Himachal Journal of Agricultural Research 48(2): 145-150 (2022)

Alternative herbal medicine in bovine mastitis: A comprehensive review

Dinesh Kumar Sharma and Pardeep Sharma*
Department of Veterinary Pharmacology and Toxicology
* Department of Veterinary Medicine
DGCN College of Veterinary and Animal Sciences
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062. India.
*Corresponding author: docpradeepsharma@gmail.com
Manuscript Received: 21.09.2022; Accepted: 10.10.2022

Abstract

Bovine Mastitis is the most widespread disease in dairy cattle occurring throughout the world. The disease is causing huge economic losses particularly in developing countries like India in terms of reduced milk production, costs on treatment, culling of chronically infected cows and occasional deaths. Moreover, mastitis has a serious zoonotic potential associated with shedding of bacteria and their toxins in the milk. The treatment of choice for mastitis is the administration of antibiotics. However, antibiotics used for the treatment of this disease are causing antibiotic resistance and its residues passes in milk. Phytochemicals isolated from plants have exhibited potent activities against bacteria causing mastitis. These agents can act alone or in combination with antibiotics to enhance the antibacterial activity against a wide range of bacteria. The drugs from plants are less toxic; side effects are scanty and are also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. This comprehensive review was prepared to know different studies on usage of various alternative medicines in relation to bovine mastitis.

Key words: Antibiotic resistance, Bovine mastitis, Phytochemicals, Zoonotic

India is the world's largest milk producer, with contribution of 16 percent to global production (Smith *et al*. 1985). The annual economic losses due to mastitis in India, United States, UK and worldwide have been estimated at $1.1 billion, $2 billion, $371 million and $35 billion, respectively (Reshi *et al*. 2015). Mastitis is a multi-etiological complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical, and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits *et al*. 2010). The disease is caused mainly by bacterial infections and is classified into two types based on epidemiology, namely contagious and environmental mastitis (Garcia 2004). The former is caused by contagious bacteria including *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*) and *Mycoplasma* spp. that spread from infected cow to a healthy cow usually at the time of milking through the hands, towels and/or the milking machine acting as the bacterial reservoirs. Conversely, environmental mastitis is caused by bacteria that spread primarily outside of the milking parlor, *i.e.*, the causative bacteria come from the cow's environment such as bedding material, soil, manure, faeces, and stagnant water (Garcia 2004). Mastitis may be classified as clinical and subclinical. In contrast to visible changes in the clinical form of mastitis, there is absence of gross abnormalities in the milk or udder in case of subclinical mastitis. The average incidence of clinical mastitis varies from 10 to 20% in most of the herds and the prevalence of intra mammary infection is around 50% of quarters (Radostits *et al*. 2007). Further, the subclinical mastitis (SCM) is 3–4 times more prevalent than clinical mastitis (Bhanderi & Garg 2012). Therefore, milk production loss is more in subclinical mastitis as compared to clinical form because of increase in undesirable milk components like proteolytic enzymes, salts and increase in somatic cell count,

thereby resulting in decrease in the desirable components such as protein, milk fat and lactose (Pyorala 2003). Mastitis causes financial losses to dairy industries through reduced milk yield, poor milk quality, increased culling and also due to additional expenses on treatment and extra labour (Radostits *et al*. 2007). Those animals, which suffered from mastitis even once in life, will never return to their natural milk production state resulting in a great economic loss (Hamadani *et al*. 2013).

