Comparative efficacy of different herbal extract on subclinical endometritis in postpartum cows

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Abstract
The objective of the present research work was to study the prevalence of subclinical endometritis in an around Akola city and to study the therapeutic efficacy Achyranthes aspera and Azadirachta indica on subclinical endometritis in postpartum cows. Total 87 cows 30-60 days in milk were examined from surrounding farms of Akola city with cytobrush technique. Cows with ≥5% PMN cells in endometrial cytology were regarded as affected by subclinical endometritis and divided into three groups comprising ten cows in each group. In T1 group cows treated with a 25 ml sterile hydromethanolic extract of Azadirachta indica intra uterine for three consecutive days. In T2 group cows treated with a 20 ml (10mg/ml) sterile hydromethanolic leaf extract of Achyranthes aspera (200 mg) intrauterine for three consecutive days and T3 group cows showing PMN ≥5% in postpartum cows in uterine cytology were taken as a positive control group and given no treatment. In the present study the overall prevalence of subclinical endometritis at 30-60 days postpartum was 35.63%. The curative efficacy was higher in Achyranthes aspera treated cows followed by Azadirachta indica treated cows as compared to untreated control cow. The first service conception rate was higher in Azadirachta indica treated cows followed by Achyranthes aspera as compared to untreated cows. It was concluded that there was higher prevalence of subclinical endometritis in postpartum cows in surrounding farms of Akola city. The therapeutic efficacy was higher in Achyranthes aspera treated cows whereas first service conception rate was higher in Azadirachta indica treated cows.

Keywords: Subclinical, endometritis, cattle, Achyranthes aspera, Azadirachta indica

1. Introduction
Subclinical endometritis is the common cause of infertility and subfertility in high producing dairy cattle delaying the onset of ovarian cyclic activity after parturition, extending luteal phase and reducing conception rate is (Sheldon et al., 2009) [1]. Uterine cytology or ultrasonography can be used for the diagnosis of subclinical endometritis. Cytobrush method (Barlund et al., 2008; Kasimanickam et al., 2004 and Kasimanickam et al., 2005) [2, 3] can be used for the collection of samples for uterine cytology. An increased proportion of PMN is prognostic for impaired subsequent reproductive performance (Barlund et al., 2008; Gilbert et al., 2005 and Kasimanickam et al., 2004) [2, 3]. Four to eighteen percent of PMN cells (Barlund et al., 2008; Galvao et al., 2009; Gilbert et al., 2005; Kasimanickam et al., 2004) [2, 3]. is the threshold value that defines subclinical endometritis.

(Plontzke et al., 2010) [7] reported the overall prevalence of subclinical endometritis with endometrial cytology was 38% with more than 5% neutrophils. (Nihret and Jebra, 2012) [8] observed that the prevalence of subclinical endometritis in, cows with lack of hygienic condition, conceived heifers, cows those were inseminated, cows having good body condition score and cows in small scale farms were 71.4%, 100%, 69.8%, 75% and 86.7%, respectively with statistically non significant difference (p>0.05) among parity, hygienic condition, body condition score, method of service and farm scales, respectively.

In India as traditional ayurvedic and folkore medicine, Neem (Azadirachta indica) has been widely used for the treatment of various diseases (Bandyopadhyay et al., 2002) [9]. It has been proved to have immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, and antioxidant properties (Subapriya and Nagini, 2005) [10]. Due to its better antibiotic and immunomodulatory property of hydro alcoholic extract of neem oil, it can be used as a therapy for endometritis in repeat breeding cows and also observed as a better clinical recovery and conception rate with hydro alcoholic extract of neem (Harendra et al., 2013) [11].
Achyranthes aspera (Aghada) is distributed as a weed throughout India, tropical Asia and other parts of the world. The plant is reported to be used as oestrogenic, antimicrobial, immunostimulant, anti-inflammatory, antioxidiant, diuretic, cardiac stimulant, antispasmodic and hepatoprotective (Dey 2011 and Sharma et al., 2013) [12, 13]. Immunomodulatory activity of the plant on elicitation of antigen-specific murine antibody response has been reported (Vasudeva et al. 2002) [14]. The oestrogenic and urotrophic activity of the extracts has successfully detected in mice (Vasudeva and Sharma, 2007) [15] & Shibeshi et al., 2006 [16]. Achyranthes aspera is a potent immunostimulating plant for traditional medicine (Srivastava, 2014) [17].

