



## Research Article

# Efficacy and Effects of Various Allopathic and Herbal Immunopotentiating Agents for Curing of Subclinical Mastitis in Dairy Cows

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**Abstract** | The major purpose of this study was to evaluate the efficacy of some allopathic and herbal ingredients for treatment of subclinical mastitis and to test out how these ingredients can be used for enhancement of milk quality as well as their effects on the haematological values. Total 30 cows were selected for this study kept in different dairy farms in the premises of Lahore. Out of these 30 cows, 24 had subclinical mastitis and 6 were healthy. Cows suffering from subclinical mastitis were divided into 5 groups. Each group consists of 6 cows. Cows kept in group one was treated with Vitamin E+ Se for five days. Cows placed in group two was treated with Garlic for five days. Group three cows were treated with Black pepper for five days and group four cows were treated with Lemon for five days. Group five having 6 healthy cows were used as negative control so they can be used for comparing blood values and milk composition of healthy cows with animals under trial. Our study concluded that Vitamin E+ Se and Garlic were the most efficient drugs as 66% cows suffering from subclinical mastitis were recovered by treatment of each of them. Cows suffering from subclinical mastitis showed a 50 % recovery percentage when treated with lemon for five days and 33 percent cows recovered from subclinical mastitis when treated with Black Pepper for five days. Udder health status and milk quality can be enhanced with use of Garlic and Vitamin E + Se combination in cows.

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## Introduction

Mastitis is a very devastating disease of dairy animals which influences the quality and quantity of milk (Akhtar et al., 2012). The pathogenic microorganisms invade the teat canal and attack the delicate mammary tissues, resulting in the inflammatory response that leads to changes in the milk (Ebrahimi et al., 2007). These changes in the milk depend on the invading pathogen and inflammatory response (Adane et al., 2012). According to the reports of livestock diseases in Pakistan, mastitis is ranked as the most prev-

alent disease of dairy animals in all areas of Pakistan (Muhammad et al., 2010; Bachaya et al., 2005). In case of mastitis dairy industry suffers economic losses because of low quality milk that is not fit for human consumption, decrease in milk yield, premature culling of animals and replacements (Batavani et al., 2007).

Most studies only consider the losses caused by clinical mastitis and snubbed the losses caused by sub-clinical mastitis, that is only detectable by measuring the milk somatic cell counts (SCC) (Shahid et al., 2006). Subclinical mastitis is 15 to 40 times more prevalent

than the clinical mastitis (Schultz et al., 1978). In case of mastitis the amounts of many blood components, such as serum albumin, sodium ions and chloride ions increase in milk (Hussain et al., 2012). For cows' milk the upper limit of the normal SCC is  $0.5 \times 10^6$  cells/ml (Hameed et al., 2012). Mastitis has an effect on the milk quality as milk from affected udder quarter has decreased milk protein, fat, sugar (lactose) contents and increased somatic cell count (Smith et al., 1997). The processing of such low quality milk results in poor quality output of finished fermented products like yogurt and cheese (Korhonen and Kaartinen, 1995). The shelf life of milk from affected udder is also less than milk from normal and healthy udder (Urech et al., 1999). The extent of variety of changes in composition depends on the inflammatory response (Kitchen, 1981).

Vitamin E and Selenium (Se) are among the most stabilized antioxidants present in living cells (Pirestani et al. 2013). Vitamin E is a fat soluble antioxidant and fundamental component of lipid membrane structure of the cells (Qureshi et al., 2010). Vitamin E plays a major role in the maintenance of membrane integrity in all cells of the body and protects cells from injury caused by oxygen free radicals (Batra et al., 1992). Herbal medicines are used in many parts of the world for treatment of different diseases (Dilshad et al., 2009). *Allium sativum* cloves (Garlic), *Piper nigrum* (Black Pepper) and *Citrus limonum* (Lemon) are famous for their therapeutic properties in the Asian and African countries (Khan et al., 2012).

Different studies have reported that *Allium sativum* (Garlic) has antiseptic and antibacterial properties because of a component named as 'allicin' (Bilal et al., 2009; Deeba et al., 2009). The use of ethno-veterinary medicine (EVM) has the ability to substitute the use of synthetic medicine. Ethno-veterinary medicines are cheaper as compared to synthetic medicines (Azaizeh et al., 2003; Fielding, 1998). The present study was planned to determine the effectiveness of above mentioned agents in dairy cows those have the ability to moderate immune response, their impacts on hemogram and milk compositional contents pre and post treatment.