Reactive oxygen species (ROS) are normally produced via aerobic metabolism in the mitochondrial electron transport chain (Decoursey *et al*. 2005). These are balanced with host antioxidant defense systems to maintain cellular homeostasis. Large amounts of ROS production that exceed host antioxidant capabilities can cause oxidative stress and consequent inflammatory reactions and diseases (Shi *et al*. 2016). High milk yield can produce oxidative stress in the mammary tissue. High milk yield is also known to be associated with bovine mastitis (Bae *et al*. 2017). Intensive growth of the mammary gland and the synthesis of milk are causes of oxidative stress and inflammation in the mammary gland (Jozwik *et al*. 2012). Both lipid peroxidation and total amount of antioxidants have been found to increase in lactating dairy cows (Castillo *et al*. 2006). When oxidative stress is occurring in cells, antioxidant systems are normally reinforced for a host defensive mechanism. Haeme oxygenase-1 (HO-1) is an antioxidant enzyme that catalyses the conversion of haeme into biliverdin, carbon monoxide, and free iron (Wang *et al*. 2008). Expression of HO-1 is regulated by oxidative stress in cells. HO-1 and the haeme-degradation byproducts play protective roles against cellular oxidative injury (Loboda *et al*. 2016).

The role of cytokines in the pathophysiology of bovine mastitis has been the subject of many studies. Cytokines play a central role in the regulation of immune responses against different infections and variations in their expression are often associated with disease activity in immune-mediated or inflammatory disorders. Modulation of cytokine secretion may offer novel approaches in the treatment of a variety of diseases. One strategy in the modulation of cytokine expression may be using herbal medicines. A class of herbal medicines, known as immune modulators, alters the activity of immune function through the dynamic regulation of inflammatory molecules such as cytokines (Loboda *et al*. 2016).

Antibiotics are used for the treatment of bovine mastitis, but these are associated with the problem of antibiotic resistance. The use of a post-milking germicide is the most effective hygiene practice the dairy farmer can use for the prevention of new intramammary infections (IMI). The number of bacteria residing on teat skin has a direct correlation to the incidence of new intramammary infections. Teat germicides reduce this teat skin bacterial population and, consequently, reduce the number of new intramammary infections (Boddie *et al*. 1993). Commonly used germicides are iodine, chlorhexidine, and sodium hypochlorite. Iodine-based teat products are mostly used to disinfect teats before and after milking (Benavides *et al*. 2016).

The prevalence of mastitis pathogens and their antimicrobial resistance have been investigated in numerous studies around the world. Teat disinfection, both pre- and post-milking has been successfully used for many years to prevent new intramammary infections (Pankey *et al*. 1984). These disinfectant residues pass on to the consumers and effect their health. Drug residues in milk have a potential health hazards effects for the consumer and may cause allergic reactions, interference in the intestinal flora and resistant populations of bacteria in the general populations, thereby rendering antibiotic treatment ineffective (Bharti *et al.* 2012).

Ethnoveterinary medicine for Mastitis: The use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice from time immemorial. It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Of the total 252 drugs in WHO's essential medicine list, 11% is exclusively of plant origin. India has a rich and diversified flora. Almost 75% of the medicinal plants grow naturally in different states of India. In India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine (Sahoo *et al*. 2010). Ethnoveterinary medicine, the scientific term for traditional animal health care, encompasses the knowledge, skills, methods, practices, and beliefs

about animal health care found among the members of a community (Matekaire *et al*. 2004). Veterinary herbal medicines comprise plant-based medicines and their therapeutic, prophylactic, or diagnostic application in animal health care. The side effects of the synthetic drugs such as presence of antibiotic residues leads to antibiotic resistance in humans; the toxic metabolites remain in milk and the byproducts of synthetic drugs become a matter of concern in the long-term usage of such drugs in mastitis. Issues like these have prompted the search for the use of alternatives such as herbal preparations, as these are cheap and safe as compared to modern animal health-care systems (Rastogi *et al*. 2015).

