

Intramammary Honey Infusion: A New Trend in the Management of Bovine Subclinical Mastitis

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Abstract: The objective of this study was to determine the most suitable and effective regimen of intramammary honey infusion in bovine subclinical mastitis. A total of twenty five subclinical mastitic cows from two farms were divided into two groups; group a: 10 lactating cows were infused 10 mL fennel honey solution 10% in sterile saline/quarter daily for three successive doses; group b: 15 lactating cows were infused day by day for three successive doses guarded with intramuscular antihistaminic drug. Milk samples of both groups showed a decrease in total bacterial count at the 3rd and 10th day and the reduction percent was 99.6 and 99.8%, respectively. Milk cytological results showed a significant increase ($p < 0.05$) in lymphocyte and significant ($p < 0.05$) decrease in neutrophil percentages at the 3rd day up to the 10th day in group b while the same results were achieved at the 10th day in group a. Milk yield records in group a showed decrease by the 3rd day post intramammary infusion and then gradually increased up to the 10th day reaching nearly the same levels. Milk yield of group b showed a slight decrease by the 3rd day and gradually increased to level higher than that before infusion. Haematological study revealed that the percentage of blood neutrophil significantly decreased ($p < 0.05$) from the 3rd up to the 10th day in both groups while the lymphocytic percentage increased significantly ($p < 0.05$). In group a, the eosinophil cells' percentage was significantly increased ($p < 0.05$) at the 3rd day. It was concluded that intramammary 10% honey infusion day by day for three doses guarded by antihistaminic drug could treat bovine subclinical mastitis. Moreover, milk cytology could be used instead of California mastitis test during and after treatment with honey.

Key words: Apitherapy, subclinical mastitis, intramammary honey infusion, milk cytology, treatment, drug

INTRODUCTION

The bees were quoted by Surat in the Holy Quran, There are issues from within their bodies a drink of varying colours where in is healing for men, verily in this is a sign for those who give thought Surat-Al-Nahl, Aya 69 (Pckthali, 1981).

Subclinical mastitis is often undetected and has the greatest economic consequences because of long term effects on milk yields which may lead to development of clinical mastitis (Ott, 1999; Wu *et al.*, 2007), rather than milk production reduced by 25, 33 and 48% in quarters with CMT scores 1+, 2+ and 3+, respectively (Tsfaye *et al.*, 2010).

Classic treatment managements of such cases have serious drawbacks; great economic losses from milk withdrawal period and initiation of bacterial antibiotic resistance (Ruegg *et al.*, 2008). Immunomodulation is the goal of several alternative therapies advocated for mastitis treatment. Recently in alternative medicine intramammary

treatment of mastitis in dairy cows with a live lactococcal culture (Crispie *et al.*, 2008; Beecher *et al.*, 2009) or with bee venom (Han *et al.*, 2009) could boost the mammary defense.

Antimicrobial fennel honey activity against subclinical mastitic pathogens was studied by Ali *et al.* (2005) also fennel honey raised B and T-lymphocytes activities in the blood stream (Neveen *et al.*, 2008). The present research aimed to enhance the natural host defensive mechanisms and initiating the udder innate immunity through intramammary fennel honey infusion and to determine the most suitable and effective regimen of intramammary honey infusion in bovine subclinical mastitis.

MATERIALS AND METHODS

Animals: About 25 Holstein dairy cows have no clinical mastitis but at least with one quarter subclinically infected were selected from two different dairy farms. All cows

were in the mid lactation period, weighed 579 ± 24 kg and were handily milked twice daily throughout the study.

Honey: Egyptian fennel honey was used in the study which was collected from an apiary in Assiut Governorate where a wide area of fennel was cultivated. Unprocessed honey was diluted with sterile normal saline solution to achieve 10% honey solution and then it was filtered under complete aseptic conditions using sterilized filter papers to remove any debris, wax or large particles (Al-Waili, 2003) rial count. Milk yield was also recorded. Blood samples were collected from each cow for haematological study.

