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https://doi.org/10.61900/SPJVS.2023.04.10

THERAPEUTIC ALTERNATIVES IN MAMMARY GLAND INFECTIONS IN COWS

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Abstract

Mastitis caused by infectious pathogens is still considered a ravaging disease of dairy cattle, affecting animal welfare. Economically, this condition appears in the dairy industry through reduced production performance and increased culling rates. Bovine mastitis is a mammary gland inflammation, most commonly caused by bacterial pathogens. Routine diagnosis is based on detecting clinical and subclinical forms of the disease. This highlights the importance of rapid detection of etiological agents at the farm level, for which several diagnostic techniques have been developed. Due to the predominance of bacterial etiology, treatment in mastitis is mainly based on the use of antibiotics. Nevertheless, antibiotic therapy has some limitations due to antimicrobial resistance, treatment efficacy, and costs at the farm level. Research needs to be directed toward developing new therapeutic agents/techniques that can replace conventional methods and address the problem of antibiotic resistance. The objective of the article is to briefly describe the current findings and results of herbal therapy as an adjuvant in the management of mammary gland infections.

Key words: bovine mastitis; treatment; herbs; therapy

INTRODUCTION

Mastitis is one of the bovine diseases, among the pathologies that particularly affect animal welfare and the economy. It adversely affects the profit benefits of livestock producers/farmers and leads to a large loss of production in the dairy sector worldwide (Bardhan, 2013; Sinha et al., 2014; Izquierdo et al., 2017; Aghamohammadi et al., 2018; Das et al., 2018). Mastitis in cattle is the mammary gland inflammation (intramammary inflammation, IMI) in cows. The disease is mainly caused by bacterial infections and is classified into two types based on epidemiology, namely contagious mastitis and environmental mastitis (Garcia, 2004). The former is caused by contagious bacteria, including *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. which are transmitted from an infected cow to a healthy cow, usually at the time of milking, via hands, towels, and the milking machine, serving as a reservoir for the bacteria. In contrast, environmental mastitis is caused by bacteria that are mainly spread outside the milking parlor, i.e. the causative bacteria originate from the cow's environment, such as bedding material, soil, manure, feces, and stale water (Garcia, 2004). Bovine mastitis leads to an increase in the cost of animal husbandry in terms of milk production. In addition, it also harms milk composition as well as milk value (Halasa et al., 2007; Kalinska et al., 2017). Environmental mastitis is strongly influenced by management practices (Garcia, 2004) and therefore requires better technical and biological tools along with appropriate incentives and encouragement. Farmers and field veterinarians need to work according to official guidelines for using approved antimicrobials (Klaas and Zadoks, 2018). Over the past century, significant progress has been made to keep mastitis under control; but due to changing population dynamics, herd structure, and more stringent processor standards that make mastitis is a complicated disease and remains a major problem in the dairy industry. Thus, further extensive research is called for (Ruegg, 2017a).

In cattle and buffalo, mastitis is an important economic problem worldwide, including in India (Das et al., 2018), Canada (Aghamohammadi et al., 2018), Germany (Hamann, 2001), the United

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Kingdom (Bennett et al., 1999), the Netherlands (Hogeveen et al., 2011) and the United States (Hadrich et al., 2018). Bovine mastitis is related to a daily loss that ranges from 1.0 to 2.5 kg of milk in the first two weeks after onset and a total loss of 110 to 552 kg throughout lactation, depending on parity and time of onset. Mastitis also has a long-lasting effect on milk production, as cows will not regain their maximum milk yield during the remaining lactation period (Rajala-Schultz et al., 1999). Despite various advanced dairy cattle and buffalo husbandry management practices, mastitis is still a threatening disease and is among the major economic problems of dairy farmers and dairy farm owners. India ranks first among the top milk-producing countries (cow and buffalo milk combined). Economic losses due to mastitis in India are about Rs. 575 million per year and reduce milk by 21% (Bardhan, 2013). In addition, consumption of milk that has been affected by mastitis can be harmful to humans because antimicrobial-resistant pathogens can be transmitted through contaminated unpasteurized milk; therefore, it is also a major public health concern/hazard (Oliver and Murinda, 2012). In addition, health risks associated with increasing microbial resistance and antibiotic residues in milk have led to increased consumer demand for organic products, as consumers consider food produced by conventional farming systems to be healthier and safer for consumption (Ruegg, 2009). Due to zoonotic threats, mastitis milk cannot be consumed and also cannot be sold; thus, contributing to major economic losses. Infected udder reduces the market price of animals and imposes an economic burden on the owner due to treatment costs (Gonzalez and Wilson, 2003; Seegers et al., 2003).

