COMPARATIVE THERAPEUTIC EFFICACY OF HOMEOPATHIC COMPLEX, HERBAL EXTRACT AND ANTIBIOTIC IN THE TREATMENT OF SUBCLINICAL MASTITIS IN DAIRY BUFFALOES

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ABSTRACT

Comparative therapeutic efficacy of three drugs; homeopathic complex, herbal (Neem seed extract) and antibiotic in the treatment of subclinical mastitis in dairy buffaloes was evaluated. Thirty six Surf Field Mastitis Test (SFMT) positive lactating animals were randomly divided into four equal groups named A, B, C and D. Group A was treated with homeopathic complex (Mastitojin Gold™), Group B was treated with hydro-methanolic extract of Neem seeds (*Azadirachta indica*), Group C was treated with Procaine Penicillin and Group D was kept as untreated control. Bacteriological cure rate, milk pH, milk yield, cost effectiveness and SFMT cure rate were evaluated in groups. The pH was significant ($P<0.01$) in Group C at 7th and 14th day post treatment. Percent cure rates of mastitic quarters on the basis of SFMT was highest in Group C followed by Group B, Group A and Group D at 28th Day Post Treatment (DPT), respectively. The quarter based bacteriological cure rate was highest in group C (83.33%) followed by Group B, Group A and Group D. Total milk yield of Group B was improved significantly ($P<0.05$) at 28th DPT as compared to other treatment groups. Comparison of treatment cost showed that neem seed extract is the cheapest source in the treatment of subclinical mastitis in dairy buffaloes. It was observed that the antibiotic therapy was better than the herbal (neem seed extract) therapy and homeopathic complex therapy.

Keywords: mastitis, treatment, homeopathic, herbal, antibiotic, buffaloes, *Bubalus bubalis*

INTRODUCTION

Pakistan is gifted with massive population of livestock. The buffalo has been designated as 'black gold' of Pakistan (Bilal *et al*., 2006). Buffalo population is nearly 31.7 million that produces 28.694 million tons of milk yearly. The buffalo shares 61.8% in total milk production (GOP, 2011). Among many diseases, mastitis is one of the most

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important diseases in Pakistan (Cady *et al*., 1983; Ajmal, 1990; Akram, 2002). Mastitis is described as the inflammation of parenchyma of udder tissue resulting from injury to the udder tissue, lowers milk production, decreases milk quality along with increased somatic cell count. Mastitis is of two types i.e., sub-clinical and clinical mastitis (Radostitis *et al*., 2007).

Mastitis is major production limiting disease of dairy animals. It not only affects the animal health but also deteriorates the quality and quantity of milk produced. Despite various efforts, it has remained the most important disease of dairy animals (Lightner *et al*., 1998).

National Mastitis Council Inc. of USA has reported that 70 to 80% milk losses are due to sub-clinical mastitis (NMC, 1996). In Pakistan, losses due to sub-clinical mastitis may be much higher than clinical mastitis as sub-clinical mastitis is 15 to 40 times more prevalent than the clinical form (Schultz *et al*., 1978; Athar *et al*., 2007; Sharif *et al*., 2007).

Mastitis causes huge financial losses in Pakistan in form of high medication cost and low milk production (Arshad *et al*., 1995; Ahmad *et al*., 1991; Soomro *et al*., 1997; Ahmad*,* 2001). In subclinical mastitis obvious signs are not observed but somatic cell count is high (Radostitis *et al*., 2007). In Pakistan, the hurdle behind the high cost losses due to mastitis may be due to lack of implementation of reasonable mastitis control program (Arshad, 1999).

Ethno-veterinary practice is rife for curing various veterinary clinical ailments. Using various practices of ethno-veterinary medicine, better therapeutic solutions to various animal diseases have been developed. It is estimated that plant materials have provided nearly 50% of western drugs (Robbers, 1996).

