Pharmacological Study

Evaluation of a topical herbal drug for its *in-vivo* immunomodulatory effect on cytokines production and antibacterial activity in bovine subclinical mastitis

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Abstract

Background: Antibiotics have been in use in the treatment of bovine mastitis since decades; however, their use is associated with cost issues and human health concern. Use of herbal drugs does not generally carry these disadvantages. Many plants/herbs have been evaluated in the treatment of bovine mastitis with additional property of immunomodulation in affected mammary gland. **Aim:** To evaluate a topical herbal drug in two breeds of cattle for its *in-vivo* immunomodulatory effect on cytokines production and antibacterial activity in bovine subclinical mastitis. **Materials and Methods:** The response to treatment was evaluated by enumerating somatic cell count (SCC), determining total bacterial load, and studying the expression of different cytokines (interleukin [IL]-6, IL-8, IL-12, granulocyte macrophage-colony stimulating factor, interferon (IFN)–γ and tumor necrosis factor [TNF]-α). **Results:** The pre- and post-treatment SCC in mastitic quarters statistically did not differ significantly, however, total bacterial load declined significantly from day 0 onwards in both the breeds. Highly significant differences ($P < 0.01$) were observed in all the cytokines on day 0, 5, and 21 postlast treatment in both the breeds. The expression level of all the cytokines showed a significant increase on day 5, while a decrease was noticed on day 21 in both the breeds of cattle. The comparison of cytokine expression profiles between crossbred and Gir cattle revealed a significant difference in expression of IL-6 and TNF-α. However, other cytokines exhibited a similar pattern of expression in both breeds, which was non-significant. **Conclusion:** The topical herbal drug exhibited antibacterial and immunomodulatory activities in subclinical mastitis and thus the work supports its use as alternative herbal therapy against subclinical udder infection in bovines.

Key words: Herbal drug, immunomodulation, subclinical mastitis

Introduction

Mastitis is one of the most prevalent and costly diseases affecting the dairy industry worldwide[1-3] and is associated with economic losses of nearly $35 billion annually. Although abnormal milk is readily detected in clinical mastitis, no apparent changes are detected in sub-clinical mastitis. Nevertheless, both forms of the disease result in decreased milk production. The reduction in milk production alone contributes 70–80% of the economic losses incurred due to sub-clinical mastitis. Decreased milk production as well as quality, loss of milk due to milk withholding time, and veterinary expenses contribute to the economic losses.[4]

The conventional antimicrobial agents have been the mainstay of mastitis therapy over the last many decades, and these drugs have potential high cure rate, when the treatment is well-targeted. However, use of antibiotics is associated with cost, the possibility of development of acquired drug resistance, drug residues in the milk, and disruption of symbiotic gut flora of the host when systemic administration is used.[5,6] Given the

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seriousness of such problems, researchers, and clinicians have been trying to find effective therapeutic agents using alternative medicine. Traditional healthcare of animals (ethno-veterinary medicine) includes use of medicinal plants/herbs, surgical techniques and management practices to prevent and treat a range of diseases and problems encountered by livestock keepers. Research has shown that many of the plants used to prepare indigenous medicines do contain valuable active ingredients, however, much research remains to be done in this area.[7]

A study has revealed efficacy of 21 different species of medicinal plants in the treatment of bovine mastitis in Ethiopia.[8] Similarly, in another study, the evaluation of therapeutic potential of a topical herbal gel has indicated elimination of bacteria and significant reduction in SCC in around 80% cases of subclinical mastitis treated with the drug.[9]

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[10] and variations in their expression are often associated with disease activity in immune-mediated or inflammatory disorders.[11,12] 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 through the use of herbal medicines. A class of herbal medicines, known as immunomodulators, alters the activity of immune function through the dynamic regulation of inflammatory molecules such as cytokines. Therefore, a study was undertaken to evaluate the effect of herbs on the somatic cell count (SCC), total bacterial load, and cytokine profile in Gir and crossbred cattle harboring subclinical mastitis.

Materials and Methods

Selection of animals and collection of quarter milk samples

Of 10 crossbred (Holstein Friesian X Jersey X Kankrej) and 30 Gir (*Bos indicus*) cows, seven crossbred and 12 Gir cows affected with subclinical mastitis (SCC >5 × 10^5 cells/ml) were taken as experimental material. A volume of 300 ml of milk from each cow was collected in sterile wide mouth glass stopper bottles. Udder was thoroughly washed with potassium permanganate solution (1:1000) and the teats were wiped with 70% ethyl alcohol before sample collection. Milk was collected before treatment (day 0) and thereafter on day 5 and 21 postlast treatment. Milk samples from the quarters were subjected to SCC using an electronic SCC (Foss, Denmark) and to bacteriological culture examination for 3 consecutive days. Based on the results, quarters with subclinical mastitis were identified.[13]

Treatment

The affected lactating cows were treated with *Mastilep* topical herbal gel (Dabur Ayurvet Ltd., Ghaziabad, India). Each 10 g of *Mastilep* contained *Eucalyptus globulus* - 0.20 g, *Glycyrrhiza glabra* - 0.20 g, *Curcuma longa* - 0.04 g, *Cedrus deodara* - 1.00 g, *Paedaria foetida* - 0.04 g and sulphur - 1.00 g in a gel base. Approximately, 5 g herbal gel was topically applied on each affected udder quarter including the teats, after the morning and evening milking for 5 consecutive days.

Isolation of milk somatic cells

Aliquots of 25 ml milk were used to determine SCC using an electronic SCC. The remainder of the milk was centrifuged at $1000 \times g$ for 15 min at room temperature. Fat layer and the supernatant were discarded and the cell pellets were washed twice in 50 ml phosphate buffered saline.

