Efficacy of a mixed herbal essential oils as a treatment option for clinical endometritis in dairy cattle

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Research

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Abstract

Background: The endometritis form of uterine infection is considered as a common reproductive disorder deleterious to the reproductive performance of dairy herds. A wide variety of treatments with controversial results have been reported for endometritis, including local or systemic administration of antibiotics or disinfectants as well as hormone application. These treatments have side effects on endometrium, and antibiotic residues are found in milk following intrauterine and systemic antibiotic therapies.

Objective: The aim of this study is to evaluate the possible effects of the mixed essential oil of Satureja bachtiarica Bunge, Artemisia Aucheri Boiss and Syzygium aromaticum (L.) Merr. & L.M.Perry on treatment of clinical endometritis in dairy cattle.

Materials and methods: One hundred and twenty cows with clinical endometritis were selected and randomly assigned to one of the following three groups: the HM group intrauterinely received mixed herbal essential oils, the OX group received 2.5 g oxytetracycline HCl, and the EX group received 1 g of ceftiofur sodium.

Results: The cleaning and first service conception rate was significantly higher in HM group than the EX, whereas the mean open days were lower in HM than the EX group. The number of service per conception was also significantly lower in HM group than OX and EX groups. In general, reproductive performance after herbal treatment was quite comparable to chemical antibiotic therapy and even better in some other reproductive indices.

Conclusion: The mixed essential oils treatment represents an effective potential alternative to postpartum therapy for cows with clinical endometritis.

Key words: cattle; endometritis; Satureja; Artemisia; Syzygium

Background

The endometritis form of uterine infection is a common reproductive disorder detrimental to the reproductive performance of dairy herds. This infection is described as a superficial inflammation of the endometrium with a purulent or mucopurulent uterine discharge visible in the vagina at ≥ 21 days of milking [1]. Clinical endometritis adversely influences reproduction and milk production through reducing conception rate, prolonging open days, increasing culling rate and treatment fees [2–4] culminating in subfertility [5] as well as considerable financial losses [6].

A wide variety of treatments have been suggested for endometritis; results of which are debated among veterinary practitioners [7]. These therapies include local or systemic administration of antibiotics or disinfectants as well as hormone application [8]. Intrauterine (IU) antibiotic treatment has been initiated with the aim of reducing endometritis negative effect on fertility through decreasing bacterial contamination of the uterus and its concomitant inflammation [9]. Tetracycline [10, 11], penicillin [10],
cephapirin [8, 12], ceftiofur hydrochloride [13] and other compounds have been tried in the treatment of endometritis with controversial results. Most studies indicate that local and systemic administration of antibiotics has a limited success in curing endometritis and that it may interfere in uterine defense mechanisms [14, 15]. Similarly, some field trials have demonstrated that intrauterine infusions of antibiotics in various protocols have generally failed in improving reproductive efficiency over the untreated control group [10] or an alternative group treated with PGF2a [11, 16].

Although some studies have recommended PGF2a as an effective treatment for endometritis with minimum harm to uterus or residual in milk and meat [11, 16–18], it has only a limited success [19] and specific evidence for improved reproductive performance of dairy cows with clinical endometritis is lacking. Thus, uncertain efficacy of intrauterine medication, inconsistent recovery rate, possible suppression of the immune system, the problem of drug residues in milk and high cost of treatment have urged researchers to find alternative therapies.

Herbal therapies have long been used in the treatment of several human disorders [20], but information regarding the treatment of reproductive diseases in farm animals are limited. Some studies have used plant extracts in the treatment of clinical endometritis and have reported minor adverse effects on reproduction and milk production as well as low cost of the treatment, and its efficacy against a variety of disorders [21].

Three plants commonly used in herbal therapies are Artemisia Aucher Boiss, Satureja bachtiarica Bunge and Syzygium aromaticum (L.) Merr. & L.M.Perry. Artemisia Aucher Boiss has been found to have antiseptic, analgesic, anti-inflammatory, and antioxidant properties [22]. Also, its essential oil is enriched with high percentage of thymol and carvacrol with antibacterial and antifungal activities [23–25]. In ethnopharmacology, Artemisia species are frequently utilized for the treatment of diseases including bacterial, fungal and viral infections [26]. Likewise, the antimicrobial and antioxidant activities of Satureja bachtiarica Bunge have been proven [27], and Syzygium aromaticum (L.) Merr. & L.M.Perry is believed to have anti-inflammatory and antiseptic activity. The latter is traditionally used in inducing uterine contractions [28]. Satureja bachtiarica Bunge is traditionally used as an antiseptic and analgesic agent in Iran [29] and Syzygium aromaticum (L.) Merr. & L.M.Perry is widely used for treatment of infectious disease in Iranian traditional medicine [30].

