

# Use of Ethanolic Herbal Extracts and Ciprofloxacin for the Treatment of Subclinical Endometritis in Crossbred Cows

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## Abstract

*The aim of this study was to evaluate the therapeutic efficacy of ethanolic extracts of Neem, Garlic, Tulsi, and Turmeric and to compare with antibiotic Ciprofloxacin in crossbred cows suffering from subclinical endometritis. The present study was conducted on 30 crossbred cows having the history of abnormal puerperium. The cows 30 days postpartum having clear cervico-vaginal discharge but found positive for Whiteside test, were included in this study. All such cows were divided into five therapeutic groups C1 (Neem), C2 (Garlic), C3 (Tulsi), C4 (Turmeric) and C5 (Ciprofloxacin). The therapeutic efficacy of these drugs was evaluated in terms of negativity to Whiteside test and conception rate. The overall percentage of animals found negative to Whiteside test and conceived after treatment with Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin up to 3rd estrus was 66.66, 50.00, 66.66, 83.33 and 100 per cent, respectively and percentage of cows conceived after treatment with Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin up to 3rd estrus was 50.00, 33.33, 50.00, 83.33 and 83.33 per cent, respectively.*

**Keywords:** Crossbred Cows, Conception Rate, Ethanolic Extract, Herbal Plants, Subclinical Endometritis, Whiteside Test

## Introduction

Subclinical endometritis (SE) is the inflammation of the endometrium without apparent clinical manifestation and often without evidence of infection (Marino *et al.*, 2017). Alteration of the inflammatory response postpartum could be at the origin of this condition. It has been suggested that SE may be a response to unspecific uterine infections (Gartner *et al.*, 2015) or a prolonged inflammatory process that persists after bacterial elimination. Popov (1969) used Whiteside test (WST) on the cervical mucus of cows for diagnosis of occult endometritis. This test is based on correlation between the number of leucocytes present in the mucus and intensity of yellow color developed (Pateria and Rawal, 1990).

Presently, treatment of such infertility is mainly achieved using intrauterine antibiotics, antiseptics and less commonly by hormones. The inconsistent results, high cost of treatment, compulsory milk disposal and inhibition of natural uterine defense, emergence of resistant bacterial strains, after antibiotic/antiseptic treatment however makes them unethical and uneconomical (Hussain and Daniel, 1992). Now a day, the major problem faced by animal reproductive health workers is the multiple drug resistance pathogens (MDR) because of the indiscriminate use of commercial antimicrobial drugs.

Antimicrobials of plant origin have enormous therapeutic potential (Cunha, 2001). They are not only effective in the treatment of infectious diseases while simultaneously minimizing many side effects that are often associated with synthetic antimicrobials or antibiotics. There are many published reports on the therapeutic effectiveness of traditional plants against Gram positive and Gram-negative microorganisms and as a result, plants are still recognized as the basis for modern medicine to treat infectious diseases (Evans, 2002). Plants used for traditional medicine contain a wide range of bioactive molecules, making them rich sources of different types of medicine particularly antimicrobial properties (Nair *et al.*, 2005).

Neem (*Azadirachta indica*) has been extensively used in India as traditional ayurvedic and folklore medicine for the treatment of various diseases (Bandyopadhyay *et al.*, 2002). It has been demonstrated that many other herbal medicines like Tulsi (*Ocimum sanctum*), Garlic (*Allium sativum*) and Turmeric (*Curcuma longa*) exhibit immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral and antioxidant properties to resolve the problem without facing antibiotic resistance and milk disposal problem (Ghazanfari *et al.*, 2002; Subapriya and Nagini, 2005). The aim of this study was to evaluate the therapeutic efficacy of ethanolic extracts of Neem, Garlic, Tulsi, and Turmeric and to compare with antibiotic Ciprofloxacin in crossbred cows suffering from subclinical endometritis

## Materials and Methods

### Selection of Animals

The present study was conducted on 30 crossbred cows belonging to the villages of Mhow and cows presented to the Veterinary Clinical Complex, Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Mhow, District- Indore (M.P.) having the history of abnormal puerperium. The cows showing the clear cervico-vaginal discharge but found positive for Whiteside test, 30 days postpartum were included in this study. All such cows were divided into five therapeutic groups C1 (Neem), C2 (Garlic), C3 (Tulsi), C4 (Turmeric) and C5 (Ciprofloxacin), consisting 6 animals in each group.

