Testimony for Veterinary Apitherapy

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INTRODUCTION

Apitherapeutic management is reborn in the modern medicine, established documentary and be advised by physicians to wide variety of patients complaining different health problems. The concept of veterinary apitherapy must be of great concern as it highly safe of animal products extremely effective and greatly economic. As mentioned above, apitherapy has wide spectrum therapeutic activities with variable varieties of mechanism of action, of among them, our referred research works dealt with:-

Antimicrobial Activity (In Vitro Assay)

Antibiotic resistance of bacteria is on the rise, thus the discovery of alternative therapeutic agents is urgently needed [1,2]. Since the antimicrobial honey activity proved to have wide varied potencies depending on botanical, geographical and seasonal conditions [2,16] leading to differences in antimicrobial potency more than 100-fold in-between different honeys [2,17], the in vitro assessment before any honey therapeutic use is required. Fennel honey among Egyptian honey batches has highly antimicrobial activity compared with prickly, sesame and cotton honey [18] or trefoil, marjoram and cotton honey [19] showing synergistic action when adding ethanolic extract of propolis (EEP) [2,16] even only 5% of both[2]. Honey EEP synergy is proved against different Staph species [19,20] and even stubborn methicillin resistant (MRSA) [2] strains biofilm producing with much antibacterial resistance either from bovine or human sources [2]. The antimicrobial activity of crude unprocessed honey is maximized when be diluted and diminished when be autoclaved or heated [21], where this potency is highly complex due to the involvement and interaction of multiple compounds in honey [22]. Egyptian fennel honey inhibits MRSA at 7% [2] and Gram’s negative (proteus, pseudomonas, klebsiella) at 25% [18].

BACKGROUND

Nowadays by the indiscriminate use of antibiotics, multi-drug resistant pathogens (MDR) developed and spread, so searching for effective natural antimicrobials is of global concern. Apitherapy possessing various therapeutic activities (antimicrobial [1,2], anti-inflammatory [3-5], antioxidant [6-9], antiproliferative [10], immunomodulator [11] ameliorative [12,13] or wound healing factor [14,15]) proved to be a suitable tool to get the target since it is very safe, highly effective, easily applicable and extremely economic. The subject is of interesting concept for the apitherapy research team affiliating to Animal Health Research Institute, assiut and Faculty of Veterinary Medicine, Assiut University, Egypt. The team produced many research works (no 12) which were tabulated in tables 1 (in vitro antimicrobial activity), 2 (antioxidant, immunomodulative and toxicity ameliorative factors) and 3 (applied honey apitherapy in lactating dairy cows) dealt with different veterinary apitherapeutic applications of different approducts; honey, propolis and bee venom.

Keywords: Apitherapy, fennel, antimicrobial, antioxidant, immune boosting, wound healing, methoxychlor toxicity introduction

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inhibition by binding and agglutination of bacterial cells or membrane permeabilization of bacterial cells [29,30]. Moreover, the presence of methylglyoxal (MGX) exhibited in manuka honey may modify some honey proteinaceous compounds and therefore affect the glucosidase activity [31] sharing in antimicrobial action. Staphylococcus aureus is a pathogen highly sensitive to the antibacterial action of honey [32] even when be MRSA [33] producing biofilm [2] with additional different antibacterial mechanisms; inhibition of cell division, collapsing microbial cytoplasm cell membranes and cell walls, inhibition of bacterial motility, enzyme inactivation, bacteriolysis, and protein synthesis inhibition [34]. Inhibition of biofilm formation by reducing the expression of 2-fibronectin binding proteins by honey MGX [35] which is abundant component in manuka honey (the highest antimicrobial antioxidant) [36].

Bioactivities of EEP where the major constituents exhibited polyphenols, aromatic acids, terpenes and flavonoids [23] are not directly related to its concentration, but a synergistic activity [37] and interaction between these various active ingredients is believed to be a main factor in achieving the complex antimicrobial activity of propolis [23,38]. Other microbioactive components; artepillin C [39] and the flavonoid formononetin [40], while polyphenols interacts with many microbial proteins by forming hydrogen and ionic bonds, thus altering their three–dimensional (3D) structure of a protein and as a consequence their functionality [23].

Bee venom has antimicrobial activity where melittin (cationic peptides - AMPs), the major active peptide of bee venom [4,25,41] has antimicrobial activity affecting bacterial cell wall (outer membrane proteins and lipopolysaccharides) [25,42] and colistin rather than its antibiofilm activity [43].