The beneficial effects of other herbs on treatment of reproductive disorders have also been investigated. For example, several studies have demonstrated the positive effects of Zataria multiflora on reproductive disorders in human (Lopes-Lutz, Alviano et al. 2008, Abdali, Jahed et al. 2015) and in dairy cattle (Hajibemani, Mirzaei et al. 2016).

In this study, we attempted to analyze the possible impact of a mixed essential oils of Satureja bachtiarica Bunge, Artemisia Aucher Boiss and Syzygium aromaticum (L.) Merr. & L.M.Perry on clinical endometritis as well as reproductive performance in dairy cattle. At the same time, the efficacy of intrauterine injection of oxytetracycline and ceftiofur sodium commonly used in the treatment of clinical
endometritis is evaluated and compared with the herbal group. The effectiveness of these three protocols are evaluated by calculating and comparing parameters of herd reproductive performance.

**Materials And Methods**

Essential oils of Syzygium, Artemisia and Dianthus were purchased from Barij Essence (Iran, Kashan). A mix vial of these three essential oils was prepared for one intrauterine injection. Its total volume was increased to 50 ml by adding distilled water. The chemical composition of this herbal mixture derived by gas chromatography-mass spectrometry (GC-MS) is presented in Table 1. Gas chromatography-mass spectrometry was performed by Thermoquest 2000 GC (Thermo Quest, USA) equipped with Thermo Finnigan Mass system and a DB-1 capillary column (30 m × 0.25 mm; 0.25 lm film thickness). Helium was the carrier gas. Mass range was from m/z 35–375 amu, and the mass spectra were taken at 70e V.
Table 1
The chemical composition of the herbal mixture derived by gas chromatography-mass spectrometry (GC-MS). Compounds were identified by comparison with MS database spectra, retention time (RT), area percentage and pure reference chemicals, and are listed in order of elution from the column.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>RT</th>
<th>Area percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alpha pinene</td>
<td>8.8</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td>Camphene (CAS)</td>
<td>9.32</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>Beta Myrcene</td>
<td>11.25</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>Yomogi alcohol</td>
<td>11.94</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>Alpha terpinene</td>
<td>12.56</td>
<td>0.32</td>
</tr>
<tr>
<td>6</td>
<td>Cymene</td>
<td>13.1</td>
<td>6.7</td>
</tr>
<tr>
<td>7</td>
<td>1,8-Cineole</td>
<td>13.25</td>
<td>2.26</td>
</tr>
<tr>
<td>8</td>
<td>Trans-2,7-Dimethyl-4,6-octadien-2-ol</td>
<td>13.75</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>Gamma terpinene</td>
<td>14.6</td>
<td>1.77</td>
</tr>
<tr>
<td>10</td>
<td>Artemesia alcohol</td>
<td>15.73</td>
<td>0.33</td>
</tr>
<tr>
<td>11</td>
<td>Beta Thujone</td>
<td>16.42</td>
<td>10.1</td>
</tr>
<tr>
<td>12</td>
<td>Alpha Thujone</td>
<td>16.77</td>
<td>2.67</td>
</tr>
<tr>
<td>13</td>
<td>Bornanone</td>
<td>17.6</td>
<td>4.5</td>
</tr>
<tr>
<td>14</td>
<td>Verbenol</td>
<td>17.8</td>
<td>0.8</td>
</tr>
<tr>
<td>15</td>
<td>p-Menthone</td>
<td>17.9</td>
<td>0.2</td>
</tr>
<tr>
<td>16</td>
<td>Cyclopentane</td>
<td>18.35</td>
<td>0.8</td>
</tr>
<tr>
<td>17</td>
<td>Endo borneol</td>
<td>18.45</td>
<td>1.8</td>
</tr>
<tr>
<td>18</td>
<td>Menthol</td>
<td>18.66</td>
<td>0.24</td>
</tr>
<tr>
<td>19</td>
<td>3-Cyclohexen-1-ol</td>
<td>18.72</td>
<td>0.67</td>
</tr>
<tr>
<td>20</td>
<td>Beta fenchol</td>
<td>19.16</td>
<td>0.41</td>
</tr>
<tr>
<td>21</td>
<td>Chrysanthenyl acetate</td>
<td>20.74</td>
<td>0.4</td>
</tr>
<tr>
<td>22</td>
<td>Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-, acetate</td>
<td>21.32</td>
<td>0.24</td>
</tr>
<tr>
<td>23</td>
<td>m-Thymol</td>
<td>21.97</td>
<td>3.1</td>
</tr>
<tr>
<td>24</td>
<td>Carvacrol</td>
<td>22.2</td>
<td>9.57</td>
</tr>
<tr>
<td>25</td>
<td>Eugenol</td>
<td>23.24</td>
<td>36.75</td>
</tr>
<tr>
<td>No.</td>
<td>Compounds</td>
<td>RT</td>
<td>Area percentage</td>
</tr>
<tr>
<td>-----</td>
<td>---------------------------------------------------------------------------</td>
<td>------</td>
<td>-----------------</td>
</tr>
<tr>
<td>26</td>
<td>Trans-caryophyllene</td>
<td>24.26</td>
<td>5.76</td>
</tr>
<tr>
<td>27</td>
<td>Alpha Humulene</td>
<td>24.9</td>
<td>1.1</td>
</tr>
<tr>
<td>28</td>
<td>Acetyleugenol</td>
<td>26.25</td>
<td>4.85</td>
</tr>
<tr>
<td>29</td>
<td>Caryophyllene oxide</td>
<td>27.3</td>
<td>1.35</td>
</tr>
<tr>
<td>30</td>
<td>10,10-Dimethyl-2,6-dimethylenebicyle [7.2.0] undecan-5.beta.-ol</td>
<td>28.3</td>
<td>0.21</td>
</tr>
<tr>
<td>31</td>
<td>1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS)</td>
<td>34.78</td>
<td>0.58</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>99.4</td>
</tr>
</tbody>
</table>