Extraction of ribonucleic acid and complementary deoxyribonucleic acid synthesis

Somatic cells were collected by centrifugation and ribonucleic acid (RNA) was extracted as described by Chomczynski and Sacchi (2006).[14] The RNA template was qualitatively assessed and quantified using 2100 Bioanalyzer (Agilent Technologies) with the RNA 6000 Nano Labchip kit. Reverse transcription reactions were performed following the manufacturer's instructions using Sensiscript® Reverse Transcriptase kit (Qiagen, Germany) in 20-μL reactions using a fixed amount of input RNA for each complementary deoxyribonucleic acid (cDNA) reaction.

Real-time polymerase chain reaction

All real-time polymerase chain reaction (PCR) reactions were performed in optical 96 well plates using the ABI Prism® 7500 fast sequence detection system (Applied Biosystems). In each reaction, approximately, 20 ng of reverse-transcribed RNA (based on the initial RNA concentration) was used for real time PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β actin were used as endogenous control. The primers for the targeted cytokines were synthesized as described by Lee *et al.* (2006).[15] The cycling conditions included initial denaturation step 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 30 s in a 20-μL reaction volume. For each sample, a dissociation curve was generated after completion of amplification and analyzed to determine the specificity of PCR reaction. All reactions were run in triplicate and the quantification cycle (Cq) values for target genes were normalized with the internal control. To determine the amplification efficiencies of primers for reference as well as cytokines specific genes, six dilutions of cDNA preparations in triplicate were amplified to obtain standard curves.

Microbiological culture examination

Microbiological tests to obtain the bacterial load (colony-forming unit (CFU)/mL) were conducted on day 0 as well as on day 5 and 21 post last treatment with herbal drug following traditional microbiological method according to Hedges (2002).[16]

Posttreatment observation and laboratory evaluations

The SCC, total bacterial load, and expressions profiles of different cytokines, that is interleukin (IL)-6, IL-8, IL-12, granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN)–γ and tumor necrosis factor (TNF)-α were carried out by collecting milk on day 0 as well as 5 and 21 postlast treatment.

Statistical analysis

The data were analyzed using Statistical Analysis System (SAS) v4.1 (SAS institute, Inc., USA) software. The methods of

two-factor analysis of variance (ANOVA) and Pearson's correlation analysis were adopted to study the effect on various parameters used in the present investigation.

Results

Of 10 crossbred and 30 Gir cows, seven (70%) and 12 (40%) respectively were identified as subclinically infected. The real time PCR analysis was used to assess cytokine profiles in somatic cells extracted from infected cattle milk with the amplification efficiency of 79–100%. The expression of GAPDH was found consistent among different samples and breeds, however, β actin expression pattern varied among all the samples. The expression values of target genes were thus normalized with GAPDH. Compared to healthy cattle, all the cytokines were observed to be up-regulated in subclinical mastitis in both breeds [Figure 1]. The expression of all the analyzed cytokines was observed to be induced in response to herbal treatment.

Data of the transcriptional activity of all the cytokines viz. ILs-6, IL-8, IL-12, GM-CSF, IFN–γ and TNF-α, collected in the present study was analyzed to assess effect of breed as well as their response to the treatment with a herbal drug on day 5 and 21 postlast treatment in Gir and crossbred cattle. Two-way analysis of cytokines expression data was carried out using SAS

software. The statistical analysis revealed higher transcriptional level of IL-6 and TNF-α in Gir cattle; however, expression of other cytokines did not show a significant difference between the breeds [Table 1]. The scatter plot for a continuous by nominal/ordinal analysis showed vertical distribution of response points for each breed [Figure 2].

Least square means of the cytokines expression data between day 0, and day 5 and 21 postlast treatment for individual breed were compared using two-way ANOVA analysis. Highly significant difference ($P < 0.01$) was observed in all the cytokines on all the 3 days. The expression level of all the cytokines significantly increased on day 5 whereas on day 21 significant reduction was observed in both the breeds of cattle [Table 1]. The scatter plot for a continuous by nominal/ordinal analysis showed vertical distribution of response points for day 0, 5 and 21 in Gir [Figure 3] and crossbred cattle [Figure 4].

The mean SCC was observed to be 1752 ± 419, 2987 ± 447 and 2465 ± 432 cells/μl in Gir and 2262 ± 868, 3615 ± 594 and 2849 ± 527 cells/μL in crossbred cattle on day 0, 5 and 21, respectively. Total bacterial load observed was $(1.3 \pm 0.4) \times 10^7$, $(5.7 \pm 1.0) \times 10^4$ and $(2.1 \pm 1.6) \times 10^3$ CFU/mL in Gir cows, and $(1.3 \pm 0.7) \times 10^7$, $(2.0 \pm 0.4) \times 10^4$ and $(1.4 \pm 0.4) \times 10^3$ CFU/mL in crossbred

Table 1: Least square means of transcriptional activity of targeted cytokines, SCC and total bacterial count in the milk of Gir and crossbred cattle on day 0, and day 5 and 21 posttreatment