Antioxidant Activity

The imbalance between oxidative stress and antioxidant scavengers leads to damage of important biomolecules resulting in malonaldehyde (MDA) production [45] and the body has a defense mechanism against its harmful effects through neutralizing the free radicals by means of either endogenous enzymatic glutathione peroxidase (GPX) and superoxide dismutase (SOD) [46] or non-enzymatic e.g., catalase, alkaloids, ascorbate [47], flavonoids [8,48], selenium [46], vit. C and vit. E [49].

Honey antioxidant activity exhibited by different bioactive micro components [50] such as flavonoids [8,9], phenolic compounds [51], chrysin [52] and amino acids [53] but the main antioxidants are considered to be the polyphenols [54]. Different antinociceptive effects of different fractions from Chinese EEP mainly chrysin [55], but honey [6] or propolis [55] chrysinas a polyphenolic compound or galanin [56] - which is an abundant component in honey - has anti-inflammatory [3] and antioxidant properties [57] enhancing protein stability [52,58], solid lipid nanoparticle (SLN) synthesis avoiding proteolytic degradation [52] resulting antiploliferating [6,59] action. This activity is achieved on honey oral [47,60] or intravenous (I/V) [7,10] administration as increase GPX & SOD and decrease the free radical metabolites malonaldehyde (MDA) due to inhibition of the pro-inflammatory cytokines (TNF-α, IL-1β) and IL-6 [60]. Although I/V honey apitherapy proved to acquire all targeted advantages, it is not advisable avoiding risky factors [7] since daily oral administration gives all positive results [61,62] comparing with intra-peritoneal route where regular consumption of honey is appraised [61].

Boosting Immunity

There is an important correlation between high antioxidant and antimicrobial activity [11], moreover, the antioxidant components present in honey [61-63] and EEP [61,64] play a great role in their immunomodulation properties. So, the above mentioned bioactive components at least have all antimicrobial, antioxidant and immunological boosting factors. Honey increases T and B lymphocytes [62,65,66] as a result of its protection of lymphocyte DNA from oxidative damage [67], while manuka honey protect DNA of not only lymphocytes but also whole blood cells from oxidative damage [68]. It is interesting that muscular exercise with honey oral supplementation reveals an increase in T lymphocyte function [69]. Honey and EEP increase other blood cellular immunological elements; neutrophils [62] and monocytes [7,61,62] rather than serum globulin [61,62]. Moreover, apitherapeutic administration of honey [70] and EEP [64] activate lymphocytic function resulting in proliferation of lymphatic follicles [61]. The immune system response to different stimuli depends on the secretion of different metabolites from macrophages [4] and its phagocytosis might be activated with honey [71] or EEP [7,72,73] administration. Not only honey and propolis apitherapy improves the immune status, but also bee venom boost cellular immunity [61] but less activity and honey bee pollen significantly increases the globulins level and phagocytic activity rather than neutrophils, monocytes and lymphocytes percentages [61,74]. Bee venom melittin enhances the release of tumour necrosis factor-α (TNF-α), Interleukin (IL-1β), IL-6, and IL-10 cytokines [4] from macrophages affecting the immune status positively.

Amelioration Effect of EEP Against Induced Methoxychlor (MXC) Toxicity

Although MXC (organochlorine pesticide) is banned by 2004 since failure of registration with the Environmental Protection Agency [75], it is used and presented residues contaminating lakes [12] or river fish [13], then animal foodstuffs. Its toxicity affects liver [76], testis, ovary [77], kidneys [76], spleen, blood vessels, pituitary, adrenals and mammary glands [78]. Additionally to the above bioactive properties of EEP, it is proved that having protection properties (amelioration) against MXC [79], methotrextate [80], benzopyrene [81] or chlorpyrifos [79,82] induced toxicity. Due to EEP immunomodulation potency
by different mechanisms of micro-components (mainly flavonoids and phenolic acids), its antioxidative and anti-inflammatory [5] properties resulted in such different amelioration effects and chrysin [83] which inhibits induced kidney fibrosis [84]. Moreover, lactating animals excrete MXC in milk [85] with potential human health hazard. Subsequently, animals contract MXC toxicity result several toxicological implications as ovarian toxicity [77], where it causes impairment of ovarian follicular development and other ovarian function [77,86] leading to reproductive tract malformations in the male offspring [87]. MXC hepto-toxicity results in significant reduction in total serum protein rather than marked elevation in hepatic marker enzymes (ALT, AST & ALP) [76] as well as changes in inter and intra cellular signaling [88]. Histopathologically, there is changes in hepatocytes [76,89] owing to induction changes in mRNA abundance of genes in the liver and testes [90], where alterations involved in regulations of gene expression, tissue development, function and homeostasis, could also contribute to transgenerational epigenetic effects of endocrine disruptors [88]. MXC affect kidneys causing toxic nephrosis [76] and dysfunction through inhibition of renal tubular cell viability and induction of cell apoptosis via mitochondrial pathways [91].