The field approach of this study was performed on a commercial Holstein dairy farm in Shahrekord, Iran, between January and June 2015. Cows were fed a total mixed ration (TMR), milked three times a day, and received a complete herd-health service and reproductive management. They were examined for endometritis at 30 days of postpartum. The examination included vaginal inspection and ultrasonographic evaluation of ovaries and uterus. The clinical endometritis was defined according to Sheldon et al, (2009) as the presence of purulent (> 50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine exudate in the vagina, 21 days or more post-partum, without any systemic signs [31]. Cows were bred on observed estrus after cleaning, and pregnancy diagnosis was performed by ultrasonography examination around 30 days after insemination. Those that showed only opaque discharge, not purulent or mucopurulent (mild endometritis) were left out of this study.

At the end of clinical examination, 120 cows with clinical endometritis were selected and randomly assigned to one of the following groups: (1) HM group receiving the mixed herbal essential oils, (2) OX group receiving 2.5 g oxytetracycline HCl (Oxyvet® 5%, RAZAK, Iran), and (3) EX group receiving 1 g of ceftiofur sodium (Excenell® 4 g, Pfizer, Madrid, Spain) diluted in distilled water. All three groups had one intrauterine injection after endometritis confirmation by ultrasonographic and visual observation. The total volume of injection for all groups was 50 ml. Intrauterine administration of oxytetracycline and ceftiofur hydrochloride was a routine procedure for treatment of clinical endometritis at the time of our study.

After about one week, the cows were reexamined, and clean ones received hormonal treatment protocols for estrus induction based on their ovaries structure. Non-clean cows retreated (if necessary) with another antibiotic dose and inseminated on observed estrous.

The efficacy of treatment protocols was evaluated by reproductive performance parameters including cleaning rate, days open, calving to first service interval, first service pregnancy rate (FSP) and service per conception (SPC). Cleaning rate was calculated in percent by the number of detected clean cows during reexamination following treatment divided by all the treated cows in the group. First service conception rate was calculated separately for cleaned cows, and cleaned and non-cleaned cows together. This was
for evaluation of the first treatment effect on general uterine health and the result of other treatments in progress.

**Statistical analysis**

Data obtained for all three groups, open days, service per conception and calving to first service interval were analyzed with one-way analysis of variance through general linear model, and the means were compared with Tukey test. The results are presented as least square means and standard error. The cleaning rate and first service pregnancy rate were compared with Chi-square between three treatment groups. Statistical software SAS 9.1 (SAS Institute, Inc.) was used throughout analysis, and P < 0.05 was considered statistically significant.