Although the association between mastitis and pathogenic microorganisms was established in 1887, the main pathogens were not identified until the 1940s. The discovery of the multifactorial etiology of bovine mastitis in the 1960s initiated further mastitis research (Singh and Singh, 1994; Ndlela et al., 2016), including the identification of common etiological agents, which are both Gram-positive and Gram-negative bacteria, such as *S. agalactiae*, *S. aureus*, *Escherichia coli* and *Klebsiella pneumoniae*; molecular epidemiology of causal pathogens; comparative pathogen typing methods at the subspecies level; virulence gene arrays; whole genome sequencing and *in vitro* antibiotic susceptibility pattern investigations. Over time, antibiotic therapy (penicillin) became available by 1945 but was not effective against all pathogens causing mastitis. There is a need for management practices targeting the pre-calving period in heifers to reduce the likelihood of mastitis in later stages (Naqvi et al., 2018). Generally, subclinical mastitis and IMIs in heifers during calving are predominantly caused by major pathogens, namely coagulase-negative staphylococci leading to mastitis in heifers. Early in the lactation period, IMIs are influenced by many factors, including the nature of the disease, the virulence of the pathogen, time of onset to calving, persistence of infection/cure, host immunity, gestation stage, and management practices, including risk associated with the season and herd location. Short-term antibiotic treatment before calving is an effective control measure for mastitis in heifers but is hardly ever recommended due to long-lasting adverse effects on udder health and milk production, thus reducing profitability for farmers (De Vliegher et al., 2012).

Mastitis diagnosis is an important demand of the dairy industry for safe milk production, not only for economic and public health reasons but also in terms of animal welfare. Diagnosis must be performed early, rapidly, and accurately for the prevention of mastitis or early detection of mastitis for management or therapeutic purposes. This involves the application of conventional as well as advanced diagnostic tests. Conventional methods are relatively cheap, easy, readily available, and applicable in the field, but usually non-specific. Advanced tests are expensive, and require technical skills and sophisticated infrastructure and facilities, but are usually accurate and specific for different forms of mastitis (Swarup et al., 1989; Singh et al., 2013; Hussein et al., 2018; Chakraborty et al., 2019).

Blanket dry cow therapy, strategic culling, and well-established biosecurity procedures are effective measures to control and prevent reintroduction of other virulent strains of *S. agalactiae* and *S. aureus* (Kefee, 2012). In addition, the combination of antibiotic treatment and culling of cows that do not respond to treatment has been shown to decrease transmission rates and reduce IMIs (Halasa, 2012). Several types of conventional and advanced therapeutic approaches are available for the management of mastitis, which includes antibiotics, vaccination, nanoparticle therapy, herbal therapy, and bacteriocins (Gomes and Henriques, 2016). Various agents help reduce udder infections, especially mastitis in cows and also help improve milk quality (Skowron et al., 2019). Out of these, antibiotic therapy and vaccination are commonly used methods for the treatment of mastitis. The extensive and uncontrolled use of antibiotics for treatment, along with the induction and persistence of biofilm-associated antibiotic resistance in mastitis has caused a decreased response to antibiotic therapy (Park et al., 2012; Babra et al., 2013). Although vaccination is not effective against bovine mastitis because a variety of microorganisms are involved in its development, *S. aureus*, *Streptococcus uberis*, and *E. coli* have been considered to be the main targets for vaccine development (Wilson et al., 2009; Bradley et al., 2015; Collado et al., 2016; Ashraf and Imran, 2020). Although several commercial vaccines are available, most of them fail to provide sufficient protection and at the same time are expensive (Cote-Gravel and Malouin, 2019).

