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Effect of immuno-potentiating herbal agents on milk and haematological parameter of subclinical mastitis affected crossbred dairy cow

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Abstract

The main purpose of this trail was evaluate the efficacy of herbal ingredient for treatment of subclinical mastitis and to test out how these ingredients effect on milk quality and yield and also effect on haematological value. Total 48 cows were selected for this study kept in conventional housing system in university dairy farm Kanpur on the bases of California Mastitis Test and Somatic Cell Count. These were divided in to 6 groups. Each group consist 6 cows. Different type of herbal paste applied in udder every morning and evening for 10 days. Out of all herbal paste turmeric powder was the most efficient drug as 75% cows suffering from subclinical mastitis were recovered by treatment of each of them. Black pepper was lowest result recovered in this trail, only 37.5% cow was recovered. Cows suffering from subclinical mastitis showed a 62.5% recovery percentage when treated with lemon for ten days and 75 percent cows recovered from subclinical mastitis when treated with Garlic for ten days. Udder health status and milk quality can be enhanced with use of Garlic and Turmeric powder combination in cows.

Keywords: Immuno-potentiating, herbal, haematological parameter

Introduction

Mastitis is one of the most expensive diseases of dairy cattle and economically among all production disease (Bhikane and Kawitkar, 2016) [5]. Mastitis is an acute or chronic disease of udder in which inflammation of mammary gland is caused by bacteria, virus or fungi pathogen. Most mastitis persists as sub-clinical infections and is not detected by the milker, only occasionally are there clinical signs with clots in the milk and inflamed quarters. Sub-clinical mastitis caused by infectious pathogens and result is an increased content of the pathogenic cell that is Streptococcus dysgalactia, Streptococcus uberis, Escherichia coli etc. Ethano veterinary medicines refer to people's belief, knowledge, skill and practice relating to care of their farm (Martin *et al*, 2001) [10]. It is affected on the milk quality from infected teat and also decreased the milk protein, lactose, fat, etc and increased SCC (Smith *et al.*, 1997) [14]. 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) [8]. These changes in the milk depend on the invading pathogen and inflammatory response (Adane *et al.*, 2012) [11].

Turmeric contains bioactive compounds with powerful medicine and antioxidant properties. Curcumin is a natural anti-inflammatory compound (KM Nelson *et al.* 2017) [13]. The main fatty acid Ricinoleic acid found in castor oil, has impressive anti-inflammatory properties. 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) [9]. Garlic contain allicin substance with powerful antiseptic and antibacterial properties (Bilal *et al.*, 2009, Deeba *et al.*, 2009) [6, 7].

Material and methods

The present investigation was carried out in the department of Animal Husbandry and Dairying and outdoor Veterinary hospital of C S A University of Agriculture & Technology, Kanpur. This study was done under conventional housing system. Milk samples were collected from each quarter before and after the treatment. The udder was initially washed with antiseptic solution and samples were collected in sterile McCartney bottles after first three streak of milk was discarded. Different physical, clinical test (CMT, WST) applied in lactating cow and found 48 lactating cow suffering from subclinical mastitis.

Select 48 lactating cow suffering from subclinical mastitis divided in to six groups (control, treatment-1 to 5) for experiment. In treatment-1 group cows was supplemented Turmeric powder @ 250gm/animal, twice a day for 10 days, in treatment-2 castor oil applied in udder 150gm/animal twice a day for 10 days (Mooventhan *et al.* 2016). In treatment-3 cows were treated with *Allium sativum* cloves (Garlic) @ 250 gm/animal twice a day for 10 days (Bilal *et al.*, 2009, Deeba *et al.*, 2009; Dilshad *et al.*, 2009) ^[6, 7]. In treatment-4 treated with *Citrus limonum* (Lemon) fruit cut into pieces @ 250 gm/animal and treatment-5 treated with black pepper grinded @ 60 gm/ animal twice a day for 10 days (Mooventhan *et al.* 2016) and in control group no supplement applied.