**Results**

A total of 120 dairy cows were included in this study, of which 39, 41 and 40 were assigned to the HM, OX and EX groups respectively. The results of cleaning rate, first service conception rate of cleaned cows and first service conception rate of cleaned and non-cleaned cows are presented in Table 1. The treatment rate was calculated for cows of each group. In HM group, 69.2%, in OX group, 56.1% and in EX group, 40% were cleaned after one intrauterine treatment. The significant difference was seen between groups HM and EX, but no such difference was observed between groups HM and OX. Considering only the cleaned cows, the first service conception rate was significantly higher for group HM than group EX but there was no significant difference between groups HM and OX. Moreover, when all the cleaned and non-cleaned cows were considered together, the results were similar, and were significant only between groups HM and EX with higher rate for HM group (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Cleaning rate (%)</th>
<th>First service conception rate in cleaned cows (%)</th>
<th>First conception rate in cleaned &amp; non-cleaned cows (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed herbal essential oil (n = 39)</td>
<td>69.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxytetracycline (n = 41)</td>
<td>56.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.27&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Excenel (n = 40)</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The mean open days were significantly lower in HM group than EX group. Similarly, the service per conception was significantly lower for HM group than groups OX and EX. There was no significant difference between the groups in terms of calving to first service (Table 2).
Table 2
Mean ± standard error of open days, calving to first service interval and number of service per conception in three treatment groups. Different letters in a column show significant difference between groups (P < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Open days</th>
<th>Calving to first service</th>
<th>Number of service per conception</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixed herbal essential oil (n = 39)</td>
<td>106 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.02 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxytetracycline (n = 41)</td>
<td>132 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Excenel (n = 40)</td>
<td>120 ± 0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>64 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Discussion**

A total of 120 dairy cows were enrolled and assigned to one of three protocols for the treatment of endometritis focusing on improvement of reproductive parameters among the herd groups. The main protocol was based on the intrauterine infusion of a mixed herbal essential oils, and a healthy control group was not included to avoid possible suffering of the animals involved and/or economic losses by the dairy herd. The mixed herbal medicine was a combination of essential oils of *Satureja bachtiarica* Bunge, *Artemisia Aucheri* Boiss and *Syzygium aromaticum* (L.) Merr. & L.M.Perry.

According to the results, intrauterine administration of mixed herbal essential oils was more effective against clinical endometritis relative to oxytetracycline and excenel. This finding is in agreement with the experiments showing antibacterial and anti-inflammatory properties of plants used in the mixed herbal medicine. According to some studies, *Artemisia Aucheri* contains analgesic, antiparasitic, antibacterial, anti-inflammatory and antiseptic agents, and is effective in treating visceral pain [32, 33]. Likewise, *Satureja bachtiarica* is an aromatic medicinal plant with antimicrobial, antioxidant and antiviral activities against several types of viruses [22, 34]. Moreover, *Syzygium aromaticum* (L.) Merr. & L.M.Perry possesses anti-inflammatory and antiseptic drugs active in treating uterine diseases through induction of uterine contractions [35].

In addition, some studies have reported that uterine bacterial contamination is low during the first weeks after calving [36] and endometrial inflammation is not always concurrent with bacterial infection [9]. Hence, it is likely that our mixed herbal essential oils act as an anti-inflammatory agent, or reduces the bacterial load to a point where inflammatory stimulus diminishes and uterine defenses gain dominance.

Animals with clinical endometritis show reduced fertility [37]. Cows with endometritis usually experience delayed resumption of ovarian cycle after calving, prolonged postpartum luteal phases [38], low conception and submission rates, long calving to conception interval and high culling rate [7, 12, 37]. All these abnormalities eventually lead to longer mean open days, which determine herd’s reproductive
management efficiency. Based on our results, calving to first service interval shows no significant
difference among groups, but open days for HM group was significantly lower than OX. Also, number of
service per conception for HM group was significantly lower than groups OX and EX. Conversely, herbal
treatment was more effective in treatment of endometritis, and led to higher first service conception (FSC)
rate for HM group. The same was also true for the total group (cleaned and non-cleaned), representing
the effectiveness of our mixed essential oils, even in the cases which needed retreatment with another
antibiotic, and probably left less negative effects on the endometrium.