The present study was therefore designed to analyze the beneficial use of ethno-veterinary medicine in mastitis treatment. In this regard the comparative therapeutic efficacy of homeopathic complex, herbal extract and antibiotic in the treatment of subclinical mastitis in dairy buffaloes was planned.

MATERIALS AND METHODS

Screening and selection of experimental animals

162 lactating buffaloes were screened for subclinical mastitis from the dairy farm of Department of Livestock Management, University of Agriculture, Faisalabad, and Sarbuland Dairy Farm 73 JB Chupal, Faisalabad. 36 buffaloes suffering from sub-clinical mastitis were selected on the basis of positive Surf Field Mastitis Test (SFMT) (Muhammad *et al*., 1995; Muhammad *et al*., 2010).

Experimental design

Thirtysix (36) lactating sub-clinically positive dairy buffaloes were randomly divided into four equal groups named A, B, C and D. Guidelines of International Dairy Federation for mastitis therapy were followed for assigning study animals in different groups (Thorburn, 1990). Group A was treated with homeopathic complex (MastitojinGold™) [Mustaqeem Herbal and Homeo, Faisalabad containing Conium 200, Calcareafluorica 200, Bryonia 200, Phytolacca 200 and Kali Mur 200], 10 ml/IM for 5 days, Group B was treatedwith lerbal product (hydro-methanolic extract of Neem seeds,*Azadirachtaindica*) 75 mg in 5 ml PBS through intra-mammary route twice a day for 5 days, Group C was treated with antibiotic (Procaine Penicillin) 2,000,000 I.U through intra-

mammary route for 5 days and Group D was kept as untreated / control group (Table 1).

Preparation of udder and teats

Before the collection of milk samples, udder and teats were cleaned and dried with paper towel. Each teat opening was then scrubbed with 70% ethanol moistened cotton gauze. Separate gauze was used for each teat.

Collection of sample

After sanitizing all teats, milk samples were collected in sterile screw caped vials labeled with date of collection, animal name and position of quarter. Left front teat was labeled as LF, left rear teat as LR, right front teat as RF and right rear teat as RR. First few streams of milk were discarded to reduce the number of contaminating bacteria in the teat canal. Sample from the nearest teat was taken first and then from the far ones to reduce the chances of contamination. Sample was collected in vials following the guidelines of National Mastitis Council, Inc., USA (NMC, 1990).

Sampling schedule

Milk samples were collected aseptically from all the experimental animals before the start of treatments i.e. at Day 0, during treatment at 14th DPT and after the end of treatment at 28th DPT.

Handling and storage of samples

Immediately after collection, milk samples were placed on crushed ice and transported to Mastitis Research Laboratory, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad and stored at 4 to 5ºC until use.

Preparation of neem seed extract

Neem seed were taken from the market and these seeds were identified from the botanical lab, University of agriculture Faisalabad. These seeds were grind into fine powder and this neem seed powder were loaded into SOXHLET apparatus (Peach *et al*., 1956), containing 70% methanol and extraction was done. After extraction, this extract was condensed under vacuum dryer. After it this

Table 1. Treatment protocol for different groups.

Group	Number of animals	Treatment	Dosage per animal	Route	Schedule	Duration of treatment
A	9	Homeopathic complex	10 Ml	Intra Muscular	Once a day	5 days
B	9	Herbal [Neem seed extract]	75 mg in 5 mL PBS	Intra-Mammary Infusion	twice a day	5 days
C	9	Antibiotic [Procaine penicillin]	20 lacs I.U	Intra Mammary Infusion	Once a day	5 days
D	9	Untreated / control	--	--	--	--

condensed extract was used in the preparation of 75 mg dose in 5 ml PBS for intra-mammary infusion.

Parameters of study

The parameters of SFMT score, SFMT based cure rate, Bacteriological Examination of Milk, pH of milk samples as described by Cruickshank *et al.* (1975) and total milk yield of every group was recorded. The data thus collected was analyzed and results were compared among treatment groups. The comparison of cost of treatment among groups was also calculated.