Treatment design: Ten dairy cows (group a) were intramammary infused through the teat canal. The udders were firstly cleaned then following the complete milking process each quarter was stripped (emptied by usually hand-milking) and 10 mL of 10% honey solution was infused once daily for three successive days.

The other 15 dairy cows (group b) were intramammary infused with the same dose day by day for three successive times guarded with intra muscular administration of antihistaminic (0.92 mg kg^{-1} body weight Avil Retard, Aventis-Egypt). All infused cows were observed and inspected closely for any abnormal manifestations especially udder.

Milk samples examination: Milk samples were collected at the morning milking from each infused quarter post the 3rd and 10th day of the 1st intramammary infusion. Each sample was conducted for the field tests (CMT and chloride test).

CMT: The foremilk was discarded then one or two squirts of milk from each quarter were drawn into the paddle dish and an equal volume of CMT reagent was added to the remaining milk. Results were recorded and scored (Schalm *et al.*, 1971; Rice, 1997).

Field test for chlorides: About 5 mL silver nitrate solution was added to 1 mL of milk followed by two drops of potassium chromate solution. Development of yellow color indicates positive and the chloride level $>0.14\%$ (AMP, 2007).

Bacteriological testing: About 1 mL of milk sample from each cow was diluted (10 fold serial dilutions) with sterile saline. About 1 mL of each dilution was plated in sterile plate, mixed with 10 mL of sterile melted standard plate count agar (Oxoid Ltd., Basingtoke, Hampshire, United Kingdom) and incubated at 37°C for 48 h. The number of colonies per milliliter was determined (APHA, 1992).

Cytological examination: Milk samples were smeared, prepared and stained with Levowitz Weber modification of the Newman Lampert stain using 0.5 g of methylene blue chloride, 56 mL of ethyl alcohol, 40 mL of xylene and 4 mL glacial acetic acid for neutrophils and lymphocytes count (Wehr and Frank, 2007).

Milk yield: Daily milk yield of the cow was calculated as the sum of morning (08:30) and afternoon (18:00) milk yield per weight (kg).

Heamatological study: Blood sampling was obtained prior to the experiment and post the 3rd and 10th day of IMM infusion. Total leucocytic count (TWBCs), percentages of band cells, segmented neutrophils, lymphocytes, eosinophils, basophils and monocytes percentages were determined.

Statistical analysis: Statistical data analysis was done using ANOVA one way Mstat-C software. 49 posthoc analysis at $p < 0.05$. The significant values of the treatments with the control group are expressed in (*).

RESULTS AND DISCUSSION

Bacterial count mean declined in group a from 1×10^6 to 4×10^4 and 2×10^4 by the 3rd and 10th day while that of group b was declined from 4×10^5 to 1×10^3 and 1×10^3 by the 3rd and 10th day, respectively (Fig. 1). Milk cytology revealed a significant increase in the lymphocyte percentage (Fig. 2) and significant decrease in milk neutrophil percentage (Fig. 3). Milk yield results clarified non significant decrease by the 3rd day post IMM infusion in group a and then it was gradually increased up to the 10th day reaching nearly the initial levels while in group b, slight decrease was noticed by the 3rd day and gradually increased to level higher than that was

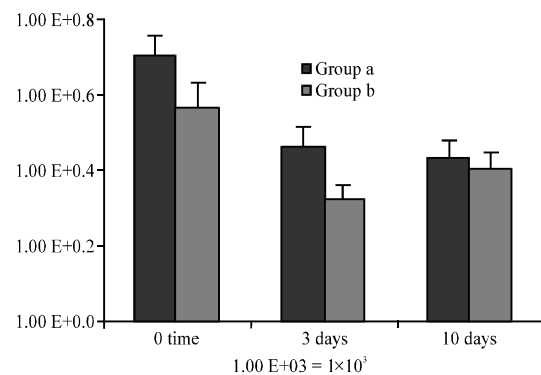


Fig. 1: Effect of intramammary infusion of fennel honey on the total bacterial count of the milk