Plants used medicines that are extracted from the herbs are used as intramammary infusion in the dry animals. They are "natural" and usually safe and have clinical and economical value in treating resistant bacteria (Buhner *et al*. 2014). The herbal treatment tested for dairy cows in the dry period is found to be an alternative to antibiotics (Mulled *et al*. 2013). Herbal preparation had a positive effect on the time to recovery from mastitis and increased the rate of bacteriological cure together with improving the reduction of somatic cell count in dairy cows. Pinedo *et al*. (2013) reported that the herbal treatment tested did not negatively affect milk production or somatic cell count and were just as successful as conventional dry cow therapy in curing infections during the dry period. Cows treated with the herbal preparation at dry off had fewer new infections (35%) than no treatment (49%). The consumption of mastitis-affected milk may be harmful to humans as antimicrobial resistant pathogens may be transmitted by contaminated unpasteurized milk; hence is also a major public health concern (Oliver *et al*. 2012). The emerging potential of drug resistance of pathogens coupled with high cost and more side effects of antibiotics have drawn the interest of researchers and general population towards ethnomedicinal plants for the potential discovery of useful compounds (Hassan *et al*. 2014). This review article is an overview of the different medicinal plants used in the treatment of bovine mastitis and highlights their importance as an alternative natural product therapeutic resource.

Role of Essential oils in the treatment of mastitis: Essential oils are natural oils typically obtained by distillation and having the characteristic odour of the plant or other source from which they are extracted. Essential oils are widely prescribed by traditional systems of medicine, all over the world. These have various pharmaceutical and biological activities like, antibacterial, antifungal, anticancer, antimutagenic, antidiabetic, antiviral, anti-inflammatory and antiprotozoal properties (Raut and Karuppayil, 2014).

Dalchini (*Cinnamon cassia* oil): *Cinnamon cassia* oil has the antimicrobial activity against major pathogens causing bacterial bovine mastitis, its miscibility in milk and possible antimicrobial mechanisms. *C. cassia* oil has inhibitory activity against all tested pathogen isolates from bovine mastitis. In disk diffusion assay it was found that discs with 20 µL of 2% (*v/v*) *C. cassia* oil solution resulted in inhibition zones of 29.6, 19.1, 27.0, 33.3 and 30.7 mm for *S. aureus, S. epidermidis, S. hyicus, S. xylosus* and *E. coli* 29, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. cassia* oil was 0.00625% and 0.025% (v/v) for *S. hyicus*, 0.025% and 0.10% (*v/v*) for *E. coli* 29, and 0.0125% and 0.05% for *S. aureus, S. epidermidis* and *S. xylosus*, respectively (Zhu *et al*. 2016). The antimicrobial effect of plant-derived antimicrobials including trans-cinnamaldehyde (TC), eugenol, carvacrol, and thymol on major bacterial mastitis pathogens was investigated in milk. All four plant-derived molecules exhibited antimicrobial activity against *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus,* and *Escherichia coli* (Baskaran *et al.* 2009).

Neem (*Azadiracta indica*): The oil of neem is effective against udder infections. The bark, seeds, leaves and roots of *Azadiracta indica* are used as an insect repellant. Livestock insects such as horn flies, flow flies and biting flies are controlled traditionally using *Azadiracta indica. Azadiracta indica* oil is effective against *E. coli* and mastitis. *Azadiracta indica* can be used as anti-inflammatory and antibacterial drug and can be an alternate therapy against bovine mastitis (Ogbueniu *et al*. 2011).

Haldi (*Curcuma longa*): The *in vitro* antimicrobial activity of different fractions obtained from rhizome of *Curcuma longa* was investigated against standard strain and clinical isolates of *Staphylococcus aureus*. The clinical isolates were found more sensitive for

different fractions, than the standard strain of *S. aureus* (Gupta *et al*. 2015).

Kalojira (*Nigella sativa*): Kalojira extract has potential as a therapeutic agent for *Staphylococcus aureus* infection causing subclinical mastitis of dairy cows and may contribute to the cow's recovery from mastitis. It has been reported that milk somatic cell count of the quarters infected with *Staphylococus aureus* decreased after injection of *Nigella sativa* extract in Holstein cows (Azadi *et al*. 2010).