Different type of milk composition parameter analyzed following the AOAC (2005) ^[3] procedure. Blood sample analyzed in local private laboratory. Statistical analyses of data were done by one-way ANOVA and paired T test using described by Snedecor and Cochran (1989) ^[15] with SPSS 20 package software. A probability levels ($p < 0.05$) was considered as statistically significant.

Estimation of lactose in Milk-

First of all taken 10 ml milk sample and 20 ml hot water, 2 ml ammonium acetate and 2 ml acetic acid was added, now the solution is transferred in 100 ml volumetric flask and mentioned the volume, now filtered the solution by 42 no. filter paper and filtrate filled in burette. In a conical flask take 5 ml Fehling solution – A, 5 ml Fehling solution –B and 40 ml distilled water. Now titration start on sprit lamp (Boiling condition) when the solution become brick red 2 ml ethyl blue added and continued the titration till the permanent brick color appeared.

$$\text{Lactose \%} = \frac{0.0678 \times 100 \times 100}{F \times \text{weight of sample taken}}$$

Where

F – Amount of filtrate (ml) required to reduce 10 ml of Fehling solution.

California Mastitis Test

The test was done as per the standard procedures described by Pandit and Mehta (1969).

Reagent: Sodium hydroxide – 15 gm, Teepol – 5 ml
Bromothymol blue – 0.1 gm Distilled water – 1000 ml

All the ingredients were mixed in a flask and mixed thoroughly and filtered. The reagent was stored in colored glass bottle.

Method

About 3 ml of milk from each quarter was drawn in to one of the four shallow cups to the paddle and equal volume of test reagent was added to the milk sample. The milk and reagent was mixed by gentle circular rotation of paddle held horizontally.

The results are calibrated as under

- Trace Slight precipitate formed which dissolved with mixing.
- 1⁺ Slimy gel formed.
- 2⁺ Gel becomes thick and flocculent.
- 3⁺ Gel becomes viscous and tacky.

Somatic cell count

The reactions occurring in CMT have been correlated with cell counts as follows.

- 1⁺ 400,000 – 1500,000 cells/ml
- 2⁺ 800,000 – 5000,000 cells/ml
- 3⁺ > 500,000 cells/ml

Result and Discussion

From table 1, it is evident that in control group 8 cows, none have recovered whereas in the treatment group turmeric powder and garlic highest recovery 75% among all treatment. Use of Turmeric powder was best option in this trail. Turmeric powder showed highly significantly ($P < 0.05$) effect on RBCs, Lymphocytes, Hb, PCV, CMT, SCC. Same result also observed by Thangadurai *et al.* (2017) ^[17]. It is less significant in MCHC, MCV, specific gravity, Electrical conductivity, Fat. Black pepper was lowest recovery percentage among treatment group. Same percentage also reported by Dilshad *et al.* (2009), Mooventhan *et al.* (2016). In this trail black pepper was used as alternative drug of antibiotic agent. Black pepper showed significantly ($p < 0.05$) effect on Lymphocytes and WBCs and milk protein but did not good response on RBCs, HB, MCV, pH and lactose. Black pepper showed less effect on subclinical affected cross bred dairy cow. Same result also observed by Bilal *et al.* (2009) ^[6]. Use of *citrus limonum* (Lemon) was third best option followed by turmeric powder and garlic for treatment of subclinical mastitis in cross bred dairy cow. Pirestani *et al.* (2013) also found that citrus was third best option. Bila *et al.* (2009) experiment that lemon with ammonium chloride 10% was the most favored drug. It was most found significant ($p < 0.05$) protein, Fat, RBCs, HB, MCHC. Garlic (*Allium stivum*) used in study was second best option which showed better results in curing subclinical mastitis, increase immune system. It showed significantly ($P < 0.05$) increased in milk composition, blood parameter, lymphocyte number and percentage. Dilshad er al. (2009) also same value observed in previously use garlic for subclinical mastitis treatment. These effect are due to antiseptic and antibacterial properties of allicin ingredient of the garlic. Garlic showed less significant ($P < 0.05$) in Electrical conductivity, MCHC. Castor oil showed average result in this study, only 50% dairy cow recovered. Castor oil showed significantly ($P < 0.05$) increases milk composition except pH and in blood parameter significantly increase in RBCs Lymphocytes, HB, and PCV after treatment and significantly ($P < 0.05$) decrease in WBCs, pH, MCV and EC. Same result also observed by Mushtaq *et al.* (2018) ^[12].