Whiteside test was done twice, one at time of screening of the animals and other before insemination. To perform this test equal volume of cervico-vaginal mucus and 5% NaOH were mixed in a test tube and heated up till boil. Development of yellow color was considered as positive as described by Popov (1969).

### Preparation of Herbal Ethanolic Extracts

#### Neem Extract

Neem bark was collected, cleaned and grind to make fine powder. Powder was filled up in filter paper thimble and the thimble was fixed in the soxhlet assembly, extraction was carried out with ethanol at 80°C temperature. The extract was recovered and extra ethanol was evaporated, finally obtained extract was weighed and dissolved in the

normal saline.

### **Garlic Extract**

Garlic cloves were dried and grinded in the mixer to make fine powder. Ethanolic extract was obtained as per the method described above for the neem extract.

### **Tulsi Extract**

Tulsi leaves were collected and dried under shade. Dried leaves were grinded and subjected for soxhlet extraction as per the procedure followed in the Neem extract.

### **Turmeric Extract**

Dried rhizome of turmeric was grinded to make fine powder. Rest of the procedure was followed similar to that of Neem extract. Ethanolic extract of turmeric seems to be hydrophobic in nature and it is not easily dissolve in normal saline so to facilitate its dissolve in normal saline few ml of tween 80 were added.

### **Treatments of Animals**

The dose rate of herbal extracts was decided on the basis of minimum inhibitory concentration suggested by different researchers (Neem - Nayak *et al.*, 2011, Garlic - Srinivasan *et al.*, 2009, Tulsi - Bhatt *et al.*, 2012, Turmeric- Nikambule, 2008). Each cow of the therapeutic groups C1, C2, C3, C4 was treated with intrauterine ethanolic extract of neem, garlic, tulsi and turmeric extract thrice at 24 hrs interval dissolved in 30 ml normal saline @ dose rate of 7.5 mg/ml, 20 mg/ml, 65 mg/ml and 20 mg/ml by intrauterine route, respectively. The cows of therapeutic group C5 were treated with intrauterine Ciprofloxacin dissolved in 30 ml distilled water @ dose rate of 4mg/kg body weight by intrauterine route thrice at 24 hrs. interval. Artificial insemination was performed at the next successive estrus. Pregnancy diagnosis was done by per-rectal examination 60 days post insemination, to evaluate conception rate in all the animals.

## **Results and Discussion**

In the present study therapeutic efficacy of ethanolic extract of neem, garlic, tulsi, turmeric and an antibiotic Ciprofloxacin was judged on the basis of response to Whiteside test and conception rate.

### **Response to Whiteside Test**

All the animals before treatment were positive to the whiteside test. The subclinical endometritic cows were treated with Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin and successively found negative for whiteside test presented as in Table 1, Fig. 1. The percentage of cows found to be negative to Whiteside test on 1<sup>st</sup> successive estrus was 33.33, 16.66, 33.33, and 50.00 per cent in Neem, Garlic, Tulsi and Turmeric extract treated cows, respectively, whereas 83.33 per cent cows were negative for Whiteside test in Ciprofloxacin treated group. The percentage of Whiteside test negative animals on 2<sup>nd</sup> successive estrus increased by 33.33 percent in Neem extract treated animals and 16.66 percent in remaining all the four treatment group cows whereas on 3<sup>rd</sup> successive estrus the negativity to Whiteside test observed was 16.66 percent in cows treated with Garlic, Tulsi and Turmeric extract. The overall percentage of animals found negative to Whiteside test up to 3<sup>rd</sup> successive estrus were 66.66, 50.00, 66.66, 83.33 and 100 per cent to Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin treatment, respectively.