Keeping in view the economic losses due to subclinical endometritis faced by the farming community & lack of cost-effective treatment for subclinical endometritis the present study was designed with the following objective.

2. Materials and Methods

The study was conducted on post partum cows between 30 to 60 days in milk were selected from ILFC, PDKV, Akola and surrounding Gorakshans of Akola city and were screened for subclinical endometritis. A total 87 cows 30-60 days in milk were examined from surrounding farms of Akola city with cytobrush technique. Cows with ≥5% PMN cells in endometrial cytology were regarded as affected by subclinical endometritis.

Out of 87 cows, total of 30 cows showing PMN cells ≥ 5% threshold level in postpartum cows were selected and divided into three groups comprising ten cows in each group. In T1 group cows treated with a 25 ml sterile hydromethanolic extract of Azadirachta indica intra uterine for three consecutive days. In T2 group cows treated with a 20 ml (10mg/ml) sterile hydromethanolic leaf extract of Achyranthes aspera (200 mg) intrauterine for three consecutive days and T3 group cows showing PMN ≥5% in postpartum cows in uterine cytology were taken as positive control group and given no treatment. For curative efficacy endometrial cytology was performed in all 30 cows from all the groups on consecutive oestrus by using cytobrush technique. Cows exhibited spontaneous estrus from all groups were artificially inseminated as per AM and PM rule. The conception rate in all the groups was determined by per rectal (P/R) examination on 60 day post A.I.

3. Results and Discussion

3.1 Prevalence of Subclinical Endometritis in Postpartum Cows

A total 87 cows from 30-60 days in milk (DIM) without any vaginal discharge were enrolled from different farms and Gorakshans sanstha around Akola city.

The prevalence of subclinical endometritis in different herds is given in Table 1. The overall prevalence of subclinical endometritis with 5% threshold level from 30-60 days in milk was 35.63% (31/87). Herd level prevalence was 30.00% (6/20), 44% (11/25), 27.27% (3/11), 33.34% (4/12), 30% (3/10) and 44.45% (4/9) on farms ILFC, PDKV, Gorakshan akola, Rashtria shala, Nimbi shetki shala, Guldhi and Gow Sanstha Dabki road, respectively.

Table 1: Prevalence of subclinical endometritis in different farms and in and around Akola city

<table>
<thead>
<tr>
<th>Name of Farm</th>
<th>No of animals screened</th>
<th>Positive for SCE</th>
<th>Herd level prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILFC, PDKV</td>
<td>20</td>
<td>6</td>
<td>30.00%</td>
</tr>
<tr>
<td>Gorakshan, Akola</td>
<td>25</td>
<td>11</td>
<td>44.00%</td>
</tr>
<tr>
<td>Rashtria shala,</td>
<td>11</td>
<td>3</td>
<td>27.27%</td>
</tr>
<tr>
<td>Nimbi shetki shala</td>
<td>12</td>
<td>4</td>
<td>33.34%</td>
</tr>
<tr>
<td>Guldhi</td>
<td>10</td>
<td>3</td>
<td>30.00%</td>
</tr>
<tr>
<td>Gow sanstha Dabki road</td>
<td>09</td>
<td>4</td>
<td>44.45%</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>31</td>
<td>35.63%</td>
</tr>
</tbody>
</table>