RESULTS

The effect of treatments on different parameters in different groups is as under:

SFMT score

The Mean SFMT score ± SE of Group A at Day 0, 14th DPT and 28th DPT was 3.00±0.210, 0.929±0.355 and 0.643±0.289 respectively. The Mean SFMT score ± SE of Group B at Day 0, 14th DPT and 28th DPT was 2.933±0.182, 0.867±0.336 and 0.333±0.187 respectively. The Mean SFMT score ± SE of Group C at Day 0, 14th DPT and 28th DPT was 2.833±0.241, 0.583±0.313 and 0.167±0.167, respectively. While the Mean SFMT score ± SE of Group D (Control) at Day 0, 14th DPT and 28th DPT was 2.588±0.228, 2.588±0.285 and 3.059±0.303 respectively (Table 2).

SFMT based cure rate in groups

SFMT based cure rates of sub-clinically affected mastitic quarters of Group A at 14th DPT and 28th DPT was 64% and 71%, respectively. Cure rate of Group B at 14th DPT and 28th DPT was 67% and 80%, respectively. Cure rate of Group C at 14th DPT and 28th DPT was 75% and 91 %, respectively. While cure rate of Group D (control group) at 14th DPT and 28th DPT was 11% and 11 %, respectively (Table 3).

Bacteriological cure rate in groups

Bacteriological based cure rates of sub-clinically affected mastitic quarters of Group A at 14th DPT and 28th DPT was 57.14% and 64.28%, respectively. Bacteriological Cure rate of Group B at 14th DPT and 28th DPT was 60% and 73.33%, respectively. Cure rate of Group C at 14th DPT and 28th DPT was 66.66% and 83 %, respectively. While cure rate of Group D (control group) at 14th DPT and 28th DPT was 5.88% and 5.88%, respectively (Table 4).

pH of milk

The Mean pH ± SE of Group A at Day 0, 14th DPT and 28th DPT was 7.186±0.017, 7.134±0.016 and 6.975±0.044, respectively. The Mean pH ± SE of Group B at Day 0, 14th DPT and 28th DPT was 7.213±0.018, 7.141±0.022, 6.935±0.011, respectively. The mean pH ± SE of Group C at Day 0, 14th DPT and 28th DPT was 7.183±0.024, 7.136±0.025 and 6.973±0.019, respectively. While the mean pH ± SE of Group D (control) at Day 0, 14th DPT and 28th DPT was 7.206±0.021, 7.257±0.025, 7.328±0.027, respectively (Table 5).

Milk yield

Effect of the treatment on milk yield of all the groups was recorded at 0 and at 28th DPT. The Mean ± SE for milk yield of Group A at Day 0 and at 14thDPT was 4.19±0.52 and 4.44±0.52, respectively. The Mean ± SE for milk yield of Group B at Day 0 and at 14thDPT was 6.08±0.72 and 6.44±0.70, respectively. The Mean ± SE for milk yield of Group C at Day 0 and at 14th DPT

was 5.64±0.21 and 5.78±0.21, respectively. While the Mean ± SE for milk yield of Group D (control group) at Day 0 and at 14th DPT was 5.54±0.29 and 5.63±0.29, respectively (Table 6). While the overall mean ± SE for total milk yield of Group A, B, C and D were 4.31±0.36, 6.26±0.49, 5.59±0.20 and 5.71±0.14, respectively. The mean ± SE for total milk yield of all experimental dairy buffaloes at Day 0 was 5.37±0.26 while this figure was 5.57±0.26 at 28th DPT.

Comparison of treatment cost in groups

The total cost of treatment in Group A, B, and C was Rs. 1350/-, Rs.62/- and Rs.1125/- respectively. While the Group D was untreated control (Table 9).