<table>
<thead>
<tr>
<th>Bact. Spp.</th>
<th>Source</th>
<th>Honey</th>
<th>Method</th>
<th>MIC</th>
<th>MBC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>Bovine purulent endometritis</td>
<td>Cotton</td>
<td>Broth dilution in tubes</td>
<td>10%</td>
<td>UD</td>
<td>Abdul-Hafeez et al. 2001[44]</td>
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<td>S. intermedius</td>
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<td>S. epidermidis</td>
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<td>S. saprophyticus</td>
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<td>Strept. agalactiae</td>
<td>Bovine subclinical mastitis</td>
<td>Fennel*</td>
<td></td>
<td>12.50%</td>
<td>25%</td>
<td>Ali et al. 2005[18]</td>
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<td>S. aureus</td>
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<td>Ps. aeruginosa</td>
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<td>Proteus spp.</td>
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<td>Kl. pneumoniae</td>
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<td>S. aureus</td>
<td>Bovine subclinical mastitis</td>
<td>Fennel honey with 10% propolis extract**</td>
<td>8%</td>
<td>15%</td>
<td>Aamer et al. 2014[19]</td>
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<td>S. intermedius</td>
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<td>S. epidermidis</td>
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<td>S. saprophyticus</td>
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<td>MRSA</td>
<td>Dairy food</td>
<td>Fennel honey</td>
<td>Agar dilution</td>
<td>7%</td>
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<td>Hamouda et al. 2018[2]</td>
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<td>Meat</td>
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<td>10.60%</td>
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<td>Throat (dairy food workers)</td>
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<td>9.30%</td>
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<td>Fingernail (dairy food workers)</td>
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<td>8.20%</td>
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<td>Dairy food</td>
<td>EEP</td>
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<td>Throat (dairy food workers)</td>
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<td>12%</td>
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<td>Fingernail (dairy food workers)</td>
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<td>16%</td>
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<td></td>
<td>Dairy food</td>
<td>5% Fennel honey*** with 5% EEP</td>
<td>7.10%</td>
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<td></td>
<td>Meat</td>
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<td>Throat (dairy food workers)</td>
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<td>8.20%</td>
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<td>Fingernail (dairy food workers)</td>
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<td>7.70%</td>
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HONEY APITHERAPY IN LACTATING DAIRY COWS

Among different lactating Holstein dairy farms, four papers of the referred works [44,92-94] applied honey apitherapy were dealt with three dairy farm problems.

Bovine Endometritis

Well managed and profitable dairy farming requires animal with good reproductive performance [95], but uterine postpartum infection with MDR pathogens which is so common threaten fertility [96] causing major cause of economic loss [97]. Bovine endometritis and prolonged luteal phase leading to repeat
breeder (infertility complain) is significantly increased in cows with persistent infections [96]. Alternative intrauterine (I/U) infusion was widely documented and tried; with own affected cow hyper immune serum [98], polymorph nuclear leucocytes, exotoxine of \( E. \) coli lipopolysaccharides [99], multitherbal extract [100], chitosan [101], low dilutions of \( \text{H}_2\text{O}_2 \) [102], silver nanoparticles [103], Momordica charantia [104] garlic, neem, ashwagandha, and turmeric [105] or tulsi and giloy herbal EOs. Even lactic acid probiotic \( \text{Pediococcus acidilactici} \) and \( \text{Lactobacillus reuteri} \) [107] therapy was tried concluded that treatment remains to be confirmed [102] as it may increase the pregnancy rate in cows [104].