The findings of studies on the effects of intrauterine administration of antibiotics on the reproductive
parameters of dairy cattle are controversial. Some have reported that intrauterine infusion of either
oxytetracycline or penicillin has no influence on time interval to pregnancy relative to untreated cases
[10]. Kutti et al (2000) have observed no significant effect on conception rate and calving to conception
interval in the cases of severe endometritis in comparison with untreated cases [39]. In contrast, some
studies have reported that infusion of cepahpirin benzathine and ceftiofur hydrochloride to cows with
subclinical and clinical endometritis improves reproductive performance, but has no significant effect on
pregnancy per artificial insemination, and parturition interval to pregnancy [8, 12, 40, 41]. In another study,
inhauterine cephapirine had no significant effect on resolution of clinical signs compared to untreated
animals, but resulted in shorter time to pregnancy [8]. On the other hand, antibiotic treatment is presumed
to interfere in normal uterine defense mechanisms via lowering bacterial antigens, leading to disruption
of neutrophil migration, the release of inflammatory mediators and chemotactic factors into the lumen
and endometrium [36]. Irritation and coagulation necrosis of endometrium have been reported as the side
effects of oxytetracycline therapy, which negatively affect uterine defense mechanisms and self-healing
ability [18, 42].

Furthermore, residues of some antibiotics (such as oxytetracycline) administered during peak milk
production appear in milk causing deleterious effects on the milk quality [43]. Of course, presence of drug
residues in food products is unpopular with the public health and thus, the risk of antibiotic resistance
and the economic losses due to milk withdrawal make the intrauterine antibiotic therapy for endometritis
unprofitable [42]. However, endometritis is a localized inflammation of the uterine lining, which interferes
in embryo nutrition, implantation and survival, and its treatment is necessary to reduce the load of
pathogenic bacteria, halt and reverse inflammatory changes, and enhance regeneration.

A well-known advantage of traditional medicine is its ability to provide holistic therapy for interrelated
diseases [44]. Indeed, natural materials such as plant extracts as stimulators of uterus defense
mechanisms have been considered as an alternative therapy for the treatment of reproductive disorders
like infections. Schnellbach (1990) reported that an intrauterine infusion of Eucalyptus compositum
solution improves first service and overall conception rate in cows with mild signs of endometritis
compared with an untreated control group [45]. An alternative therapy with Zataria multiflora has been
introduced for treatment of clinical endometritis in dairy cattle, which is as equally effective as penicillin-
streptomycine therapy [46]. Similarly, Esparza et al., (1995) found that a combination of plant extracts
provides the necessary agents for the treatment of clinical endometritis without any deleterious effect on
the milk quality [21]. Also, Kumar et al., (2006) successfully applied garlic extract for the treatment of endometritis [47]. In this regard, our mixed herbal essential oils significantly reduced the open days, and can be considered as a new therapeutic approach. Unfortunately, no reliable scientific information on clinical trials of treating cattle endometritis by essential oils of Satureja bachtiarica, Artemisia Aucheri and Syzygium aromaticum (L.) Merr. & L.M.Perry is available to compare our findings.

Conclusions

The results of this study indicate that intrauterine administration of mixed herbal essential oils of Satureja bachtiarica Bunge, Artemisia Aucheri Boiss and Syzygium aromaticum can effectively treat dairy cows diagnosed with clinical endometritis, and improve their reproductive performance. The results were completely comparable to those of chemical antibiotics and even better in some reproductive indices. Thus, this herbal treatment represents an effective potential alternative to postpartum therapy for cows with clinical endometritis.

Declarations

Ethics approval and consent to participate

All animal experimental procedures were approved by the Ethics Committee of Shahrekord University, Shahrekord, Iran.

Consent for publication

Not applicable.

Availability of data and materials

Please contact the corresponding author for data requests.

Competing interests

The authors declare that they have no competing interests

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Authors contributions:

Ali Kadivar: Designed and performed experiments; Raziyeh Elahi: performed experiments and co-wrote the paper; Najmeh Davoodian: co-wrote the paper and analysed data; Naser Shams Esfandabadi: co-wrote the paper and analyzed data; Rohollah Dehghani Tafti: performed farm experiments; Habib Allah
Rashidzade: collected reproductive data and analyzed data; Mohammd Javad Behzadi Shahrbabak: performed farm experiments; Taghi Taktaz Hafshejani: performed farm experiments and collected reproductive data

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