DISCUSSION

There is no precise definition of alternative therapy. An appropriate explanation is that alternative therapy of animals comprises ways of treatment that are not incorporated in the conventional veterinary therapy, as accepted and experienced in the curricula of veterinary institutions. It is a substitute to "school medicine" (Persson *et al*., 1999). Homeopathic strategy is based mainly on the law of similarities. This basic principle is expressed by the Latin motto "Similiasimilibuscurantur", i.e. the like is cured in like manner. This also marks the fundamental difference between homeopathy and allopathy. The allopathy is a system of therapeutics in which diseases are treated by producing a condition antagonistic to the condition to be cured (Silahava *et al*., 2005). Homeopathic concept of health, disease and treatment was first described by Hahnemann in 1982. The homeopathic drugs of plant, mineral or animal origin are administered to patients in highly diluted form (Hektoen *et al*., 2004). The efficacy of homeopathic treatment for mastitis is the alternative to the use of antibiotics (Werner *et al*., 2010). The ethno-botanical knowledge is under severe threat of disappearing due to rapid urbanization, socio-economic, environmental, technological and cultural changes (Nfi *et al*., 2001; Khan *et al*., 2012). So it is dire need to collect and systematically analyze the data to pay due consideration to conserve wild plants having medicinal importance (Habib *et al*., 20114).

In our study homeopathic, herbal and antibiotic therapies were used for the treatment of mastitis and results were interpreted based on SFMT score, SFMT cure rate, bacteriological cure, pH and milk yield in experimental animals.

The SFMT score is a precious tool for semi-quantitative evaluation of milk cell concentration and a good indicator of udder infection (Fazal-ur-Rehman, 1995; Busato *et al*., 2000). In our study at 14th DPT the SFMT score of Group C was less than 1+ and 90% animals were recovered. Other researchers had also reported 85% recovery rate with less than 1+ CMT score in sub-clinical mastitis (Dhakal, 2006).

On the basis of SFMT cure rate, the percentage cure rate in Group C (90%) was the highest, followed by Group B (80%), Group A (71%) and Group D (11%) (Table 3). Among the different treated groups, the group treated with procaine penicillin showed the highest cure rates percentages at 14th and 28th DPT. The SFMT based cure rate of Group C was 75% and 91% at 14th and 28th DPT, respectively. It was followed by Neem seed extract (Group B) which showed 67% and 80% at 14th DPT and 28th DPT, respectively. Homeopathic complex (Group A) had shown 64% at 14th and 71% at 28th DPT, respectively. While

Table 2. Mean ± SE for SFMT based cure rates (at Day 0, 14th and 28th DPT) in groups.

Group	SFMT cure rate		
	Day 0	14th DPT	28th DPT
A	3.000±0.210[a]	0.929±0.355[b]	0.643±0.289[b]
B	2.933±0.182[a]	0.867±0.336[b]	0.333±0.187[b]
C	2.833±0.241[a]	0.583±0.313[b]	0.167±0.167[b]
D	2.588±0.228[a]	2.588±0.285[a]	3.059±0.303[a]

Mean values with different superscripts differ significantly (P>0.05).

Table 3. SFMT based cure rate at 14th and 28th DPT in groups.

Group	Quarters infected at Day 0	Quarters cured at 14th DPT	Percentage cure rate at 14th DPT	Quarters cured at 28th DPT	Percentage cure rate at 28th DPT
A	14	9	64%	10	71%
B	15	10	67%	12	80%
C	12	9	75%	11	91%
D	17	2	11%	2	11%

Table 4. Bacteriological cure rate at day 14th and 28th DPT in groups.

Group	Cured quarters out of total at 14 day	Percent cure rate at 14th DPT	Cured quarters out of total at 28th DPT	Percent cure rate at 28th DPT
A	8/14	57.14%	9/14	64.28%
B	9/15	60%	11/15	73.33%
C	8/12	66.66%	10/12	83.33%
D	1/17	5.88%	1/17	5.88%

Table 5. Mean ± SE of pH of milk in groups.

Group	Day 0	7th DPT	14th DPT
A	7.186±0.017[a]	7.134±0.016[b]	6.975±0.044[b]
B	7.213±0.018[a]	7.141±0.022[b]	6.935±0.011[b]
C	7.183±0.024[a]	7.136±0.025[b]	6.973±0.019[b]
D	7.206±0.021[a]	7.257±0.025[a]	7.328±0.027[a]

Mean values with different superscripts differ significantly (P>0.01).