Table 1: Recovery % by Immuno-potentiating Herbal agents in subclinical mastitis affected cross bred dairy cow

Group	No. of affected animals	No. of recovered animals	% of recovered animals
Control (T ₀)	8	0	0
Turmeric Powder (T ₁)	8	6	75
Castor oil (T ₂)	8	4	50
Garlic (T ₃)	8	6	75
Lemon (T ₄)	8	5	62.5
Black pepper (T ₅)	8	3	37.5

Table 2: (Mean \pm SEM) of compositional changes in milk before and after treatment in cross bred dairy cows

Treatment	pH		Fat (%)		Protein (%)		Lactose (%)	
	Before	After	Before	After	Before	After	Before	After
Control	6.9 \pm 0.01	6.9 \pm 0.01	3.81 \pm 0.02	3.81 \pm 0.02	3.80 \pm 0.01	3.80 \pm 0.01	4.64 \pm 0.02	4.64 \pm 0.02
Turmeric Powder	6.9 \pm 0.03	6.6 \pm 0.01	3.31 \pm 0.03	3.58 \pm 0.01	3.48 \pm 0.03	3.82 \pm 0.02	4.16 \pm 0.04	4.58 \pm 0.03
Castor oil	6.9 \pm 0.01	6.7 \pm 0.02	3.28 \pm 0.02	3.50 \pm 0.03	3.45 \pm 0.01	3.72 \pm 0.03	4.28 \pm 0.02	4.54 \pm 0.01
Garlic	6.8 \pm 0.01	6.5 \pm 0.02	3.32 \pm 0.04	3.52 \pm 0.01	3.44 \pm 0.03	3.76 \pm 0.04	4.38 \pm 0.04	4.62 \pm 0.03
Lemon	6.8 \pm 0.02	6.6 \pm 0.03	3.30 \pm 0.02	3.48 \pm 0.03	3.42 \pm 0.01	3.87 \pm 0.02	4.32 \pm 0.03	4.56 \pm 0.01
Black pepper	6.7 \pm 0.03	6.5 \pm 0.01	3.27 \pm 0.04	3.49 \pm 0.03	3.46 \pm 0.02	3.69 \pm 0.02	4.32 \pm 0.02	4.61 \pm 0.02

Means having different superscripts within the same row differs significantly ($P < 0.05$)

Table 3: (Mean \pm SEM) of Immuno-Potentiating Herbal agents effect on milk before and after treatment

Treatment	Somatic cell count (cell/ml) in lakhs		CMT		Electrical conductivity (μ g/cm)		Specific gravity	
	Before	After	Before	After	Before	After	Before	After
Control	2.73 \pm 2.01	2.73 \pm 201	Clumping noticed	Clumping noticed	1 \pm 0.05	1 \pm 0.05	1.01 \pm 0.17	1.01 \pm 0.17
Turmeric Powder	2.72 \pm 1.76	1.91 \pm 1.17	Clumping noticed	No clump	1 \pm 0.02	0.8 \pm 0.04	0.98 \pm 0.21	1.027 \pm 0.02
Castor oil	2.70 \pm 2.10	1.92 \pm 1.06	Clumping noticed	No clump	1 \pm 1.0	0.9 \pm 0.03	0.97 \pm 0.14	1.030 \pm 0.01
Garlic	2.74 \pm 1.05	1.95 \pm 1.54	Clumping noticed	No clump	0.98 \pm 0.12	0.81 \pm 0.02	1.01 \pm 0.12	1.028 \pm 0.02
Lemon	2.71 \pm 1.80	1.90 \pm 1.15	Clumping noticed	No clump	1.07 \pm 0.01	0.92 \pm 0.03	0.99 \pm 0.01	1.027 \pm 0.12
Black pepper	2.68 \pm 2.10	1.87 \pm 1.47	Clumping noticed	No clump	1.11 \pm 0.2	0.95 \pm 0.03	1.0 \pm 0.01	1.03 \pm 0.02