Mastitis-causing pathogens. The vast majority of pathogens causing clinical bovine mastitis are of environmental or ubiquitous origin. In comparison, contagious agents are mainly related to subclinical infections (Abebe et al., 2016; Klaas and Zadoks, 2018). Mastitis is a multi-etiological infection and some bacteria are mainly responsible for clinical, subclinical, contagious, and environmental mastitis. The most common bacteria involved are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Trueperella pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacter aerogenes*, and *Pasteurella* spp. (Levison et al., 2016; Abdalhamed et al., 2018; Shinozuka et al., 2018; Zhang et al., 2018). Out of these, the contagious pathogens are *S. aureus*, *S. dysgalactiae*, and *S. agalactiae*. *S. aureus* is the predominant organism, while the main environmental pathogens are members of the Enterobacteriaceae, notably *E. coli* and *S. uberis* (Petersson-Wolfe et al., 2010). *S. agalactiae* is the most common Gram-positive bacteria in clinical mastitis, followed by *S. aureus*, while *Klebsiella* spp. and *E. coli* were the most isolated Gram-negative bacteria in clinical mastitis (Cortinhas et al., 2016). *S. agalactiae* and *S. aureus* disseminate mainly through contact, so herd biosecurity can be considered an important preventive measure to minimize and eliminate the reservoirs (Kefee, 2012).

Both clinical and subclinical forms of mastitis can be attributed to most bacterial pathogens. However, *T. pyogenes* is exclusively responsible for causing clinical forms of mastitis (Malinowski et al., 2006). In primiparous cows, the highest milk loss is due to *S. aureus*, *Klebsiella* spp., and *E. coli*. In older cows, substantial losses are due to infections with *Streptococcus* spp., *T. pyogenes*, *S. aureus*, *Klebsiella* spp., and *E. coli* (Grohn et al., 2004). In general, *S. aureus*, *S. agalactiae*, and *S. uberis* are common pathogens causing mastitis, while *Mycoplasma bovis* and *Corynebacterium bovis* are less often involved (Wernicki et al., 2014; Vakkamaki et al., 2017). Coagulase-negative staphylococci and their role in causing mastitis should also be seriously considered (Krukowski et al., 2001). Wilson et al. (1997) reported that *S. agalactiae* and various pathogens, including *Prototheca* spp., *Streptococcus* spp., and *T. pyogenes*, are associated with most cases of mastitis. Mastitis shows its most severe form when it is associated with infections due to coliforms, CAMP-negative *Streptococcus* spp., *T. pyogenes*, *S. agalactiae*, fungi (yeast-like), and *Prototheca* spp. (Wilson et al., 1997; Malinowski et al., 2006). *Corynebacterium* spp. (40%) and *S. aureus* (32%) were the most frequent isolates found by Steele and McDougall (2014) in subclinical mastitis cases in New Zealand. *Prototheca* spp. are pathogenic algae and opportunistic pathogens that cause mastitis in dairy herds and pose zoonotic potential (Alves et al., 2017; Dos Anjos et al., 2019).

S. aureus was the most common pathogen identified in cases of mastitis (McParland et al., 2019). Methicillin-resistant *S. aureus* (MRSA) CC22-MRSA-IV was reported as an intramammary pathogen by Magro et al. (2018). On genotyping by DNA microarrays, MRSA was noted as an epidemic UK-EMRSA 15 grouping in CC22. These isolated strains had resistance genes for β-lactams and macrolides. The isolates were obtained from milkers and dairy cows, suggesting reverse zoonosis. Routine milk sampling and evaluation identified the presence of mastitis-causing pathogens in 13% of all samples obtained from dairy herds. Out of the pathogens isolated, *S. aureus*, *Streptococcus* spp., *T. pyogenes*, and *C. bovis* were found to be the most common pathogens (Cvetnic et al., 2016).