## Materials and Methods

### Selection and Grouping of Cows

A total of 30 cows were selected from government

and private farms located near Lahore for this study. Out of these 30 cows, 24 cows were suffering from subclinical mastitis and 6 cows were healthy. Those cows that have subclinical mastitis were grouped in 4 separate groups, 6 cows in each group. All healthy cows were placed in group 5.

### Experimental Procedure

Group 1, cows were given Vitamin E + Selenium @ 10 ml/day (50 mg Vit E/ml and 0.5 mg Na selenite/ml) I/M for 5 days as described by Pirestani et al. (2013). Group 2, cows were treated with *Allium sativum* cloves (Garlic) @ 250 gm/animal PO twice a day for 5 days (Bilal et al., 2009; Deeba et al., 2009; Dilshad et al., 2009). Group 3, cows were treated with *Piper nigrum* (Black Pepper) grinded @ 60 gm/animal PO twice a day for 5 days (Bilal et al., 2009; Deeba et al., 2009). Group 4, cows were treated with *Citrus limonum* (Lemon) fruit cut into pieces @ 250 gm/animal PO twice a day for 5 days (Bilal et al., 2009; Dilshad et al., 2009). Group 5, cows were kept as negative control means healthy animals.

**Table 1:** Recovery % by Immuno-potentiating Agents in Subclinical Mastitis after treatment in cross breed cow groups

Treatment Group	Treated	Cured	Recovery %	P-Value
Garlic	06	04	66.00268	0.0268
Vitamin E + Se	06	04	66	0.0268
Lemon	06	03	50.00436	0.0436
Black pepper	06	02	33.00776	0.0776
Negative Control	06	00	0000	

**Vit E + Se, Garlic, Lemon:** In these treatments are differ significantly ( $P < 0.05$ ) after treatment Vitamin E + Se and Garlic showing equal maximum recovery after treatment while Lemon shows less recovery after previous two agents; **Black Pepper and Negative control group:** treatment not differ ( $P > 0.05$ ) after treatment

### Samples Processing

Analysis of the blood before and after the treatment was performed using Automated Haematology Analyzer at University Diagnostic Laboratory of UVAS, Lahore. Following blood parameters were recorded: Total leukocyte count, Erythrocyte count, Haemoglobin, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Platelets count. Before the start of the study and at the end of study lactose, fat and protein percentage of milk was recorded along with pH of milk by using Lactoscan MCC50 in University Diagnostic Lab of UVAS, Lahore.

**Table 2:** Haematological indices (Mean ± SEM) before and after treatment in cross bred cow groups

Treatment Group	WBCs (x10 <sup>9</sup> /L)		Lymphocytes (x10 <sup>9</sup> /L)		Lymphocytes %	
	Before	After	Before	After	Before	After
Vitamin E + Se	9.47±0.54 <sup>a</sup>	7.70±0.53 <sup>a</sup>	4.87±0.20	6.20±0.54 <sup>a</sup>	56.05±1.72	81.47±4.11 <sup>a</sup>
Garlic	8.42±0.36 <sup>b</sup>	6.72±0.37 <sup>b</sup>	3.95±0.35	5.90±0.20 <sup>b</sup>	51.03±2.83	73.93±2.72 <sup>b</sup>
Black Pepper	9.27±0.41 <sup>c</sup>	7.15±0.28 <sup>c</sup>	5.03±0.21	7.61±0.48 <sup>c</sup>	65.68±3.14	76.35±3.86 <sup>c</sup>
Lemon	9.15±0.35 <sup>d</sup>	7.04±0.36 <sup>d</sup>	4.83±0.29	6.41±0.58 <sup>d</sup>	54.15±3.24	68.41±4.75 <sup>d</sup>
Negative control	6.57±0.18 <sup>a</sup>	6.57±0.18 <sup>b</sup>	5.51±0.10	5.51±0.10 <sup>ab</sup>	90.63±0.17	90.63±0.17 <sup>ab</sup>

