मास्टिटिस टेस्ट (सीएमटी) आणि सोमॅटिक सेल काउंट सारख्या निदान साधनांचा वापर केला गेला. डेअरी फार्ममधील सुप्त अवस्थेतील स्तनदाह रोगातील गायींवर हा अभ्यास करण्यात आला. दुग्ध अवस्थेतील तीस

गायींची यादृच्छिकपणे निवड करण्यात आली आणि त्यांना पाच समान-उपचार गटांमध्ये विभागले गेले जसे की टी 0 निरोगी गायींचा समावेश होता, टी 1 गटास गोमूत्र अर्क देण्यात आले होते, टी 2 ला आंबेहळद अर्क देण्यात आला होता (@20mg / किलो बीडब्ल्यूटी), टी 3 ला गोमूत्र संकरित आंबेहळद अर्क (@20mg / किलो बीडब्ल्यूटी), गोमूत्र अर्क (@0.15 मिली / किलो बी.डब्ल्यू.टी.) पाण्यात मिसळून तोंडी देण्यात आले 28 दिवसासाठी दिले गेले. गोमूत्र अर्क (@0.15 मिली / किलो बीडब्ल्यूटी) पाण्यात मिसळून तोंडी 28 दिवसासाठी दिले गेले होते. गट टी 4 ला मॉर्बोफ्लोक्सासिन अँटिबायोटिक्सचे मानक उपचार दिले गेले. एकूण ५२ गायींवर सीएमटी करण्यात आली त्यापैकी २४ जनावरे सुप्त अवस्थेतील स्तनदाह रोगातील आढळली. सुप्त अवस्थेतील स्तनदाह चे प्रमाण ४६ टक्के होते. अभ्यासात सुप्त अवस्थेतील स्तनदाहतील गायींच्या दुधाच्या नमुन्यांच्या भौतिक गुणधर्मांमध्ये महत्त्वपूर्ण बदल दिसून आले, ज्यात पीएच, एकूण प्रथिने, अल्ब्युमिन आणि लॅक्टेट डिहायड्रोजनेज एंजाइमची पातळी वाढणे आणि दुधाची चरबी, घनता आणि चिकटपणा कमी होणे समाविष्ट आहे. एकूण पांढर्या रक्त पेशींची संख्या, मोनोसाइट्स आणि ग्रॅन्युलोसाइट्स आणि हिमोग्लोबिन, पॅक्ड पेशींचे प्रमाण, एकूण लाल रक्त पेशींची संख्या आणि लिम्फोसाइट्स कमी झाल्यामुळे रक्त तपासणी मध्ये देखील महत्त्वपूर्ण बदल आढळले. गोमूत्र संकरित आंबेहळद अर्कची अँटी-मास्टिटिक क्रिया एकट्या गोमूत्र अर्क किंवा आंबेहळद अर्क उपचार गटांपेक्षा चांगली आढळली. तथापि, मानक औषध मॉर्बिफ्लोक्सासिन अँटिबायोटिक्सने प्राप्त केलेल्या गटाने सुप्त अवस्थेतील स्तनदाहतील प्रभावित गायींमध्ये चांगले अँटी-मास्टिटिक क्रियाकलाप दर्शविले. हा अभ्यास सुप्त अवस्थेतील स्तनदाहतील उपचारांमध्ये गोमूत्र संकरित आंबेहळद अर्कची कार्यक्षमता दर्शवितो, दुग्ध उद्योगातील या प्रचलित समस्येवर आश्वासक उपाय प्रदान करतो.