The overall prevalence of subclinical endometritis in the present study is in accordance with previously published data from confinement housing systems. Kasimannickam et al. (2004) [3] diagnosed subclinical endometitis in clinically healthy dairy cows and described a prevalence of 35% and 34% for 20 to 33 days postpartum and 34 to 47 days postpartum, respectively. Green et al. (2009) [18] reported a prevalence of 37% subclinical endometritic cows. Cheong et al. (2011) [19] reported a herd-level prevalence of SCE of 36.1%. Senosy and Osawa (2013) [20] revealed the prevalence of SCE to be 33.3% and 30.4% in cold and temperate season, respectively at ≥5% threshold level. Lima et al. (2013) [21] observed prevalence of SCE of 29.5% with 5% threshold level. Melcher et al. (2014) [22] revealed the prevalence of SCE of 33% by counting 100 cells. Carneiro et al. (2014) [23] reported a prevalence of SCE of 26%. Singh et al. (2016) [24] observed the prevalence of SCE was 29.4% at ≥4% threshold level. Dini et al. (2015) [25] reported the prevalence of SCE 30 days postpartum as 38.5% using the threshold of 18%. Syed (2016) [26] reported prevalence of 36.14% in post partum cows in and around Akola city showing herd-level prevalence of 30% (6/20), 46.66% (7/15), 40% (8/20), 30% (3/10), 33.34% (4/12) and 33.34% (2/6) (in ILFP), Dr Punjabrao Deshmukh

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In contrast to present findings, a lower prevalence of SCE obtained by Dubuc et al. (2010) [27] who reported 13.5% prevalence for cytological endometritis at ≥6% threshold level of PMN cells. Kaufmann et al. (2010) [28] who observed the overall prevalence of SCE was 12.4% at 18% threshold level of PMN cells. Ribeiro et al. (2013) [29] reported 13.4% prevalence in subclinical endometritic cows at ≥5% threshold level and Barrio et al. (2015) [30] who observed 14.9% prevalence of SCE in 35-45 days in milk.

The difference in percentage of prevalence with earlier findings may be due to different threshold level of PMN cells, difference in postpartum day of diagnosis, variation in season, variation in Body Condition Score and different counting methods for PMN cells (Senosy and Osawa 2013, Carneiro et al., 2014 and Melcher et al., 2014) [20, 23, 22].

3.2 Therapeutic Efficacy of Azadirachta indica and Achyranthes aspera in Subclinical Endometritic Cows

The therapeutic efficacy of Azadirachta indica and

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Achyranthes aspera in subclinical endometritic cows were shown in Table 2. The therapeutic efficacy was measured on the basis of curative percentage by endometrial cytology on the subsequent heat after the treatment.

Table 2: Therapeutic efficacy of Azadirachta indica (T1) and Achyranthes aspera (T2) in subclinical endometritic cows

<table>
<thead>
<tr>
<th>Treatments Groups (n=6)</th>
<th>No of Animals Treated</th>
<th>No of Animals Negative in endometrial cytology</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>10</td>
<td>7</td>
<td>70.00%</td>
</tr>
<tr>
<td>T2</td>
<td>10</td>
<td>8</td>
<td>80.00%</td>
</tr>
<tr>
<td>T3</td>
<td>10</td>
<td>2</td>
<td>20.00%</td>
</tr>
</tbody>
</table>

The therapeutic efficacy on the basis of curative percentage in groups Azadirachta indica and Achyranthes aspera were 70.00% (7/10) and 80.00% (8/10) after the treatment and in untreated group it was 20.00% (2/10), respectively. From the present study it was revealed that the curative efficacy was higher in Achyranthes aspera treated cows followed by Azadirachta indica treated cows as compared to untreated control cows. The higher curative percentage of Achyranthes aspera and Azadirachta indica might be due to immunomodulatory, anti inflammatory and antibacterial as well as estrogenic properties of Achyranthes aspera.

The therapeutic efficacy of Azadirachta indica observed in the present study is in accordance with Kumar et al. (2013) who reported 75% therapeutic efficacy with hydro acetoin neem bark extract, whereas higher therapeutic efficacy was observed by Kumar et al. (2013) [11] and Harendra et al. (2013) [11] 96.02% with hydroalcoholic neem bark and methanolic extract of neem oil, respectively which are in not accordance with present study.