Target genes	Relative expression (least squares means±SE)			Overall means±SE
	0th day	5th day	21st day	
IL-6				
Gir	$(4.1\pm0.9)\times10^3$ [b]	$(1.1\pm0.1)\times10^6$ [a]	$(4.6\pm1.6)\times10^4$ [b]	$(3.8\pm1.2)\times10^5$ [x]
Crossbred	$(4.5\pm1.1)\times10^2$ [b]	$(3.4\pm0.8)\times10^5$ [a]	$(2.5\pm0.5)\times10^4$ [b]	$(1.2\pm0.4)\times10^5$ [y]
IL-8				
Gir	$(3.4\pm0.6)\times10^3$ [b]	$(2.9\pm1.2)\times10^6$ [a]	$(4.7\pm2.2)\times10^5$ [ab]	$(1.1\pm0.4)\times10^6$ [x]
Crossbred	$(2.0\pm1.2)\times10^5$ [b]	$(2.0\pm0.6)\times10^6$ [a]	$(2.0\pm0.6)\times10^5$ [b]	$(8.1\pm2.8)\times10^5$ [x]
IL-12				
Gir	$(6.0\pm0.7)\times10^3$ [b]	$(3.2\pm1.0)\times10^5$ [a]	$(3.9\pm1.1)\times10^4$ [b]	$(1.2\pm0.4)\times10^5$ [x]
Crossbred	$(7.1\pm1.1)\times10^2$ [b]	$(1.2\pm0.3)\times10^6$ [a]	$(2.3\pm0.6)\times10^4$ [b]	$(4.2\pm1.6)\times10^5$ [x]
GM-CSF				
Gir	$(2.6\pm0.9)\times10^4$ [b]	$(3.9\pm1.2)\times10^6$ [a]	$(2.0\pm0.9)\times10^5$ [b]	$(1.3\pm0.5)\times10^6$ [x]
Crossbred	$(0.8\pm0.2)\times10^2$ [b]	$(8.3\pm2.1)\times10^6$ [a]	$(4.9\pm2.2)\times10^4$ [b]	$(2.8\pm1.1)\times10^6$ [x]
TNF-α				
Gir	$(2.8\pm0.4)\times10^3$ [b]	$(4.8\pm1.0)\times10^5$ [a]	$(1.1\pm0.3)\times10^5$ [b]	$(2.0\pm0.5)\times10^5$ [x]
Crossbred	$(3.8\pm0.7)\times10^3$ [b]	$(9.3\pm3.3)\times10^4$ [a]	$(2.1\pm0.5)\times10^4$ [b]	$(3.9\pm1.3)\times10^4$ [y]
IFN-γ				
Gir	$(0.5\pm0.1)\times10^3$ [b]	$(1.2\pm0.2)\times10^7$ [a]	$(9.4\pm2.4)\times104$ [b]	$(4.5\pm1.5)\times10^5$ [x]
Crossbred	$(4.7\pm1.0)\times10^2$ [b]	$(1.1\pm0.3)\times10^6$ [a]	$(1.5\pm0.7)\times104$ [b]	$(3.8\pm1.5)\times10^5$ [x]
SCC				
Gir	$(1.7\pm0.4)\times10^3$ [a]	$(2.9\pm0.4)\times10^3$ [a]	$(2.4\pm0.4)\times103$ [a]	$(2.4\pm0.2)\times10^3$ [x]
Crossbred	$(2.2\pm0.8)\times10^3$ [a]	$(3.6\pm0.5)\times10^3$ [a]	$(2.8\pm0.5)\times103$ [a]	$(2.9\pm0.3)\times10^3$ [x]
Total bacteria				
Gir	$(1.3\pm0.4)\times10^7$ [a]	$(5.7\pm1.0)\times10^4$ [b]	$(2.1\pm1.6)\times10^3$ [b]	$(4.6\pm2.1)\times10^6$ [x]
Crossbred	$(8.8\pm0.7)\times10^6$ [a]	$(2.0\pm0.4)\times10^4$ [b]	$(1.4\pm0.4)\times10^3$ [b]	$(4.3\pm2.6)\times10^6$ [x]