Senjana (*Moringa oleifera*): Methanol extract (ME) of *Moringa oleifera* leaves has beneficial effects in bovine mammary epithelial cells through its anti-inflammatory, antioxidant and casein production properties. Radical scavenging capacities and anti-inflammatory properties of ME were examined using lipopolysaccharide (LPS)-challenged MAC-T cells. ME showed significant radical scavenging activities. In addition, ME decreased reactive oxygen species produced by LPS in cells. ME also attenuated inflammatory cyclooxygenase-2 expression induced by LPS by down-regulating NF-κB signaling cascade. Moreover, ME ameliorated LPS-induced pro-inflammatory cytokines including tumor necrosis factor-α, interleukin-1β, and interleukin-6. Furthermore, ME up-regulated mRNA expression levels of heme oxygenase-1, NAD(P)H: quinone oxidoreductase-1, and thioredoxin reductase 1. Importantly, ME promoted differentiated MAC-T cells by increasing mRNA expression levels of α-casein S1, α-casein S2, and α-casein. In conclusion, ME has beneficial effects in bovine mammary epithelial cells through its anti-inflammatory, antioxidant, and casein production properties (Cheng *et al*. 2019).

Tulsi (*Ocimum sanctum*): Immunotherapeutic potential of aqueous extract of *O. sanctum* leaf in bovine sub-clinical mastitis (SCM) was investigated. Somatic cell count (SCC), total bacterial count (TBC), milk differential leukocyte count (DLC), phagocytic activity and phagocytic index and leukocyte lysosomal enzymes like myeloperoxidase and acid phosphatase content were evaluated after intramammary infusion of aqueous leaf extract of *O. sanctum*. The results revealed that the aqueous extract of *O. sanctum* treatment reduced the TBC and increased neutrophil and lymphocyte counts with enhanced phagocytic activity and phagocytic index. Similarly, the lysosomal enzymes contents of the milk polymorphonuclear cells (PMNs) were also enhanced significantly in animals treated with the extract. The results suggested that the crude aqueous extract of *O. sanctum* (leaf) possesses some biologically active principles that are antibacterial and immunomodulatory in nature. As such, the present work substantiates the therapeutic use of medicinal herb and also emphasizes on the potential of the commonly available non-toxic substances to enhance the mammary immunity (Mukherjee *et al*. 2005).

Ghrit Kumari (*Aloe vera*): The results show that treatment with *Aloe vera* gel extract disrupted the cell membrane causing lysis in 75% of *Staphylococcus aureus*, in 88% of *E. coli*, in 97% of *Streptococcus uberis*, and in 88% of MRSA cells. Cell membrane disruption is attributed to the presence of anthraquinones (Forno-Bell *et al*. 2019).

Essential oils can substitute antibiotics, being immune enhancers and fight bacterial infections without any potential side effects and leaving harmful residues in the animal body. As alternative to antibiotic treatment, essential oils-based phytochemical still needs to clarify composition and mechanism of action in order to assess the appropriate dose and dosage regimen that should be safely used in cows with diagnosed intramammary infections such as mastitis.

Conclusion

Mastitis is one of the major diseases affecting the productivity of livestock. The antibiotics used for the treatment are showing antimicrobial resistance and affecting the health of people by its residues. Medicinal plant products can be a good alternative to them. Various *in vitro* studies have been conducted and antimicrobial activities of plants have been established however, few *in vivo* or clinical studies have been conducted against mastitis. Phytochemical studies, identification of active compounds and their testing by *in-vitro* and *in-vivo* studies would help in identifying new drug molecules and standardized phytochemicals could find antibiotics place in different pharmaceutical forms. Mitigation methods for the tissue irritation by essential oils is also required. Herbal teat dips, herbal ointments, herbal

disinfectants could be developed to combat mastitis. These novel drugs would be effective in treating mastitis and would overcome the problem of antibiotic resistance. The milk so obtained will be safer for consumption.

Conflicts of interest: The authors declare no conflicts of interest in this review article.

References

Azadi HG and Farzaneh N. 2010. Comparison of two regimens of *Nigella sativa* extract for treatment of subclinical mastitis caused by *Staphylococcus aureus*. American Journal of Applied Sciences **7** (9): 1210-1214.