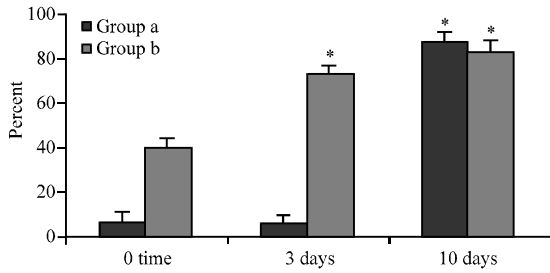


Fig. 2: Effect of intramammary infusion of fennel honey on the percentage of lymphocytes in milk

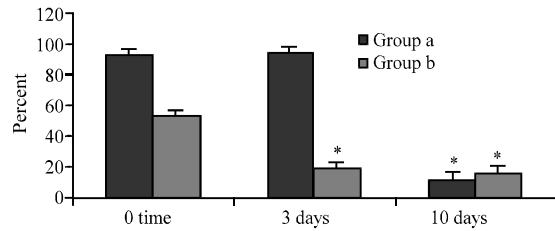


Fig. 3: Effect of intramammary infusion of fennel honey on the percentage of neutrophils in milk

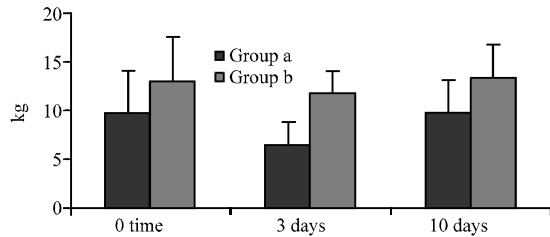


Fig. 4: Effect of intramammary infusion of fennel honey on the milk yield

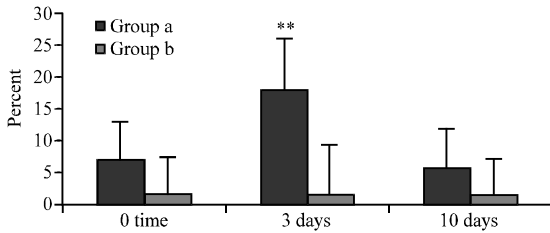


Fig. 5: Effect of intramammary infusion of fennel honey on the percentage of eosinophils in blood

before treatment (Fig. 4). Blood picture clarified highly significant increase ($p < 0.01$) of the percentage of blood eosinophils in group a while it was normal in group b (Fig. 5). Significant increase ($p < 0.05$) in blood lymphocytes (Fig. 6) and significant decrease in neutrophil percentage was recorded (Fig. 7). The leucogram results revealed that TWBCs showed a significant decrease ($p < 0.05$) at the 3rd day followed by a significant increase ($p < 0.05$) at the 10th day in both

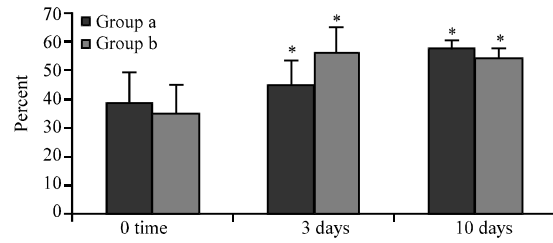


Fig. 6: Effect of intramammary infusion of fennel honey on the percentage of lymphocytes in blood

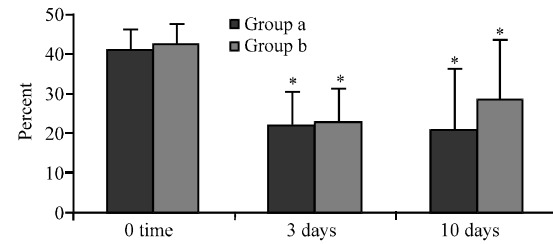


Fig. 7: Effect of intramammary infusion of fennel honey on the percentage of neutrophils in blood