Bovine affection

Table 2: Apitherapy in normal healthy animals as antioxidant, immunomodulative and toxicity ameliorative factors

<table>
<thead>
<tr>
<th>Studied parameter</th>
<th>Animal</th>
<th>Apiproduct</th>
<th>Mode of application</th>
<th>Conclusions</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Antioxidant activity and immunomodulation</td>
<td>Goats</td>
<td>Fennel honey</td>
<td>Rapidly intravenously infused 20% honey solution (70-80 drops/min) in normal saline solution</td>
<td>In 75% of infused goats: ↑ the activity of antioxidants (GPX &amp; SOD) and ↓ free radicals metabolites, improving haemo-ideces (↑ WBCs, ↑ lymphocytes, ↑ monocytes and ↑ serum globulins). While the rest 25% had anaphylactic shock and died.</td>
<td>Abdel-Mooty et al. 2018[7]</td>
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<td>Boosting immunity prior experimental induced infection</td>
<td>Sprague dawley rats</td>
<td>Fennel honey</td>
<td>Oral &amp; intraperitoneal 10% honey solution administration for 60 days</td>
<td>All were immunized where oral honey administration showed the highest immunization rate</td>
<td>Sayed et al. 2009[61]</td>
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<td>EEP</td>
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<td>Oral &amp; intraperitoneal administration of aqueous (AEP) &amp; ethanolic extract (EEP) for 60 days</td>
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<td>Bee venom (bee sting)</td>
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<td>Single bee sting for 5 successive times</td>
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<td>Ameliorative effects against MXC toxicity</td>
<td>Sprague dawley rats</td>
<td>EEP</td>
<td>Orally EEP (200 mg/L, drinking water) for 12 months</td>
<td>Propolis protected MXC-induced hepato renal toxicity</td>
<td>Sayed et al. 2009[61]</td>
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<td></td>
<td>EEP</td>
<td></td>
<td>Orally EEP (200 mg/L, drinking water) for 10 months</td>
<td>Propolis decreased MXC-induced ovarian toxicity</td>
<td>Eman El Sharkawy et al. 2014[77]</td>
</tr>
</tbody>
</table>

Table 3: Application of honey apitherapy in lactating dairy cows; I/U : intrauterine, I/Mm : intramammary, Honey*: showed healing manifestations earlier than other medical herbal (\( T. \) vulgaris, \( O. \) vulgare and \( M. \) chamomilla) lotion and ointments

<table>
<thead>
<tr>
<th>Bovine affection</th>
<th>Mode of application</th>
<th>Conclusions</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Purulent endometritis (with repeat breeder)</td>
<td>I/U infusion 0ml honey day by day three successive doses</td>
<td>75% of treated cows got conception</td>
<td>Abdul-Hafeez et al. 2001[44]</td>
</tr>
<tr>
<td>Subclinical mastitis</td>
<td>1/Mm infusion of 10ml of 50% fennel honey day by day three successive doses</td>
<td>Milk yield increased significantly</td>
<td>Abdul-Hafeez et al. 2005[92]</td>
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<td>I/Mm infusion of 10ml 10% fennel honey day by day three successive doses guarded by antimistaminic administration</td>
<td>Milk yield increased significantly</td>
<td>Nahed Wahba et al. 2011[93]</td>
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<tr>
<td>Surgical claw affections (digital dermatitis and inter digital necrobacillosis)</td>
<td>Pure honey* bandage for 5 consecutive days; 2 days without management; then once daily for other 3 days only up to 14 days</td>
<td>Complete healing repair and hair developed by 14 days</td>
<td>Ali et al. 2017[94]</td>
</tr>
</tbody>
</table>
complain. Despite the treatment success is judged by total conception rate not only clearance of clinical signs, it was 75% (83.8% in cows did not accept any antibiotics & 70% in cows previously antibiotic treated) just post three I/U infusions of 100 ml pure undiluted honey day by day. In mare endometritis, MDR infection appears to be more difficult since it is commonly associated with biofilm [108], so biofilm was targeted alternatively in vitro [109] and in vivo with N-acetylcycteine, H₂O₂, ozone and hypochlorous acid giving no reducing in biofilm [108]. Authors recommended to use these non-antibiotics combined with antibiotic infusion where must be administered with separate syringes [108]. By 70% honey I/U infusion in mares [110], positive results to both targets (antimicrobial and antibiofilm- as mentioned above) against endometritis pathogens but not as in bovine, where treatment success is judged by endometrium ultrasound and cytological examinations showing clinically clearance of endometritis, but fertility was not improved.