<sup>a,b,c,d</sup> superscript differ among the treatment groups (rows) differ significantly ( $P < 0.05$ ); **Vitamin E + Se, Garlic, Black pepper, Lemon:** In these treatment groups all variables are differ significantly ( $P < 0.05$ ) in before and after treatments; **Negative control group:** All variables are not different ( $P > 0.05$ ) in before and after treatments

**Table 3:** Haematological indices (Mean ± SEM) before and after treatment in cross bred cows

Treatment Group	RBCs(x10 <sup>12</sup> /L)		Hb(x g/dL)		MCV(x fl)	
	Before	After	Before	After	Before	After
Vitamin E + Se	6.51±0.46 <sup>b</sup>	7.50±0.39 <sup>b</sup>	8.08±0.29	8.67±0.33 <sup>b</sup>	44.50±0.71	46.83±0.79 <sup>b</sup>
Garlic	6.82±0.29 <sup>b</sup>	7.78±0.30 <sup>b</sup>	7.95±0.34	8.88±0.25 <sup>b</sup>	44.50±0.56	46.33±0.61 <sup>b</sup>
Black Pepper	7.24±0.39 <sup>ab</sup>	8.13±0.32 <sup>b</sup>	8.91±0.38	10.01±0.45 <sup>a</sup>	44.83±0.54	45.33±0.61 <sup>b</sup>
Lemon	8.00±0.07 <sup>a</sup>	9.28±0.14 <sup>a</sup>	8.83±0.36	10.23±0.17 <sup>a</sup>	44.00±0.58	46.83±0.68 <sup>a</sup>
Negative control	8.03±0.32 <sup>a</sup>	8.03±0.32 <sup>b</sup>	8.70±0.07	8.70±0.07 <sup>b</sup>	44.66±0.76	44.66±0.76 <sup>ab</sup>

<sup>a,b,c,d</sup> superscript differ among the treatment groups (rows) differ significantly ( $P < 0.05$ ); **Vitamin E + Se, Garlic and Lemon:** In these treatment groups, variables (RBCs, Hb, MCV) are differ significantly ( $P < 0.05$ ) in before and after treatments; **Negative control group and Black pepper:** All variables are not different ( $P > 0.05$ ) in before and after treatments

**Table 4:** Milk compositional changes before and after treatment in cross bred cows

Treatment Group	Fat%		Lactose%		Protein%		pH	
	Before	After	Before	After	Before	After	Before	After
Vitamin E + Se	3.28±0.03 <sup>b</sup>	3.50±0.02 <sup>b</sup>	4.17±0.05	4.55±0.06 <sup>b</sup>	3.48±0.05	3.82±0.07 <sup>b</sup>	6.7±0.03	6.5±0.03 <sup>b</sup>
Garlic	3.30±0.03 <sup>b</sup>	3.50±0.03 <sup>b</sup>	4.37±0.05	4.60±0.04 <sup>b</sup>	3.45±0.04	3.77±0.03 <sup>b</sup>	6.7±0.04	6.4±0.04 <sup>b</sup>
Black Pepper	3.30±0.03 <sup>b</sup>	3.52±0.01 <sup>b</sup>	4.27±0.07	4.57±0.05 <sup>c</sup>	3.43±0.08	3.68±0.04 <sup>c</sup>	6.7±0.03	6.5±0.03 <sup>c</sup>
Lemon	3.30±0.04 <sup>b</sup>	3.48±0.04 <sup>b</sup>	4.28±0.06	4.52±0.05 <sup>b</sup>	3.37±0.05	3.90±0.04 <sup>ab</sup>	6.8±0.03	6.5±0.02 <sup>b</sup>
Negative control	3.78±0.03 <sup>a</sup>	3.78±0.03 <sup>b</sup>	4.63±0.03 <sup>a</sup>	4.63±0.03 <sup>b</sup>	3.83±0.02 <sup>a</sup>	3.83±0.02 <sup>bc</sup>	6.5±0.03 <sup>a</sup>	6.5±0.03 <sup>b</sup>

<sup>a,b,c,d</sup> superscript differ among the treatment groups (rows) differ significantly ( $P < 0.05$ ); **Vitamin E + Se, Garlic and Lemon:** In these treatment groups, variables (RBCs, Hb, MCV) are differ significantly ( $P < 0.05$ ) in before and after treatments; **Negative control group and Black pepper:** All variables are not different ( $P > 0.05$ ) in before and after treatments

### Statistical Analysis

The data on chemotherapy, hemogram and milk composition was analyzed by one-way analysis of variance (ANOVA) and paired T test using SPSS 20.0. A probability levels <0.05 was considered as statistically significant.