Means were compared using two-way ANOVA. Means bearing similar superscript each row (a, b) and column (x, y) do not differ significantly (*P*≥0.05). ANOVA: Analysis of variance, SCC: Somatic cell count, IL: Interleukin, GM-CSF: Granulocyte macrophage-colony stimulating factor, TNF-α: Tumor necrosis factor-alpha, IFN: Interferon, SE: Standard error
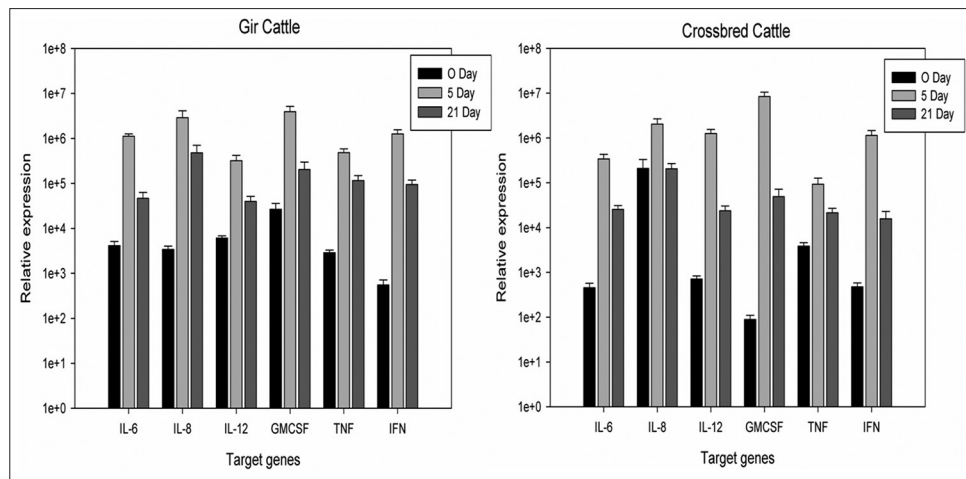
Figure 1: Relative quantification (means ± S.E.) of transcriptional level of target genes on day 0, and day 5 and 21 posttreatment in Gir and crossbred cattle compared to healthy control. Fold expression/cell was converted to fold expression/µL of milk by multiplying fold expression/cell value with the somatic cell count/µL
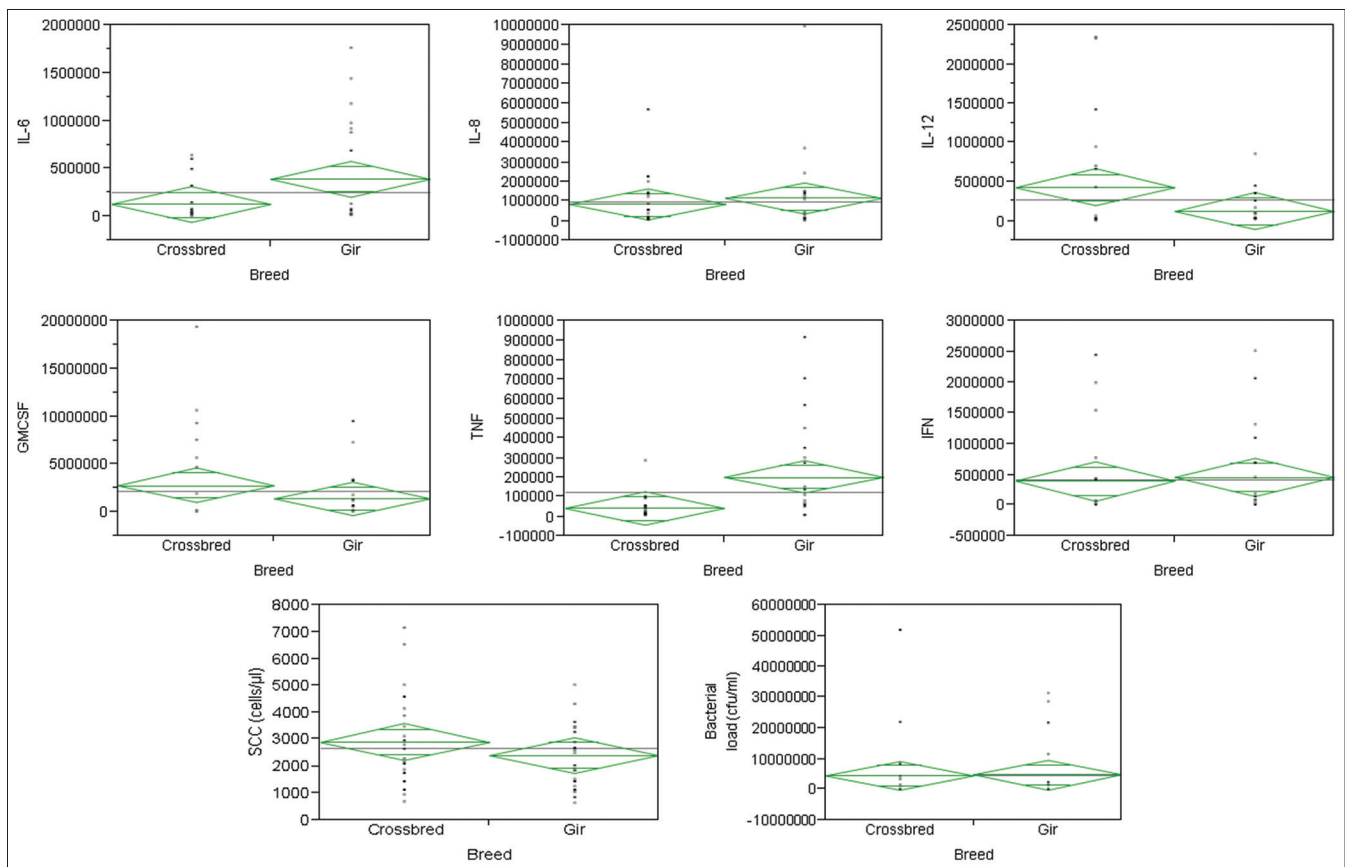

Figure 2: The continuous by nominal/ordinal scatter plot with means diamonds shows significant higher expression in interleukin-6 and tumour necrosis factor-α in Gir cattle. Line across each diamond represents the group mean. The vertical span of each diamond represents the 95% confidence interval for each group

cattle on day 0, 5 and 21, respectively. The total bacterial load significantly decreased from day 0 to day 21 posttreatment, however, the mean differences in SCC between 0, 5 and 21 days in both breeds of cattle were non-significant [Figure 5].