Bovine Subclinical Mastitis

Mastitis imposes considerable economic losses on the dairy industry all over the world [111], where it is one of the most vital noteworthy monetary risks to dairy ranchers and affects reproductive performance in dairy cattle [112]. The overwhelming usage of antibiotics and prolonged infection treatment has led to emergence of MDR pathogens [103]. The emergence of antibiotic resistant microorganisms is a great public health concern and has triggered an urgent need to develop alternative antibiotics [113]. Based on the antibacterial properties of agents from plants, the antimicrobial action of essential oils (EOs) of medicinal plants and its feasibility to be intramammary infused is recommended [114], but as the mammary gland is very susceptible tissue to irritation, any intramammary infusions should be non-irritating [115]. Following the in vitro testing, clinical trials intramammary infusion (I/Mm) of any alternative medicinal product must be warranted to determine safety and possible withdrawal time in milk before its recommendation for use [116]. The in vitro assessment of EOs; Origanum vulgare [41], Thymus vulgaris and Lavandula angustifolia [117], fruit guttiferone-A and 7-epiclusianone [118], onion and black cumin [119] or chitosan hydrogel [120] against bovine mastitis pathogens and Salvia officinalis [116] against ewe subclinical mastitis pathogens was documented. EOs I/Mm application [116,117] concluded variable results away from its in vitro assessment as external massage with EO ointment had activity more than that of I/Mm [117] or I/Mm had significant results when be combined with antibiotics[116] even chitosan hydrogel is recommended only for uninfected cows as it is not a complete replacement for antibiotic dry cow therapy [120]. Despite of the positive in vitro antimicrobial potency of probiotic lactic acid bacteria due to produced bacteriocin and elicit substantial innate immunity [121], the I/Mm infusion of Lactobacillus lactis [121-123], Weissella confusa [124,125] or Lactobacillus acidophilus [126] concluded no increase in intramammary cure rate [126] where S. aureus infections did not improve, and coagulase negative staphylococcal infections tended to relapse [123] with abnormal milk secretions and udder inflammation. Thus its I/Mm should not be adopted [124], but I/Mm of 10% solution of thymus and lavender Eos [117] showed synergistic action which the only study recommended EOs I/Mm infusion.

Otherwise, the in vitro testing of honey against pathogens of bovine [2,18,19] and ovine mastitis [119,127,128] encouraged its I/Mm infusion where by 20% fennel honey followed by 30% then 40% finally 50% (the in vitro MIC) for three successive doses [92] concluded decline in total bacterial count (TBC) and highly significant increase in milk yield. But regardless to the in vitro MIC concerning boosting immunity [61], only 10% fennel honey infusion day by day for three doses guarded by antihistaminic drug significantly decreases milk TBC, increase milk yield and improves blood immunological parameters [93]. Subsequently, I/Mm honey infusion is the only successful trend among alternative medicinal approach with unique drawback since extreme CMT positive results [92,93] owing to the highly increased lymphocytes and total leucocytes post honey administration [61,62,65,66,74]. Moreover, milk cytology could be used instead of CMT during and after treatment with honey [93]. Beyond mastitis negative impact on milk yield and milk components, it has a detrimental effect on reproductive performance in dairy cows [112,129] rather than the mastitis following exudative metritis resulting in mastitis ascending infection.

Bovine Claw Affections

Topical honey application has a beneficial effect on the healing of cutaneous wounds [130], ulcers [131] or infected chronic wounds showing synergism with Nigilla sativa EO [132]. There is good evidence for honey also having bioactivities that stimulate the immune response promoting tissue growth and wound repair, moreover suppress inflammation [133].

Twenty lactating cows suffering from claw lesions either digital dermatitis or interdigital necrobacillosis (infected wound in highly infected media) showing sever lameness were divided to equal four groups of five cows of each. Three groups were treated with medicinal plant extract of Thymus vulgaris, Matricaria chamomilla and Origanum vulgare (as lotion and ointment) with the obtained in vitro MIC value against the isolated bacteria. The rest group was dressed only with undiluted honey as honey used in wound care can withstand dilution with substantial amounts of wound exudate maintaining enough activity to inhibit the growth of bacteria [133] accelerating healing process[15]. All groups had different degrees of cure post 30 days but honey group was by 14 days with complete healing[95] concluded that honey is the most effective valuable economic alternative tool than herbal EOs for its healing superiority and application feasibility.
CONCLUSION

Basing on the former justifications of the wide spectrum antimicrobial efficacy and promoting wound healing as well as tissue growth, honey apitherapy is advisable to be used.

Undiluted in cases of direct exposure with the pathogen even MDR either septic wounds & purulent metritis or food additive as a preservative especially those of dairy sources acceptable to be sweetened.

In cases of boosting immunity of healthy non affected animals or subclinical mastitis, 10% honey solution would be enough fully effective.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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Abdul-Hafeez: Testimony for Veterinary Apitherapy


Chamani G, Zarei MR, Mehrabani M, Mehdavinezhad A,