Type of mastitis and clinical relevance. Epidemiologically, mastitis can be classified into contagious and environmental mastitis and is caused by a broad spectrum of pathogens. Furthermore, mastitis can also be categorized as clinical or subclinical forms (Garcia, 2004; Abebe et al., 2016). Any increase in humidity and pollution in the barn environment also increases the load of bacterial pathogens in the environment. One study showed a 74.7% prevalence of mastitis in the herd and 62.6% in cows. Regarding subclinical and clinical mastitis, the former type seems to be responsible for the majority of cases (59.2%) compared to the latter (3.4%) (Abebe et al., 2016). Clinical mastitis can be easily identified based on obvious symptoms in terms of inflammation of the udder, which shows redness in the affected part or the whole udder, increased temperature, swelling, pain on touch, milk clots, discoloration, and changes in milk consistency. General symptoms are pyrexia (> 39.5 C) and loss of appetite. Environmental pathogens, including coliforms, induce major causes of clinical mastitis. Of the 20,000 cases of clinical mastitis in the Netherlands, 40% were caused by *S. uberis* and *S. dysgalactiae*, 30% by *S. aureus*, and 30% by *E. coli* (Steeneveld et al., 2011). Cow udder can show decreased susceptibility as well as resistance to the inflammatory state under certain conditions. These conditions include the administration of antibiotics to the udder for prolonged periods, a higher incidence of udder mycosis due to mineral-vitamin and antioxidant deficiencies, dietary imbalance, poor environmental conditions, and climate change (Wawron et al., 2010).

Kumar et al. (2010) have studied the incidence and economics of clinical mastitis. Compared to the clinical form, in subclinical mastitis, there are no clinically visible symptoms, although a change in milk composition may be an indicator. It is therefore recognized and confirmed by laboratory examination of milk or by animal tests such as the California mastitis test (CMT) followed by laboratory isolation of the aetiological agent.

The somatic cell count (SCC) in milk should be less than 200,000 per ml in a healthy cow. Somatic cells are mostly white blood cells (WBC), i.e. infiltrating neutrophils, as well as macrophages into mammary gland tissue as a result of inflammation (Akers and Nickerson, 2011). *S. agalactiae* localizes mainly in the udder and causes persistent infections with higher SCC (Kefee, 2012).

Mastitis is the result of the host's immune response to infectious agents affecting the udder (Gurjar et al., 2012). Usually, there is a balanced microflora in a healthy udder. The intramammary microbiota is made up of a complex community of various bacteria (Rainard, 2017; Andrews et al., 2019). The commensal mammary microbiota present in the healthy udder plays a major role in immune homeostasis (Derakhshani et al., 2018). As a result, a disruption of the diversity of the microbiota in the udder (dysbiosis) can have an important effect on the udder. The normal microbiome of the udder is an important factor to consider when making a diagnosis of mastitis, as healthy quarters also contain some bacteria. Different bacterial genera such as *Ruminococcus, Oscillospira, Roseburia, Dorea, Prevotella, Bacteroides, Paludibacter,* and *Bifidobacterium* are usually present in the udder. Any injury or congenital anomaly of the udder or teat, such as teat fistula, leaky teat, and udder injury that exposes the udder to external microbes or milk retention tends to cause mastitis (Rambabu et al., 2011).

In one study, tissue affected as a result of mastitis showed significant inflammation with marked decreases in alveolar epithelium and lumen, while histopathologically, an increase in stromal connective tissue was reported along with leukocytosis (Nickerson et al., 1995). These types of conditions either expose the udder to external pathogens or weaken internal resistance. In the clinical form of mastitis, *Staphylococcus* spp. or *E. coli* predominates and the normal microbiota is disrupted. The researchers proposed that because of either alteration of the normal microbiome due to pathogens or prolonged use of antibiotic therapy, mastitis is induced and established (Falentin et al., 2016). In a detailed molecular epidemiological study, most dairy cattle in the U.S. were found to carry more than 10 species of coagulase-negative staphylococci (CNS), and the bacteria were isolated at various stages of lactation (Jenkins et al., 2019).

Mastitis is a complex of negative outcomes of various factors acting together at the host level. These factors include pathogens, their growth pattern in the udder parenchyma, signaling pathways for the establishment of clinical manifestations, and various molecular mechanisms mediated by pathogen-associated molecular patterns (PAMPs). Doing so is possible through various host pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-like receptors (RIGs), which trigger inflammation of the udder due to microbial infections, along with several environmental factors. Therefore, it requires a collaborative approach to diagnosis as well as control of this important disease (Bhattarai et al., 2018).