Bae H, Jeong CH, Cheng WN, Hong K, Seo HG and Han SG. 2017. Oxidative stress-induced inflammatory responses and effects of N-acetylcysteine in bovine mammary alveolar cells. Journal of Dairy Research **84** (4): 418-425.

Baskaran SA, Kazmer GW, Hinckley L, Andrew SM and Venkitanarayanan K. 2009. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens *in vitro*. Journal of Dairy Science **92** (4): 1423-1429.

Benavides ML, Lombard J, Raymond J, Hemling T and Timms L. 2016. Trends in the USA that are challenging the status quo in milking routines. Proceedings of the New Zealand Milk Quallity Conference, ISSN 2253-5349.

Bhanderi BM and Garg MR. 2012. A study on reducing the incidence of sub-clinical mastitis in dairy cows by feeding a vitamins and minerals based strategic feed supplement. Indian Journal of Dairy Science **65** : 388-392.

Bharti SK, Sharma NK, Gupta AK, Murari K and Kumar A. 2012. Pharmacological actions and potential uses of diverse galactogogues in cattle. International Journal of Clinical Pharmacology and Therapeutics **2** (1): 24-28.

Boddie RL, Nickerson SC and Adkinson RW. 1993. Evaluation of teat germicides of low iodine concentrations for prevention of bovine mastitis by Staphylococcus aureus and *Streptococcus agalactiae*. Preventive Veterinary Medicine **16** (2): 111-117.

Buhner SH. 2014. Herbal antibiotics: an effective defense against drug resistant 'Superbugs'. *Mother Earth News*, https://www.motherearthnews.com/natural-health/herbal-antibiotics-defense-zm0z13djzsor/.

Castillo C, Hernandez J, Valverde I, Pereira V, Sotillo J, Alonso ML and Benedito JL. 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Research in Veterinary Science **80** (2): 133-139.

Cheng WN, Jeong CH, Seo HG and Han SG. 2019. Moringa extract attenuates inflammatory responses and increases gene expression of casein in bovine mammary epithelial cells. Animals **9** (7): 391.

Decoursey TE and Ligeti E. 2005. Regulation and termination of NADPH oxidase activity. Cellular and Molecular Life Sciences CMLS **62** (19): 2173-2193.

Dragana ZT, Jovan BS, Ivan MG, Zoran NR, Tijana BK, Nadežda BT, Blagoje PP and Zorana RK. 2022. Review of trends in essential oils as alternatives to antibiotics in bovine mastitis treatment. Matica Srpska Journal for Natural Sciences **142**: 47-60.

Forno-Bell N, Bucarey SA, García D, Iragüen D, Chacón O and San Martín B. 2019. Antimicrobial Effects caused by *Aloe barbadensis* miller on bacteria associated with mastitis in dairy cattle. Natural Product Communications **14** (12).

Garcia A. 2004. Contagious vs. Environmental Mastitis. Extension Extra. Paper 126. Access at http://agbiopubs.sdstate.edu/articles/ExEx4028.pdf.

Gupta A, Mahajan S and Sharma R. 2015. Evaluation of antimicrobial activity of *Curcuma longa* rhizome extract against *Staphylococcus aureus*. Biotechnology reports **1** (6): 51-55.

Hamadani H, Khan AA, Banday MT, Ashraf I, Handoo N, Bashir A and Hamadani A. 2013. Bovine mastitis- A disease of serious concern for dairy farmers. International Journal of Livestock Research **3** (1): 42-55.

Hassan H, Murad W, Tariq A and Ahmad A. 2014. Ethnoveterinary study of medicinal plants in Malakand Valley, district Dir (lower), Khyber Pakhtunkhwa, Pakistan. Irish Veterinary Journal **67** (1): 1-6.

Jozwik A, Krzyzewski J, Strzalkowska N, Polawska E, Bagnicka E,Wierzbicka A, Niemczuk K, Lipinska P and Horbanczuk JO. 2012. Relations between the oxidative status, mastitis, milk quality and disorders of reproductive functions in dairy cows – a review. Animal Science Papers and Reports **30** (4): 297-307.

