Formulation, Optimization and Pharmacological Evaluation of Lipid Based Therapeutic System for Bovine Mastitis

Summary

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Bovine mastitis is an inflammatory condition of mammary gland of lactating animals, characterized by pain, oedema, swelling, and polymorph neutrophils (PMN) infiltration. Mastitis is curse for dairy industry as it decreases the productivity and quality of milk, and increase the cost of herd management**.** Currently, antibiotics either alone or in combination with nonsteroidal anti-inflammatory agents (NSAIDs) are most commonly prescribed for clinical management of bovine mastitis. However, long term use of antibiotics causes bacterial resistance and have negative impact on consumer health. Therefore, alternative safer drugs with universal effectiveness, lasting benefits and fewer side effects is requisite in the area of mastitis management.

Polyunsaturated fatty acids (PUFA's) are the set of fatty acids with vital pharmacological activities. Many characteristic seed oils and few nuts comprises large quantity of PUFA's. Linseed (flaxseed) oil normally comprises of 45–55% of ω-3 PUFA's as alpha linolenic acid [ALA (ω-3:18-3)], while soybean oil, rapeseed oil, and walnuts generally contain ~10% of PUFA's as ALA (ω-3:18-3). ω-3 and ω-6 PUFA's are biologically more important fatty acids.

PUFA's and corresponding metabolites have extensive variety of physiological activity, including cell membrane structure uniformity, signalling, and regulation of proteins expression. ω-3 fatty acids are perhaps one of the most suitable examples of how diet may affect inflammation. ω-3 fats exert a remarkable variety of biological responses many of which affect inflammation and clinical conditions related to its presence. α-Linolenic acid (ALA; 18:3) is a ω-3 PUFA and is transformed into anti-inflammatory eicosanoids through series of desaturation and elongation reactions. It would be interesting to comment that the *L. usitattissimum* fixed oil, containing 57.38% of ALA (ω-3:18-3) has been found to exhibit anti-inflammatory, analgesic, and antimicrobial activity. Furthermore, the same has been reported to exhibit significant efficacy against

subclinical cases of bovine mastitis in a field based study. Considering the same, we selected the ALA as the lipid source for formulation with the cefotaxime. Total eight number of ALA based intra-mammary nanosuspension (ALA-NS) were formulated, optimized and evaluated. Only two formulation (ALA-NS; F1 and F2) were found stable after optimization. Furthermore, particle size, zeta potential, polydispersity index, sedimentation volume, stability and sterility testing were performed for both ALA-NS (F1 and F2) formulation.

Moreover, ALA-NS (F1 and F2) were evaluated for antimicrobial activity against mastitis causing pathogens like e.g., *Staphylococcus aureus* (ATCC-29737), *Streptococcus agalactiae* (ATCC-13813), *Staphylococcus epidermidis* (ATCC-12228), *Escherichia coli (*ATCC-8739, MTCC-118) *Lactobacillus sporogenes* (ATCC-31284), *Pseudomonas aeruginosa* (ATCC-25619) and *Candida albicans* (ATCC-10231). ALA-NS (F1 and F2) showed better antimicrobial activity and lower minimum inhibitory concentration (MIC) value than cefotaxime and ALA.

Considering the same, therapeutic efficacy of ALA-NS (F1 and F2) was analyzed against lipopolysaccharides (LPS) induced mastitis in female albino Wistar rats. ALA-NS (F1 and F2) were also evident for the regulation of oxidative markers like decreases the thiobarbituric acid reactive species (TBARs) and protein carbonyl level with restoration of superoxide dismutase (SOD), catalase and glutathione enzymes along with restoration of histological architecture by decreasing the inflammatory cells (leucocytes) infiltration, adipose tissue, and thickening the alveolus walls in comparison to control. Subsequently, ALA-NS (F1 and F2) also restored altered nitric oxide and hydrogen sulphide level after LPS administration.

When perceived through more stringent biomarkers through western blot analysis. ALA-NS (F1 and F2) downregulated the inflammatory markers [nuclear factor kappa

(NFκBp65), cyclooxygenase (COX), lipoxygenase (LOX), interferon- γ (IFN- γ), hypoxia and fatty acid synthesis markers [hypoxia-inducible factor-1 (HIF-1), prolyl hydroxylase-2 (PHD-2), sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FASN) in LPS induced mastitis in rats. Inflammation in mastitis leads to cell death and therefore we further evaluated the apoptotic marker in mammary gland tissue. ALA-NS (F1 and F2) favorably regulated the apoptotic markers and therefore endorsed apoptosis.

Furthermore, the ALA-NS (F1 and F2) was scrutinized through on field base study on subclinical mastitic cows. Nine mix-breed cows were divided into three groups and subjected to treatment for ALA-NS (F1 and F2) and cefoperzone intramammary suspension for 10 days. Subclinical mastitis on day 1 was confirmed through field based tests like pH, california mastitis test (CMT), white side test (WST), and bromothymol blue test score (BBT). The milk sample were collected on 1^{st}, 5^{th} and 10^{th} days of study for further evaluation. The mastitic milk was also recorded for the increased somatic cell count (SCC) and total microbial count (TMC) on day 1. Subsequently, treatment with ALA-NS (F1 and F2) decreased the milk pH along with CMT, WST, and BBT score after 10^{th} days of treatment.

Treatment with ALA-NS (F1 and F2) demarcated a significant effect on field based parameters along with curtailment of TMC, and SCC. The efficacy of ALA-NS (F1 and F2) was further affirmed using more stringent markers for inflammation (NFκB-p65), milk quality (SREBP-1c) and bacterial resistance (UCHL-1) in milk samples. The treatment with ALA-NS decreased the expression of NFκB-p65, SREBP-1c, and UCHL-1 after 10 day of treatment.

To conclude, the treatment of mastitis majorly depends upon antibiotics and NSAIDs. However, antibiotics have several limitations like resistance and secreted in milk. The

current study demonstrated that the intramammary injection of ALA-NS have anti-inflammatory and antibacterial effects with efficacy against sub-clinical mastitis. ALA being peripheral analgesic also subsides the pain, providing additional benefit. Apparently, anti-inflammatory, antibacterial, peripheral analgesic properties of ALA-NS could be accounted for the therapeutic efficacy of proposed regime.