Advances in treatment of mastitis. An effective and efficient mastitis control program involves early identification of infection through understanding the pathogenesis, development of new sensitive tests for early detection, adoption of good management practices to reduce the possibility of transmission, and prevention of uninfected transmission. The control program should include the strategic use of antimicrobials to reduce problems with antibiotic residues in milk and antimicrobial resistance (Ruegg et al., 2017a). Before drug therapy, the primary cause of udder infection should be clarified. Teat or udder conditions such as teat fistula, leaky teat, teat spider, and udder wounds require immediate attention. Because such conditions rupture the effective barrier and tend to expose the teat canal or udder to external microbes; therefore, prompt remedy is required.

Successful treatment of clinical mastitis depends on several factors: antimicrobial treatment, identification of the causative agent, parity, lactation status, history of previous SCC, clinical mastitis, and other systemic disorders (Steeneveld et al., 2011).

In a study conducted under Dutch circumstances, the cow-specific treatment recommended for clinical mastitis was not found to be economically beneficial (Steeneveld et al., 2011). Still, herd-specific interventions, such as cow-specific treatment and culling strategies against subclinical and clinical IMIs, can turn out to be highly cost-effective in managing mastitis (Gussmann et al., 2019).

Management of mastitis involves both preventive and therapeutic strategies and is mainly based on antibiotic therapy. However, recent approaches used to treat mastitis involve the usage of natural therapies, such as zeolites and propolis, which could potentially act as an alternative to antibiotic therapy (Benic et al., 2018).

Herbal therapy. Herbal therapy is a promising area in the treatment of mastitis as there are no associated adverse effects. Ethno-veterinary medicine is a branch of veterinary medicine that focuses on the treatment of diseases with herbal formulations (Tiwari et al., 2018). Herbs can be used as a therapeutic alternative or as an adjuvant in the management of mastitis in cattle. They can be used as an antibacterial, anti-inflammatory, and immunomodulatory agent for the treatment of mastitis (Mushtaq et al., 2018). The anti-inflammatory and antibacterial effects of Chinese herbs have been effectively used in the treatment of bovine mastitis (Muluye et al., 2014; Yang et al., 2019). Also, they can be used as a substitute for antibiotics and antipyretic drugs that are commonly used in the treatment of mastitis (Muluye et al., 2014). Ranjith et al. (2018) reported that methanolic extracts of herbal preparation that contain *Diploclisia glaucescens* leaves and *Curcuma longa* rhizomes in equal ratios produced analgesic activity along with anti-inflammatory activity. The analgesic activity of the herbal extract was found to be comparable to that of ibuprofen and indomethacin (Ranjith et al., 2018). Herbal therapy involves a variety of routes of administration depending on the type of formulation. Of these, topical routes (Hase et al., 2013), oral administration (Dash et al., 2016), and intramammary routes (Yang et al., 2019) are the most commonly used methods. In a comparative study performed to evaluate the efficacy of homeopathic complex therapy, herbal therapy (Neem seed extract), and antibiotic therapy for the treatment of subclinical mastitis in dairy buffaloes, antibiotic therapy was found to have superior efficacy compared to herbal therapy (Neem seed extract) and homeopathic complex therapy groups. When the cost factor was taken into account, herbal therapy was found to be the least expensive (Younus et al., 2018). Therefore, it can be effectively used as an adjuvant to antibiotics in the treatment of clinical mastitis without causing a large change in the cost factor. Some herbal extracts may have anti-inflammatory and antioxidant values that help heal udder inflammation and minimize oxidative stress. *Moringa* extract has been found to ameliorate inflammatory mediators and enhance antioxidant systems in bovine udder epithelial cells. This inhibited the expression of proinflammatory cytokines (TNF-α, IL-β, and IL-6), cyclooxygenase-2 expression and reduced NF-kβ, increased heme-oxygenase-1, NAD(P)H and quinone oxidoreductase-1, besides that *Moringa* extract increased the expression of casein proteins (Cheng et al., 2019).