The correlation of transcriptional activity of cytokines, total bacteria and SCC present in the milk of Gir and crossbred cattle revealed significant ($P < 0.05$) correlation between SCC and total bacteria in Gir, whereas in crossbred cattle the significant correlation was found between SCC and IL-8 as well as IFN-γ. In Gir cattle IL-8 showed a significant correlation with IL-12. Other cytokines were non-significantly correlated to each other in both the breeds [Table 2].
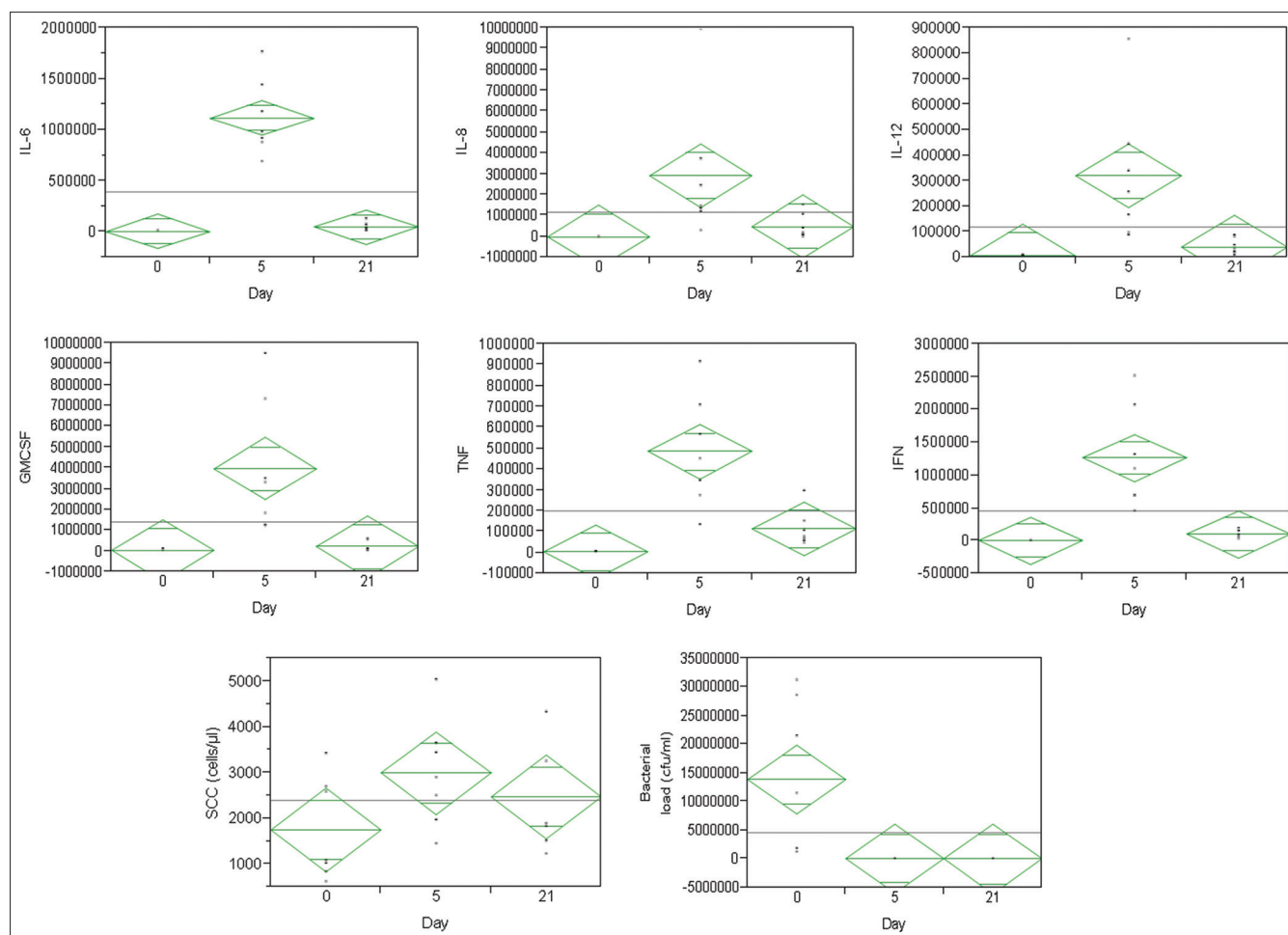
Figure 3: The scatter plot with means diamonds showed significant difference in the entire cytokines as well as bacterial load in Gir cattle. Line across each diamond represents the group mean. The vertical span of each diamond represents the 95% confidence interval for each group

Discussion

Modulation of cytokine expression may offer novel approaches in the treatment of a variety of diseases. One strategy in the modulation of cytokine expression may be through the use of herbal medicines. This may offer an explanation to the effects of herbs on the immune system and other tissues. Antimicrobial drugs can interact directly with the immune system of human and animal. Growing evidence can be found in literature that some antimicrobials exert their beneficial effects not only by killing or inhibiting the growth of bacterial pathogens, but also indirectly through immunomodulation by influencing the cytokine production.

In this study, we examined the effects of a topical herbal drug on some targeted cytokines expression profiles, which showed significantly ($P < 0.05$) higher expression of all the cytokines analyzed on day 0 as well as on day 5 postlast treatment, however, their level of expression was significantly reduced on day 21 post-treatment. *G. glabra*, *C. longa* and *C. deodara* have been reported to possess immunomodulating properties.[17] The up-regulation of cytokines expression observed in the present study on day 5 post-treatment can thus be attributed to immunomodulation by these herbs. The decline in the

cytokines expression recorded on day 21 posttreatment may have been due to reduction in the effect of the herbs. Each cytokine induces its signaling cascade through interaction with its cell-surface receptors. This may include the up-regulation and/or down-regulation of several genes and their transcription factors resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition.[18] Inflammatory cytokines are multi-potential mediators of cellular immune system and have a wide variety of biologic activities. Depending on their local concentration they can have favorable or unfavorable effects on the host immune response. Unfavorable effects may occur at very low or very high concentrations of these cytokines.[19] The balance between the production of inflammatory and anti-inflammatory cytokines is responsible for the outcome and the duration of the immune response.