Means having different superscripts within the same row differs significantly ($P < 0.05$)

Table 4: (Mean \pm SEM) of Haematological parameter before and after treatment in cross bred dairy cows

Treatment	RBCs ($10^{12}/L$)		WBCs ($10^9/L$)		Lymphocytes ($10^9/L$)	
	Before	After	Before	After	Before	After
Control	8.02 \pm 0.41	8.02 \pm 0.41	6.62 \pm 0.11	6.62 \pm 0.11	5.54 \pm 0.10	5.54 \pm 0.10
Turmeric Powder	7.64 \pm 0.24	9.10 \pm 0.14	8.42 \pm 0.16	6.75 \pm 0.31	4.01 \pm 0.18	6.14 \pm 0.54
Castor oil	7.41 \pm 0.32	8.89 \pm 0.12	8.98 \pm 0.10	7.0 \pm 0.10	4.98 \pm 0.12	7.05 \pm 0.32
Garlic	6.85 \pm 0.30	7.79 \pm 0.32	8.38 \pm 0.36	6.74 \pm 0.22	3.98 \pm 0.32	5.85 \pm 0.20
Lemon	7.85 \pm 0.12	8.91 \pm 0.15	9.10 \pm 0.24	6.97 \pm 0.036	4.72 \pm 0.28	6.01 \pm 0.41
Black pepper	7.20 \pm 0.35	8.10 \pm 0.25	9.14 \pm 0.40	7.01 \pm 0.14	4.98 \pm 0.35	7.12 \pm 0.41

Means having different superscripts within the same row differs significantly ($P < 0.05$)

Table 5: (Mean \pm SEM) of Haematological parameter before and after treatment in cross bred dairy cows

Treatment	HB (gm/dl)		MCV (fl)		PCV (%)		MCHC (g/dl)	
	Before	After	Before	After	Before	After	Before	After
Control	8.68 \pm 0.06	8.68 \pm 0.06	44.52 \pm 0.75	44.52 \pm 0.75	26.8 \pm 0.21	26.8 \pm 0.21	32.56 \pm 0.21	32.56 \pm 0.21
Turmeric Powder	8.45 \pm 0.45	9.94 \pm 0.25	44.42 \pm 0.12	45.35 \pm 0.74	27.1 \pm 0.14	31.10 \pm 0.42	32.52 \pm 0.07	32.86 \pm 0.01
Castor oil	8.91 \pm 0.38	10.05 \pm 0.44	44.82 \pm 0.54	45.24 \pm 0.61	25.98 \pm 0.54	28.02 \pm 0.02	31.48 \pm 0.12	31.95 \pm 0.47
Garlic	7.92 \pm 0.32	8.81 \pm 0.21	43.83 \pm 0.41	45.50 \pm 0.12	26.52 \pm 0.62	30.84 \pm 0.32	32.02 \pm 0.41	32.85 \pm 0.32
Lemon	8.86 \pm 0.12	10.1 \pm 0.10	43.62 \pm 0.55	45.87 \pm 0.68	28.10 \pm 0.21	32.85 \pm 0.65	32.10 \pm 0.65	32.94 \pm 0.43
Black pepper	8.88 \pm 0.38	9.98 \pm 0.12	44.52 \pm 0.14	45.33 \pm 0.45	27.5 \pm 0.41	31.84 \pm 0.12	31.92 \pm 0.24	32.26 \pm 0.36

Means having different superscripts within the same row differs significantly ($P < 0.05$)

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