Several plant species are used for the prevention and control of bovine mastitis in southern Brazil due to their anti-inflammatory, immunomodulatory, and antibiotic effects (Avancini et al., 2008; Xu et al., 2015). The leaves, bark, bulbs, and aerial parts have been used to prepare medicinal plants. The leaves, bark, bulbs, and aerial parts have been used to prepare medicinal plants. Species of plants such as *Achillea millefolium, Allium sativum, Alternanthera brasiliana*, *Baccharis trimera*, *Chenopodium ambrosioides*, *Cuphea carthagenensis, Foeniculum vulgare*, *Phytolacca dioica*, *Sambucus nigra*, *Sida rhombifolia*, *Solanum mauritianum*, *Atractylodis macrocephalae Koidz*, and *Solidago chilensis*, have been used orally, of which *Alternanthera brasiliana*, *Baccharis trimera* and *Sambucus nigra* have also been used as topical agents. *Ocimum basilicum* and *Parapiptadenia rigida* are the two plant species that have been used intramammary in bovine mastitis (Avancini et al., 2008). *Staphylococcus epidermidis* is one of the major causes of medical device infections and bovine mastitis due to its biofilm-forming ability. *Oxytropis glabra* is a Fabaceae species that is extensively used as a Chinese herbal formula in western China. *In vitro* studies performed to evaluate the effect of *O. glabra* decoction on *S. epidermidis* biofilm formation have identified a potential inhibitory mechanism that can be further explored in the development of new drugs against biofilm-associated infections (Ren et al., 2020). In one study evaluating the efficacy of *Ocimum sanctum* leaf juice as supportive therapy for the management of chronic staphylococcal-induced