Table 6. Percentage decrease of milk pH at 7th DPT and 14th DPT in groups.

Group	Percent decrease of milk pH w.r.t Day 0	
	7th DPT	14th DPT
A	0.75%	2.94%
B	0.96%	3.80%
C	0.65%	2.92%
D	0.071%	1.6%

Table 7. Mean ± SE for total milk yield at 14thDPT in groups.

Group	Day of treatment		Mean
	Day 0	28th DPT	
A	4.19±0.52	4.44±0.52	4.31 ± 0.36^{b}
B	6.08±0.72	6.44±0.70	6.26 ± 0.49^{a}
C	5.64±0.21	5.78±0.21	5.59 ± 0.20^{a}
D	5.54±0.29	5.63±0.29	5.71 ± 0.14^{a}
Mean	5.37 ± 0.26^{a}	5.57 ± 0.26^{a}	--

Mean values with different superscripts differ significantly (P>0.05).

Table 8. Percentage increase of milk yield at 28th DPT in groups.

Group	Percent increase in milk yield at 28th DPT w.r.t Day 0
A	25%
B	36%
C	14%
D	9%

Table 9. Comparison of treatment cost among groups.

Group	Cost of single dose	Cost / Dose / Day	Total treatment cost
A	Rs.15/-	Rs.15/-	1350/-
B	Rs.3.4/-	Rs. 6.8/-	Rs.62/-
C	Rs.25/-	Rs.25/-	Rs.1125/-
D	--	--	--

Group D showed 11% at 14th and 28th DPT. The survey of use of ethno-veterinary practices in Nepal also indicated that the use of about 30 to 40 gm paste of tuber of plant *Diosocrea deltoidea* is also used for treatment of mastitis in buffaloes (Birendra and Chhetri, 2012). It is also documented that the fruit of *Amomum subulatum* and leaves of *Rosa indica* are also effective against mastitis (Chakraborty and Pal, 2012). Other studies have also documented the use of *Allium sativum* (Lahsan), *Capscicum frutescens* (Surkh mirch), *Vernonia anthelmintica* (Kali ziri), *Piper nigrum* (Kali mirch) *Capscicum anmum* (Hari mirch) and *Brassica compestris* (Sarson) have also been used for treatment of mastitis in dairy animals in peri-urban areas of district Faisalabad (Pakistan) (Deeba *et al*., 2009).

In our study the bacteriological examination of milk samples was carried out in all groups of sub-clinically mastitic buffaloes at day 0, 14th and 28th DPT (Table 4). Procaine penicillin therapy (Group C) had highest bacteriological cure rate of 83.33%, followed by neem seed extract (73.33%) and homeopathic complex (64%). Whereas the percentage for untreated / control (Group D) was the lowest (5.88%). Both the antibiotics and non-antibiotic antibacterial have significant affects against mastitogens. Researchers have found that mastitogens are also susceptible to non-antibiotics like Caprylic acid and mono-caprylin (Nair *et al*., 2005). Based on bactericidal activity the antibiotics like Penicillin and Novobiocin have also shown good results against mastitogens (Thornsberry *et al*., 1997). Apart from penicillin the common pathogen of mastitis i.e. *Staphylococcus aureus*, is also sensitive to neomycin, erythromycin (36%), penicillin (54%), tetracycline (64%) and sulphamethaxazol + trimethoprim (75%) (Hawari and Dabbas, 2008). Although in some cases mastitogens exhibited resistance to some degree against cloxacilline, penicillin and streptomycin (Ebrahimi *et al*., 2008; (Turutoglu *et al*., 2006). Studies have reported that the *S. aureus* is the most important mastitogen and has responded poorly to antibiotics treatment (Gruet *et al*., 2001). The alternative medicine from homeopathic or herbal extract can be effective in cases of antibiotic resistant mastitogens, as observed in our study. Other scientists also observed that the efficiency of homeopathic complex in treatment of acute mastitis was 86.6%, while in case of antibiotic treatment it was 59.2% with a average recovery period of 4.5 days, and concluded that homeopathic complex was more effective and economical in treatment of mastitis (Varshney and Naresh, 2004). Use of Neem seed extract in management, treatment and for decrease in bacterial count of mastitis has also been observed by other researchers (Kumar and Mukherjee, 2009). In our study mastitis therapy in different study groups was carried for 5 days. The more favorable results were obtained in some other studies with extended duration of therapy with homeopathic complex for more than 5 days i.e. for 7 days and more (Varshney and Naresh, 2004). The difference of efficiency for antibiotic, homeopathic and herbal extract from our study can also be due to difference in duration of therapy, mode of administration of drug, use of adjutants / excepients, use of different solvent, method of preparation and formulation of extracts, etc. It is also observed that apart from antibiotics, *per os* administration of non-antibacterial compound i.e. trisodium citrate to buffaloes also brought cure in mastitis cases (Dhillon *et al*., 1995; Sarfaraz, 2010). Studies have also documented that the methanol extract of *Asteracantha longifolia* is effective against the Bovine mastitis caused by *Staph. aureus* and *E. coli* (Chakraborty and Pal, 2012).