### Results

In our study more number of cases were cured through the use of Vitamin E + Se and Garlic as shown in **Table 1**. Both allopathic (Vitamin E + Se) and herbal (Garlic, Black pepper and Lemon) products showed significant effect as immune-potentiating agents on

WBCs and lymphocytes (**Table 2**). Use of Vitamin E + Se, Garlic and Lemon showed the immune-potentiating characteristics by increasing lymphocyte count and decreasing the overall WBCs count. These immune-potentiating characteristics can be related to the cure of subclinical mastitis by decreasing somatic cells.

Vitamin E + Se, Garlic and Lemon showed the good response by increasing Erythrocytes count, Haemoglobin and MCV but Black Pepper didn't show the significant results on Erythrocytes count, Haemoglobin and MCV shown in **Table 3**.

Vitamin E + Se and Garlic showed the significant result by improving milk Fat %, Protein %, Lactose % and pH. Lemon showed significant result on all except Protein % shown in [Table 4](#).

Combination of Vitamin E+ Se was the most effective to treat sub-clinical mastitis in cows by improving immune system, increasing the haematological parameters and improving the milk compositional contents as shown in [Table 2, 3 and 4](#). Use of Garlic for cure of sub-clinical mastitis in the present study also showed the good recovery as shown in [Table 1 and 4](#).

## Discussion

In our present study more number of cases were cured through Vitamin E + Se and Garlic. Previously combination of Vitamin E + Se was the most effective drug as it improved the immune system and increased the haematological parameters and improving the milk composition as indicated by [Qureshi et al. \(2010\)](#). Vitamin E + Se was the most effective drug by improving the immune system and increasing the haematological parameters and improving the milk compositional contents as described by [Chishti et al. \(1992\)](#).

Garlic was used in the present study, which showed better results in curing subclinical mastitis, boosting immune system, increasing the lymphocyte number and percentage, blood parameters and milk composition. [Dilshad et al. \(2009\)](#) previously used Garlic for the treatment of mastitis also showed the same results as our study. These effects are due to antiseptic and antibacterial properties of allicin ingredient of the *Allium sativum* (Garlic).

[Pirestani et al. \(2013\)](#) also found out same findings by using Vitamin E + Se on milk composition. Use of *Citrus limonum* (Lemon) was the third best option for treatment of sub-clinical mastitis after Vitamin E + Se and Garlic. [Bilal et al. \(2009\)](#) stated that *Citrus limonum* (Lemon) with Ammonium chloride 10% was the most favoured drugs, followed by *Citrus limonum* 9% and *Veroniaanthelmintica* 8%, ground to a pestle and orally administered and this prescription proved effective in curing mastitis.

In our study, Black Pepper was used as immune-potentiating agent. Black Pepper showed the significant effect on WBCs but didn't show good response on RBCs, Hb, and MCV. This might be due to less time

duration for that dose to enhance RBCs, Hb, and MCV production. Black Pepper showed fair effect on subclinical mastitis. Previously a study conducted by [Bilal et al. \(2009\)](#) also showed the same effects of Black Pepper on subclinical mastitis.

## Conclusion

The subclinical mastitis has deleterious effects on milk composition and haematology. Vitamin E + Se, Garlic and Lemon can improve milk composition and haematological parameters. Vitamin E + Se and Garlic have excellent effects in curing subclinical mastitis and considered as first choice for the cure of mastitis. Combinations of any two of the agents may cure the disease more rapidly.

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## Conflict of interest

The authors declare that there is no conflict of interests.

## Authors' Contribution

JAK, MSK, WS and MA designed the study. MI, AH, NK, MKA and AI took part in the execution of study. MI, AH, and WS implemented the study and involved in sampling as well as testing. MI, MKA and AI drafted the manuscript. All the authors read and accepted the final manuscript before submission.

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