Laboratory studies have clearly elucidated that overall pharmacological effects and therapeutic efficacies of medicinal plants often do not derive from a single compound, but from several compounds generating synergic activity.[20,21] Many researchers have proposed that multi-component pharmacological agents that hit multiple targets impact the complex equilibrium
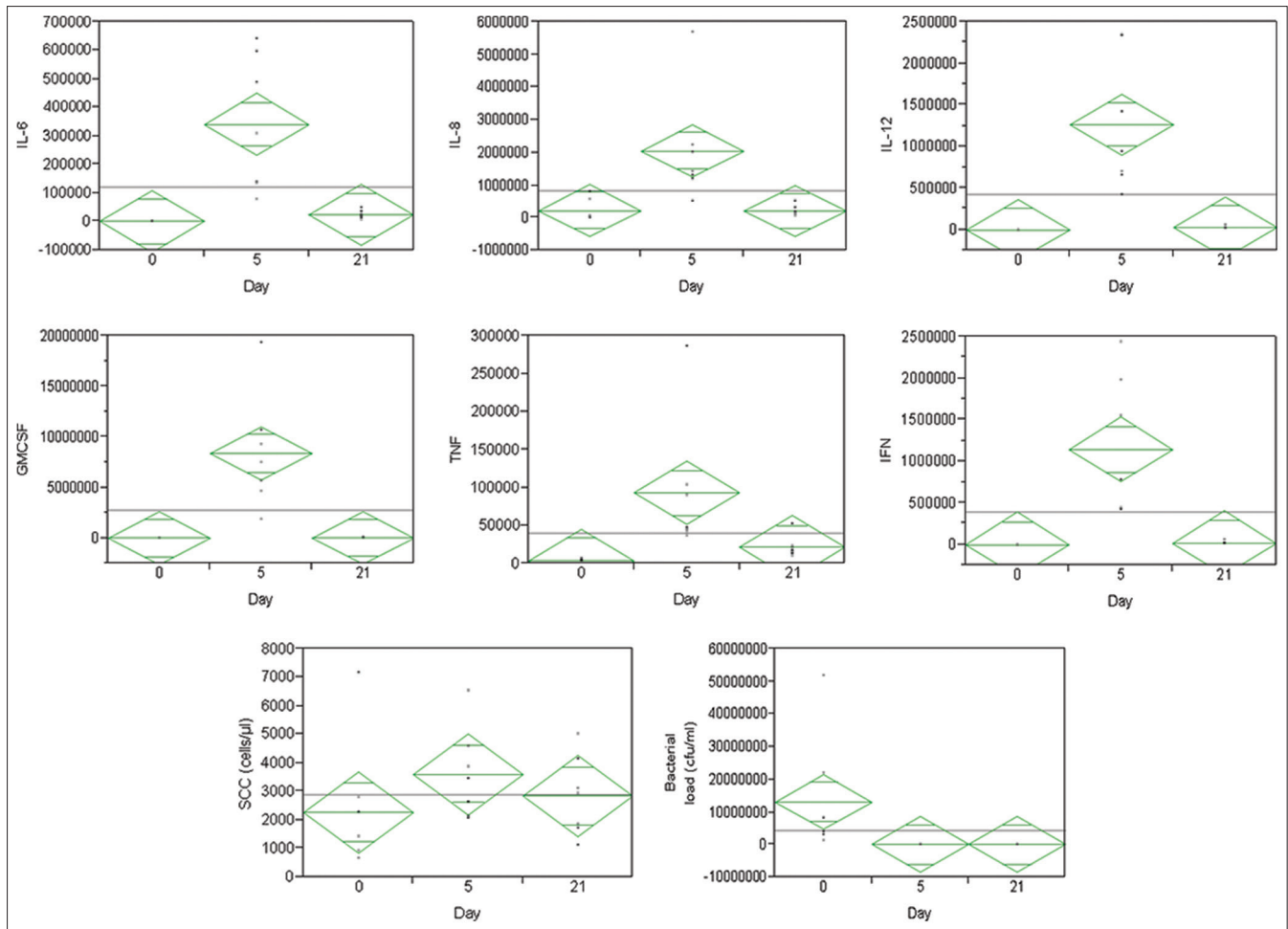
Figure 4: The scatter plot with means diamonds showed significant difference in the entire cytokines as well as bacterial load in crossbred cattle. Line across each diamond represents the group mean. The vertical span of each diamond represents the 95% confidence interval for each group
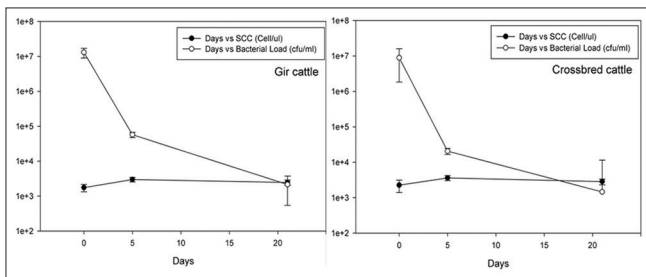

Figure 5: Somatic cell count (cells/µl) and total bacterial count (colony-forming unit/ml) present in the milk of Gir and crossbred cattle. Significant reduction of bacterial count was observed from day 0 onwards

of whole cellular networks more favorably than drugs acting on a single target.[22,23] In developing countries, the traditional ethnoveterinary medicinal practices are followed by the rural folk and a number of veterinary diseases are managed using these. The use of several antibiotics and other drugs are banned for animal health care in a number of countries because of concerns related to human health.

Results obtained in this study revealed that the bacterial load declined significantly ($P < 0.05$) on day 5 and 21 post-treatment in both the breeds, however, significant changes were not observed in SCC. Reduced load of bacteria observed after treatment may be attributed to the fact that bacteria present in the tissues readily attract phagocytes and then are ingested and killed by phagocytosis. Interestingly, phagocytes in the tissues are activated by cytokines.[24] Different cytokines have different actions on the same cell, however, there is a large degree of overlap between the action of one cytokine and another.