Loboda A, Damulewicz M, Pyza E, Jozkowicz A and Dulak J. 2016. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cellular and Molecular Life Sciences **73** (17): 3221-3247.

Matekaire T and Bwakura TM. 2004. Ethnoveterinary medicine: A potential alternative to orthodox animal health delivery in Zimbabwe. International Journal of

Applied Research in Veterinary Medicine **2** (4): 269-273.

Mukherjee R, Dash PK and Ram GC. 2005. Immunotherapeutic potential of *Ocimum sanctum* (L) in bovine subclinical mastitis. Research in Veterinary Science **79** (1): 37-43.

Mullen KA, Sparks LG, Lyman RL, Washburn SP and Anderson KL. 2013. Comparisons of milk quality on North Carolina organic and conventional dairies. Journal of Dairy Science **96** (10): 6753-6762.

Ogbueniu IP, Odomenam VU, Obikaonu HO, Opara MN, Emenalom OO, Uchegbu, MC, Okoli IC, Esonu BO and Loej MU. 2011. The growing importance of neem (*Azadirachta indica*) in agriculture industry, medicine and environment: A Review. Research Journal of Medicinal Plants **5**: 230-245.

Oliver SP and Murinda SE. 2012. Antimicrobial resistance of mastitis pathogens. Veterinary Clinics of North America: Food Animal Practice **28** (2): 165-185.

Pankey JW, Eberhart RJ, Cuming AL, Daggett RD, Farnsworth RJ and McDuff CK. 1984. Uptake on post milking teat antisepsis. Journal of Dairy Science **67** (6): 1336-1353.

Pinedo P, Karreman H, Bothe H, Velez J, and Risco C. 2013. Efficacy of a botanical preparation for the intramammary treatment of clinical mastitis on an organic dairy farm. The Canadian Veterinary Journal **54** (5): 479.

Pyorala S. 2003. Indicators of inflammation in the diagnosis of mastitis. Veterinary Research **34** (5): 565-578.

Radostits OM, Gay CC, Hinchcliff KW and Constable PD. 2007. A textbook of the diseases of cattle, horses, sheep, pigs and goats P- 2045-2050.

Radostits OM, Blood DC and Gay CC. 2010. Veterinary medicine—A textbook of diseases of cattle, sheep, pigs, goats and horses (8th ed.) Bailliere Tindall, London. P-1507–1516.

Rastogi S, Pandey MK, Prakash J, Sharma A and Singh GN. 2015. Veterinary herbal medicines in India. Pharmacognosy Reviews **9** (18): 155.

Raut JS and Karuppayil SM., 2014. A status review on the medicinal properties of essential oils. Industrial crops and Products **62** :250-264.

Reshi AA, Husain I, Bhat SA, Rehman MU, Razak R, Bilal S and Mir MR. 2015. Bovine mastitis as an evolving disease and its impact on the dairy industry. International Journal of Current Research and Review **7**(5): 48.

Sahoo N, Manchikanti P and Dey S. 2010. Herbal drugs: standards and regulation. Fitoterapia **81** (6): 462-471.

Shi H, Yan S, Jin L, Shi B and Guo X. 2016. Vitamin A affects the expression of antioxidant genes in bovine mammary epithelial cells with oxidative stress induced by diethylene triamine-nitric oxide polymer. Czech Journal of Animal Science **61** (3): 117-126.

Smith KL, Todhunter DA and Schoenberger PS. 1985. Environmental mastitis: cause, prevalence, prevention. Journal of Dairy Science **68** (6): 1531-53.

Wang X, Stavchansky S, Zhao B, Bynum JA, Kerwin SM and Bowman PD. 2008. Cytoprotection of human endothelial cells from menadione cytotoxicityby caffeic acid phenethyl ester: the role of heme oxygenase-1. European Journal of Pharmacology **591**: 28–35.

Zhu H, Du M, Fox L and Zhu MJ. 2016. Bactericidal effects of Cinnamon cassia oil against bovine mastitis bacterial pathogens. Food Control **66**: 291-299.