In our study the pH of milk decreased in experimentally treated groups as compared to untreated / control group. The percent decrease on milk pH was more marked at 14th DPT. The percent decrease of milk pH of Group A at 7th DPT and at 14th DPT was 0.75% and 2.94% respectively. The percent decrease of milk pH of Group B at 7th DPT and at 14th DPT was 0.96% and 3.80% respectively. The percent decrease of milk pH of Group C at 7th DPT and at 14th DPT was 0.65% and 2.92% respectively. The percent decrease of milk pH of Group D (untreated / control group) at 7th DPT and at 14th DPT was 0.071% and 1.6% respectively (Table 6). It is also observed in other studies that the administration of homeopathic complex *per os* in cattle and buffaloes changed the pH of milk from alkaline to acidic (Mujahid, 2010). The change in pH may serve as one of the indicators to assess the udder health status of the animal and food value of the milk. Normal value of milk pH is 6.5 (Dhillon *et al*., 1989). The lower value of pH of milk may be prohibitive to bacterial growth. While mastitic milk has increased pH, ranging from 7.2 to 7.6, which is optimal for the multiplication of most of the mastitogens (Cruickshank *et al*., 1975). As the severity of mastitis increases, the pH value increases (Haggag *et al*., 1991; Radostits *et al*., 2007). Other researchers have also documented that Neem seed extract is helpful in bringing level of milk pH to normal value (Kumar and Mukherjee, 2009). Swinging the pH to acidic not only creates the unfavorable environment for the growth of mastitic pathogens but may also render some antibiotics (e.g penicillin) more effective. Other experiment has shown that exposure of pathogens to very low pH of 1 or 2 had resulted in killing of vancomycin resistant *Enterococcus spp*., *Staphylococcus aureus*, *Klebsiella pneumonia*, *Candida glabrate*, and this killing was enhanced with non-antibacterial acidified nitrate (Rao *et al*., 2006). In the environment of low pH, the pathogens cannot acquire energy for their growth and acidic conditions make their survival difficult (Gibson, 1999). The mastitic milk having high pH is of bad taste. The bad taste of milk is also indicator of change of udder disorder. The use of leaves of *Monotheca buxifolia* has also been used to treat the abnormal taste of milk by traditional veterinary healers (Habib ul Hassan *et al*., 2014).