Several cytokines, including IL-6, IL-8, TNF-α and IFN-γ, are known to be important in eliciting the acute phase response and allowing the accumulation of leukocytes at the site of infection. In our study, the transcriptional activity of IL-6 and TNF-α was observed significantly high in Gir cattle. The role of IL-6 as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-α.[25] Such relation was observed in Gir but not in crossbred cattle. TNF-α and IL-6 have been associated with endotoxin-related inflammatory responses, which is indicated by their increased level in the milk and sera of dairy cattle.[26] Several cytokines are involved in eliciting different immune response to different mastitis pathogens. In our study, the profiles of TNF-α and IL-6 demonstrated different patterns not only between the two types of infections, but also among the animals. IL-6 also acts synergistically with GM-CSF.[27] IL-6

Table 2: Pearson's correlations between and within cytokine transcriptional activity, total bacteria, and SCC present in milk of Gir and crossbred cattle

Cattle breeds	IL-6	IL-8	IL-12	GM-CSF	TNF-α	IFN-γ	SCC	Total bacteria
IL-6								
Gir	1	−0.211	−0.068	−0.084	−0.220	−0.270	−0.753	−0.688
Crossbred	1	−0.061	0.737	0.374	0.309	−0.440	−0.300	−0.378
IL-8								
Gir	−0.211	1	0.871*	−0.060	−0.346	0.525	0.410	0.103
Crossbred	−0.061	1	−0.198	0.013	0.402	0.657	0.768*	−0.173
IL-12								
Gir	−0.068	0.871*	1	0.169	−0.266	0.694	0.022	−0.231
Crossbred	0.737	−0.198	1	0.613	−0.082	−0.092	−0.021	−0.083
GMCSF								
Gir	−0.084	−0.060	0.169	1	0.447	−0.201	−0.259	−0.046
Crossbred	0.374	0.013	0.613	1	0.087	0.369	0.237	0.102
TNF-α								
Gir	−0.220	−0.346	−0.266	0.447	1	−0.050	−0.090	−0.238
Crossbred	0.309	0.402	−0.082	0.087	1	−0.051	−0.178	−0.014
IFN-γ								
Gir	−0.270	0.525	0.694	−0.201	−0.050	1	0.129	−0.322
Crossbred	−0.440	0.657	−0.092	0.369	−0.051	1	0.910**	0.358
SCC								
Gir	−0.753	0.410	0.022	−0.259	−0.090	0.129	1	0.789*
Crossbred	−0.300	0.768*	−0.021	0.237	−0.178	0.910**	1	0.020
Total bacteria								
Gir	−0.688	0.103	−0.231	−0.046	−0.238	−0.322	0.789*	1
Crossbred	−0.378	−0.173	−0.083	0.102	−0.014	0.358	0.020	1

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed). SCC: Somatic cell count, IL: Interleukin, GM-CSF: Granulocyte macrophage-colony stimulating factor, TNF-α: Tumor necrosis factor-alpha, IFN: Interferon

showed positive though the non-significant correlation with GM-CSF in crossbred cattle, whereas in Gir it was negatively correlated.

IL-8 is a chemokine produced by macrophages and epithelial cells in response to bacterial invasion. IL-8 mediates neutrophil function by allowing neutrophils to resolve bacterial infections by migrating through the walls of blood vessels to the site of infection.[28] In the present study, negative correlation was seen between IL-8 and TNF-α in Gir cows whereas in crossbred cattle the two cytokines were correlated positively. The expression of IL-8 is induced by both exogenous and endogenous (e.g. TNF-α) proinflammatory stimuli.[29,30] Induction of IL-8 by TNF-α was observed only in crossbred cattle in the present study. IL-12 is naturally produced by dendritic cells and macrophages. It has an anti-angiogenic activity brought about by increasing production of IFN-γ.[31] IL-12 showed a positive correlation with IFN-γ in Gir, however, the same was negative in crossbred cattle. Augmentation of IFN-γ activity with IL-12 was observed only in Gir, but not in crossbred cattle. IL-12 also showed positive and significant correlation with IL-8 in Gir, and IL-6 in crossbred cattle. Polymorphonuclear neutrophils (PMNs) play an important role in host defense against microorganisms. In addition to their phagocytic and killing properties, PMNs can synthesize numerous cytokines including IL-8 and it has been postulated that IL-12 might play a role in IL-8 production and release by PMNs.[32] The

discrimination in the transcriptional activity of cytokines in two groups may be attributed to the genetic make-up of the individual breeds which could have resulted in differences in expression of different cytokines.

Csermely (2005)[23] suggested that a pharmacological strategy directed toward multiple targets could result in more efficient therapeutic outcomes. It has also been emphasized that the multi-component compounds possessing broader specificity and lower affinity, as found in botanical medicines, can be more efficient than compounds with high affinity and high specificity.[22] Moreover, the use of whole plants, instead of isolated chemicals, may offer a safer clinical strategy in the treatment of many diseases.[33,34] Cytokines operate both as a cascade and as a network, regulating the production of other cytokines and cytokine receptors, while stimulating the production of acute-phase proteins.[35]

Conclusion

So far, there is a paucity of literature on research work done on the influence of botanical medicines on cytokines and other messenger molecules. Inflammatory molecules and many of their receptors may turn out to be modulated by plants and in that event the herbal medicines would have therapeutic potential for future. The results of *in-vivo* research suggest that the botanical medicines modulate cytokines and thus it may be concluded that *Mastilep* topical herbal gel possesses

antibacterial and immunomodulatory activities against bovine sub-clinical mastitis. These facts are supported by reduction in total bacterial count and enhancement of cytokine expression of somatic cells in bovine mammary gland in response to the herbal therapy. The present work supports its use as alternative herbal therapy against bovine sub-clinical mastitis and may elucidate the mechanism of action for many of its therapeutic effects.