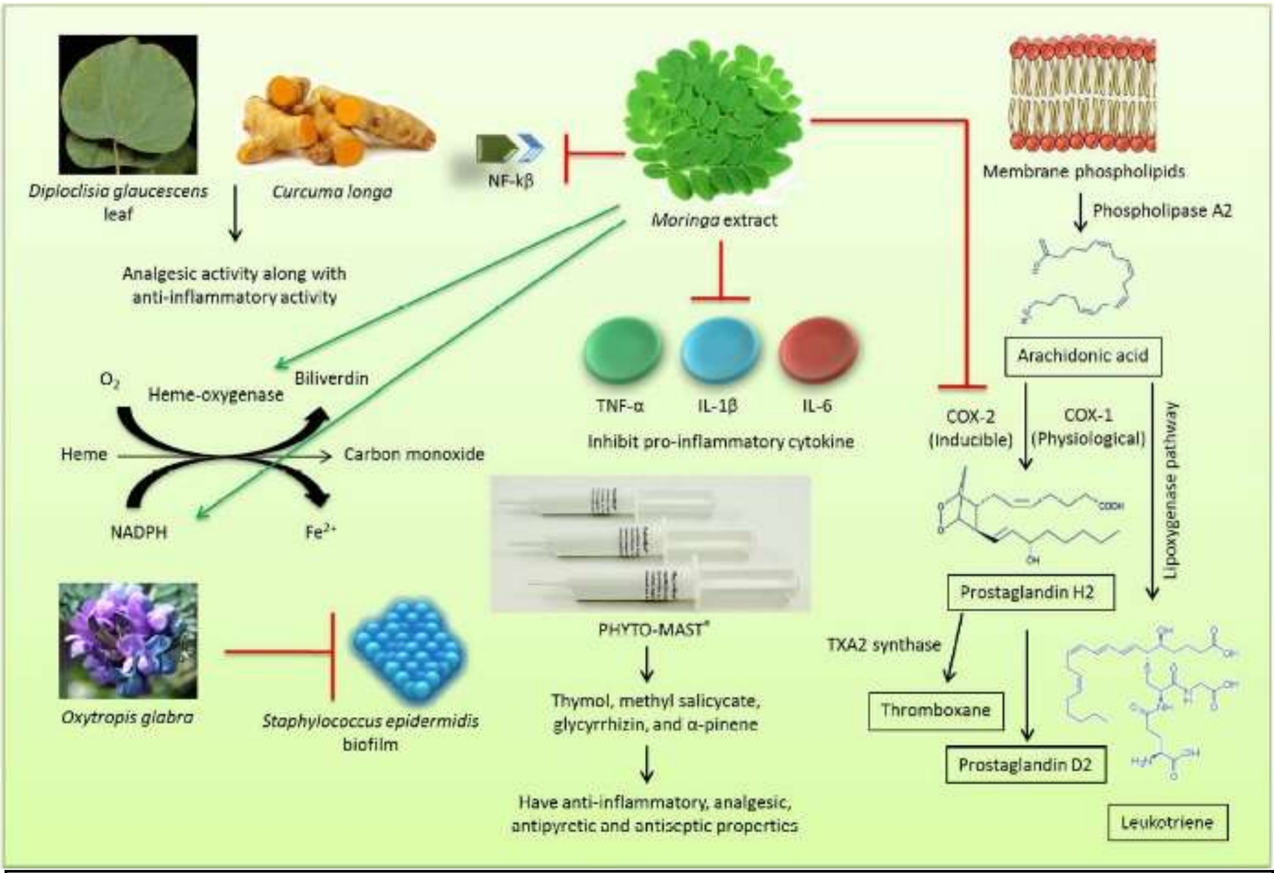

Figure 1. **Herbal therapy for the treatment of mastitis. Various herbs possess anti-inflammatory and analgesic properties**

mastitis, it was found that the leaf extract had significant bio-enhancing and antioxidant activities, which can be effectively used in combination with antibiotics (Dash et al., 2016). Therefore, instead of using herbal therapy as a single agent in the management of clinical mastitis, better results can be achieved if they are included in the treatment protocol as an adjuvant, along with other modalities of therapy. In a recent study aimed at evaluating the *in vitro* antibacterial activity of ethyl acetate extract of the *Terminalia chebula* plant against molecularly identified isolates of *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Bacillus megaterium*, it was observed that a concentration of 500 μg/mL of extract had the same antibacterial efficacy as standard amoxicillin (Kher et al., 2019). This result provides insight into the potential of plant extracts to replace antibiotics as a single agent in the management of clinical mastitis.

Botanical preparations, such as PHYTO-MAST®, have ingredients (thymol, methyl salicylate, glycyrrhizin, and α-pinene) considered within the „Generally Recognized as Safe" United States Food and Drug Administration (FDA). The ingredients have anti-inflammatory, analgesic, antipyretic, and antiseptic properties and may be effective in treating mastitis (McPhee et al., 2011). However, one study failed to demonstrate any therapeutic effect after 3 days of repeated intramammary application at 12-hour intervals (Pinedo et al., 2013).

An overview of the role and mode of action of herbal therapy for the treatment of mastitis is shown in Figure 1.

CONCLUSIONS

Mastitis affects animal well-being and causes economic and production losses through deterioration of milk quality, reduced production performance, increased culling rate, cost of treatment, and due to mortality associated with the acute form of the disease. Several strains of microorganisms can cause both clinical and subclinical forms of the disease. Subclinical mastitis is more economically important than clinical mastitis because of its ability to deteriorate the quality of milk to such a high level that it cannot be detected at first sight, but will affect the overall quality.

Once mastitis is diagnosed, the main challenge for the veterinarian or producer is to manage the animals in such a way that they do not get more severe and become an economic loss to the production system. Several therapeutic strategies, such as antibiotics, vaccines, bacteriocins, herbal therapy, immunotherapy, and nanoparticle technology, were evaluated for their effectiveness in treating mastitis, but no single technique is effective in controlling or treating the disease because of the variable response of the etiological agents to therapeutic techniques. To date, antibiotics have been widely used as the main therapeutic agent in the management of mastitis, but with the emergence of bacterial resistance, which has arisen due to the uncontrolled use of antibiotics, several other treatment options are being investigated. The development of a universal therapeutic agent/technique that can be seen as a substitute for antibiotic therapy is a necessity of this century.

One such therapeutic agent/technique can overcome the emerging problem of bacterial resistance. Future research should be directed towards advanced therapeutic strategies that can provide a solution to the current situation.

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