The difference in therapeutic efficacy of Azadirachta indica with earlier findings might be due to difference in breed, postpartum time period of treatment, season, body condition score and variation in PMN cells counting. (Sensosy and Osaka 2013, Carneiro et al., 2014 and Melcher et al., 2014) [20, 21, 22]. Therapeutic efficacy of Achyranthes aspera observed in present study is in close accordance with Syed (2016) who reported 66.66%, therapeutic efficacy of hydromethanolic extract of Achyranthes aspera in postpartum cows, whereas 20% cows observed negative for subclinical endometritis in untreated cows which are in agreement with Kumar et al. (2013) [11] and Harendra et al. (2013) [11] both of whom reported 25% cows to be negative for subclinical endometritis cows.

3.3 First Service Conception Rate in Experimental cows

All the cows exhibited spontaneous estrus in groups treated with Azadirachta indica, Achyranthes aspera including untreated groups they were artificially inseminated as per Am and Pm rule. In the present study, 5 out of ten cows were found to be pregnant with a 50 percent conception rate in Azadirachta indica group, 4 out of ten cows were found to be pregnant with 40.00 percent conception rate in the Achyranthes aspera group. Whereas, out of ten cows only two cows were found to be pregnant with 20.00 percent in untreated groups, respectively. (Table 3)

Table 3: First service conception rate in subclinical endometritic cows with different treatments

<table>
<thead>
<tr>
<th>Treatments Groups (n=10)</th>
<th>Number of Animals Treated</th>
<th>Number of Animals Inseminated</th>
<th>Number of Animals Pregnant</th>
<th>First Service Conception Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>50.00</td>
</tr>
<tr>
<td>T2</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>40.00</td>
</tr>
<tr>
<td>T3</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>20.00</td>
</tr>
</tbody>
</table>

From the present study, it was observed that cows treated with Azadirachta indica showed higher first service conception rate followed by Achyranthes aspera treated cows as compared to untreated cows.

The higher first service conception rate observed in cows from treatment groups as compared to untreated group might be due to better therapeutic efficacy of Azadirachta indica or Achyranthes aspera in subclinical endometritis which had a detrimental effect on fertility causing an increase in calving to conception and a decrease in the rate of cows who become pregnant (Ricci et al. 2016) [23].

The first service conception rate in Achyranthes aspera group observed in present study is in close agreement with Syed (2016) who recorded 50% first service conception rate in subclinical endometritis cows treated with methanolic extract of Achyranthes aspera.

The first service conception rate in Azadirachta indica observed in present study is in accordance with Kumar et al. (2013) who reported 50% conception rate in endometritic cows treated with 30 ml hydroacetonic neem bark, whereas Kumar et al. (2013) [31] and Harendra et al. (2013) [11] reported higher conception rate 62.50% and 71.42% in endometritic cows treated with hydroalcoholic and methanolic fraction of neem oil, respectively which is not accordance with present study.

A lower first service conception rate than the present study for Azadirachta indica observed by Harendra et al. (2013) [11] 25% in endometritic cows treated with ethanolic fraction of neem seed powder.

Results of untreated cow control group are in agreement with Nibret and Jebra (2012) and Ricci et al. (2015) [23] who observed first service conception rate of 21.4% and 13% in Subclinical endometritic cows, respectively. Similarly Barlund et al. (2008) [02] reported 14.2% conception rate in endometritic positive cows.

In the present study the difference in conception rate in earlier findings may be due to the variation in curative efficacy of different drugs used. Lima et al. (2013) [21] stated that the cows with subclinical endometritis from the early postpartum period have greater depression in measures of fertility at first AI.

4. Conclusion

The therapeutic efficacy was higher in Achyranthes aspera treated cows followed by Azadirachta indica treated cows as compared to untreated control cows. The first service conception rate was higher in Azadirachta indica treated cows followed by Achyranthes aspera treated cows as compared to untreated control cows.
5. References