Acknowledgment

References

1. Bradley A. Bovine mastitis: An evolving disease. Vet J 2002;164:116-28.
2. Petrovski KR, Trajcev M, Buneski G. A review of the factors affecting the costs of bovine mastitis. J S Afr Vet Assoc 2006;77:52-60.
3. Viguier C, Arora S, Gilmartin N, Welbeck K, O'Kennedy R. Mastitis detection: Current trends and future perspectives. Trends Biotechnol 2009;27:486-93.
4. Chockalingam A, Zarlenga DS, Bannerman DD. Antimicrobial activity of bovine bactericidal permeability-increasing protein-derived peptides against gram-negative bacteria isolated from the milk of cows with clinical mastitis. Am J Vet Res 2007;68:1151-9.
5. Paape MJ, Nickerson SC, Ziv G. *In vivo* effects of chloramphenicol, tetracycline, and gentamicin on bovine neutrophil function and morphologic features. Am J Vet Res 1990;51:1055-61.
6. Sandholm M, Pyorala S. Coliform mastitis. Endotoxin mastitis – Endotoxin shock. In: Sandholm M, Honkanen-Buzalski T, Kaartinen L, Pyorala S, editors. The Bovine Udder and Mastitis. Jyvaskyla: Gummerus Kirjapaino; 1995. p. 149-60.
7. MacCorkle CM, Mathias ME. Ethnoveterinary medicine in Africa. Africa 1992;62:59-93.
8. Sahle S. A study on medicinal plants used in the traditional veterinary practices for treatment of bovine mastitis in selected sites of Central Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University, DVM Thesis; 2002.
9. Verma AK, Nauriyal DS. Therapeutic potential of a topical herbal gel against bovine subclinical mastitis. Indian J Anim Sci 2009;79:275-7.
10. Campos M, Godson DL, Aughes HP, Babiuk LA. Cytokine application in infectious diseases. In: Goddeeris BM, Morrison WI, editors. Cell Mediated Immunity in Ruminants. Boca Raton, FL, USA: CRC Press, Inc.; 1994. p. 229-37.
11. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138-46.
12. Hansen PJ, Soto P, Natzke RP. Mastitis and fertility in cattle-possible involvement of inflammation or immune activation in embryonic mortality. Am J Reprod Immunol 2004;51:294-301.
13. Bovine Mastitis: Definition and Guidelines for diagnosis. Brussels: International Dairy Federation; 1987. IDF-Bulletin No. 211.
14. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. Nat Protoc 2006;1:581-5.
15. Lee JW, Bannerman DD, Paape MJ, Huang MK, Zhao X. Characterization of cytokine expression in milk somatic cells during intramammary infections with *Escherichia coli* or *Staphylococcus aureus* by real-time PCR. Vet Res 2006;37:219-29.
16. Hedges AJ. Estimating the precision of serial dilutions and viable bacterial counts. Int J Food Microbiol 2002;76:207-14.
17. Spelman K, Burns J, Nichols D, Winters N, Ottersberg S, Tenborg M. Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. Altern Med Rev 2006;11:128-50.
18. Tian B, Nowak DE, Brasier AR. A TNF-induced gene expression program under oscillatory NF-kappaB control. BMC Genomics 2005;6:137.
19. Barak V. Soluble cytokine receptors in disease. Isr J Med Sci 1995;31:565-71.
20. Spinella M. The importance of pharmacological synergy in psychoactive herbal medicines. Altern Med Rev 2002;7:130-7.
21. Spelman K, Duke JA, Bogenschutz-Godwin MJ. The synergy principle in plants, pathogens, insects, herbivores and humans. In: Kaufman PB, editor. Natural Products from Plants. Boca Raton, FL: CRC Press; 2006. p. 478.
22. Agoston V, Csermely P, Pongor S. Multiple weak hits confuse complex systems: A transcriptional regulatory network as an example. Phys Rev E Stat Nonlin Soft Matter Phys 2005;71:051909.
23. Csermely P, Agoston V, Pongor S. The efficiency of multi-target drugs: The network approach might help drug design. Trends Pharmacol Sci 2005;26:178-82.
24. Sompayrac L. How the Immune System Works. 3rd ed. Malden, MA: Blackwell Publishing; 2008.
25. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, *et al.* IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 1998;101:311-20.
26. Nakajima Y, Mikami O, Yoshioka M, Motoi Y, Ito T, Ishikawa Y, *et al.* Elevated levels of tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) activities in the sera and milk of cows with naturally occurring coliform mastitis. Res Vet Sci 1997;62:297-8.
27. Caracciolo D, Clark SC, Rovera G. Human interleukin-6 supports granulocytic differentiation of hematopoietic progenitor cells and acts synergistically with GM-CSF. Blood 1989;73:666-70.
28. Kehrli ME Jr, Harp JA. Immunity in the mammary gland. Vet Clin North Am Food Anim Pract 2001;17:495-516, vi.
29. Matsukawa A, Hogaboam CM, Lukacs NW, Kunkel SL. Chemokines and innate immunity. Rev Immunogenet 2000;2:339-58.
30. Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol 2003;284:L566-77.
31. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 2003;3:133-46.
32. Ethuin F, Delarche C, Benslama S, Gougerot-Pocidalo MA, Jacob L, Chollet-Martin S. Interleukin-12 increases interleukin 8 production and release by human polymorphonuclear neutrophils. J Leukoc Biol 2001;70:439-46.
33. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine 2001;8:401-9.
34. Ernst E. Herbal medicines put into context. BMJ 2003;327:881-2.
35. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.