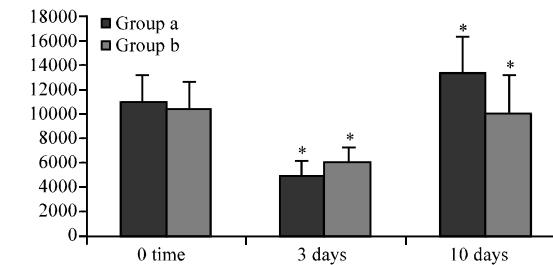


Fig. 8: Effect of intramammary infusion of fennel honey on the total WBCs in blood

groups (Fig. 8). As the increased consumer demand for organic dairy products, almost all organic farmers (95%) used some non antimicrobial compounds to treat clinical mastitis but the use of these compounds to successfully treat mastitis have not been described (Ruegg, 2009). With few exceptions (Hu *et al.*, 2003; Crispie *et al.*, 2008; Beecher *et al.*, 2009; Han *et al.*, 2009), most studies have accounted for the treatment of mastitis or subclinical mastitis referring to the antibacterial activity of antimicrobials against pathogenic organisms even those who tried apitherapy (Allen and Molan, 1997; Abdel-Hafeez *et al.*, 2005). Honey was proved as an immuno-modulating agent due to certain biological and antioxidant components (Al-Waili and Haq, 2004; Neveen *et al.*, 2008). Moreover, intramammary fennel honey infusion resulted in significant decline of the total bacterial count and highly significant increase in milk yield (Abdel-Hafeez *et al.*, 2005). Through the present

study, bacterial count mean declined in group a and b where the reduction percent was 99.6 and 99.8%, respectively.

For many years ago, manual milk cytology (leukocyte differential counts) was the standard technique used to determine the presence or absence of inflammatory processes in bovine milk with low SCC (Kitchen, 2003). In the present study, all cows (before treatment) when examined for CMT, scored from T (trace) up to ++ve while after treatment, scored extremely positive reactions (+++ve). The same results were recorded by Abdel-Hafeez *et al.* (2005) and persisted for 3 months post IMM honey infusion. It considered false positive reactions since it accompanied by a significant increase in lymphocyte percentage (Fig. 2). So, milk cytology could be used as alternative dependable diagnostic paradigms after honey treatment instead of CMT (which gave false extreme positive results) or field chloride test since the increased proportion of PMN in milk has been shown to be a more sensitive inflammatory indicator than the total SCC (Redelman *et al.*, 1988; Kelly *et al.*, 2000). Furthermore, milk cytology could be used instead of measuring lymphocyte levels in blood, since the initial increases in milk lymphocytes paralleled the initial blood elevations (Fig. 2 and 6) as well as the decrease in milk and blood neutrophil percentage (Fig. 3 and 7).

The most obvious economic merit of the intramammary honey infusion is there is no an evidence of milk discards. In commentarial farms, milk must be discarded for 5-8 (Ostera, 2000) or 9.5 days (Ruegg, 2004) due to the risk of chemical therapeutic residues in milk. In the current investigation, milk yield results clarified non significant decrease then it was gradually increased up to the 10th day reaching nearly the initial levels. Moreover, daily follow up for 2 weeks after the end of the experiment revealed increase in the milk yield.

All blood indices were improved by intramammary infusion of fennel honey in the present study. However, blood picture varied between the two groups according to the frequency regimen of administration. No adverse reactions were noticed by 10% intramammary honey infusion in group b while four cows (40%) from group a showed slight inflammatory signs (one post the first and three post the second infusion). These inflammatory signs may be due to allergic reactions indicated by a significant increase ($p < 0.05$) of the percentage of blood eosinophils (Fig. 5). Adverse mammary inflammatory reaction was also recorded post intramammary infusion of honey solution (Abdel-Hafeez *et al.*, 2005) or probiotic culture (Han *et al.*, 2009). The leucogram results revealed that TWBCs

showed significant decrease followed by significant increase both groups (Fig. 8). In an experimental study (Neveen *et al.*, 2008), oral administration of fennel honey increases the total WBCs segmented neutrophils, monocytes as well as serum globulins such remarks proved superiority of honey in modulating B and T lymphocytes.

CONCLUSION

The study shows that honey appears to have the potential to provide an alternative remedy for the treatment of subclinical mastitic udders saving milk yields and producing valuable organic milk. It could be obviated milk withholding for all organic milk producers after this apitherapeutic regimen.

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