In our study the maximum increase in the milk production was recorded in the Group B treated with Neem seed extract (36%), followed by Group A (25%), Group C (14%) and Group D (9%) (Table 8). The increase in milk production is one of the indicators of recovery from mastitis and improvement of the udder health. The normal functioning of udder parenchyma is pre requisite for maximum milk production from dairy animals. Researchers have also recorded that up to 28% increase in milk production in animals which recovered from sub-clinical mastitis (Sastry and Tripathi, 1998). Other ethno-veterinary practices involving use of plant material of *Euphorbia hirta* and *Solena heterophylla* has also been recorded to increase milk production in buffaloes (Birendra and Chhetri, 2012).

In our study homeopathic product Mastitojin Gold™ [Mustaqeem Herbal and Homeo, Faisalabad containing Conium 200, Calcareafluorica 200, Bryonia 200, Phytolacca 200 and Kali Mur 200] was used Inra-muscularly and hydro-methanolic extract of Neem seeds was used Intra-mammary in experimental groups. Other studies have also documented the use of excepients like milk, sugar, flour etc along with herbal and homeo products during practicing ethnoveterinary practices (Habib ul Hassan *et al*., 20114). Studies have also documented that ethno-

veterinary practices are used by livestock owners by preparing remedies by pulverization, soaking in water and decoctions and used orally or applied locally (Deeba *et al*., 2009). In our study extract Neem seeds was used for treatment of mastitis. Parts of plants like leaf, root, bark, bulb, stem / branch, fruit, tuber, whole plant, milk secretion and rhizome of other herbs are also used in veterinary health care. Tamiru et al. (2013) documented that cow affected with mastitis is treated by juice of grinded fruit of *Solanum dasphyllum*, bulb of *Allium sativum* and leaf of *Croton macrostachyus* orally for three consecutive days. Water squeeze of crushed root of *Cynoglossum lanceolatum* is also drenched orally for mastitis treatment in Dabo Hana district of West Ethiopia.

In our study the comparison of treatment cost showed that neem seed extract is the cheapest source (Rs.6.8/-) in the management and treatment of subclinical mastitis in dairy buffaloes, followed by homeopathic complex (Rs.15/-) and procaine penicillin (Rs.25/-) on per dose basis (Table 9). Similarly in other studies researchers also compared the treatment cost of homeopathic and antibiotic in the management of mastitis and found that treatment cost for homeopathic complex and antibiotic was Rs.21.44/- and Rs.149.20/-, respectively (Varshney and Naresh, 2005). The variation of cost is due to different combination of antibiotic and homeopathic drugs.

In recent era, the demand for an inexpensive and easily available mastitis therapy has increased that should not produce drug residues in milk of treated animals. Since drug resistance is one of the current issues of antibiotic therapy (Tamiru *et al*., 2013). Homeopathic therapies have no side effects, no drug resistance and are mostly cheap (Loken, 2001). Mostly the homeopathic therapy is used as the control measure and for cure of mastitis. The main effect of homeo-therapy is on the immune system of udder as it is assumed that this therapy boost up the immune system to cope with disease challenge (Meaney, 1993). Use of homeopathic medicine to treat mastitis is good in food producing animals as it lowers the hazard of antibiotic residues in milk and milk products (Loken, 2001). The findings of other studies are also in line with our results that homeopathic complex is also effective and economical option in treatment of mastitis (Loken, 2001; Varshney and Naresh, 2004). Other researchers have also documented that the use of ethnoveterinary medicine presents a cheaper and alternative to synthetic medicines (Dilshad *et al*., 2010). Yirga *et al*. (2012) also reported that ethno-veterinary medicines are cheap and locally available than pharmacotherapy

CONCLUSION

It was concluded that all three treatments can be used in the treatment of sub-clinical mastitis. Neem seed extract and homeopathic complex can be used as alternative treatment in the management of sub-clinical mastitis in dairy buffaloes. Neem seed extract is the cheapest source in the treatment of sub-clinical mastitis in dairy buffaloes. Neem seed extract and homeopathic complex have no side effect and no residual effect in the milk of treated animals.

In Pakistan majority of livestock farmers are poor. The use of ethno-veterinary medicine can also be an alternative to modern allopathic drugs for treatment of mastitis in developing countries like Pakistan.

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