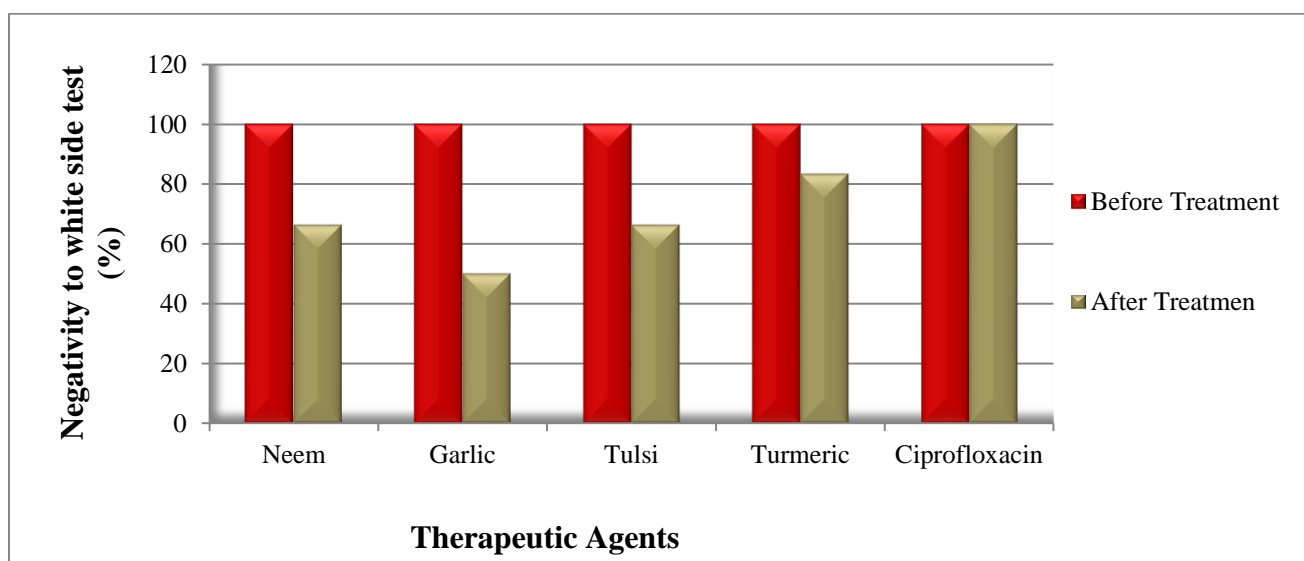
Chi - square analysis using online software WASP 1 (Web Agri Stat Package) revealed significantly greater values ( $p < 0.05$ ) for overall Whiteside test negative cases in group C5 as compared to group C1, C3 and C4. The difference between group C1, C3 and C4 was non-significant. However, significantly lower values were found in group C2 as compared to other groups. The findings regarding overall response of cows to Whiteside test after treatment in subclinical endometritic cows in the present study are in close accordance with Kumar *et al.* (2013) who reported that 75per cent endometritic repeat breeder cows became negative to Whiteside test after treatment with hydro-alcoholic extracts of the Neem bark, they further mentioned that there was significant ( $P < 0.05$ ) reduction in the proportion (from 100 to 25%) of Whiteside test positive animals treated with Neem bark extract. Similarly, Thombre

(2017) found 66.66 per cent recovery rate in endometritic repeat breeder cows after treatment with Neem seed extract.

**Table 1:** Subclinical endometritic cows found negative to Whiteside test after treatment

Treatment groups (n=6)	WST negative animals after treatment (%)			
	1 <sup>st</sup> Insemination	2 <sup>nd</sup> Insemination	3 <sup>rd</sup> Insemination	Overall
C1 (Neem)	33.33 (2)	33.33 (2)	0.00 (0)	66.66 (4)
C2 (Garlic)	16.66 (1)	16.66 (1)	16.66 (1)	50.00 (3)
C3 (Tulsi)	33.33 (2)	16.66 (1)	16.66 (1)	66.66 (4)
C4 (Turmeric)	50.00 (3)	16.66 (1)	16.66 (1)	83.33 (5)
C5 (Ciprofloxacin)	83.33 (5)	16.66 (1)	0.00 (0)	100 (6)

Values in parenthesis indicated number of animals



**Figure 1:** Overall percentages of subclinical endometritic cows found negative to Whiteside test

The findings in this study are in accordance with Tomar (2016) reported 83.33, 83.33 and 100.00 per cent Whiteside negative cases after treatment with Neem, Garlic and Ciprofloxacin in endometritic repeat breeder cows. Bhardwaz (2018) reported 70.00, 70.00 and 90.00 percent Whiteside negative cases of infectious repeat breeder cows treated with Neem, Garlic and Ciprofloxacin.

The findings in the present study are also in close accordance with Kumar (2016) who reported 83.33 per cent Whiteside negative animals after treatment with Garlic and Turmeric extract in subclinical endometritic cows. Brahmanand *et al.* (2017) also reported similar findings who reported 66.66 per cent Whiteside test negative cases after treatment with Neem bark extract in subclinical endometritic cattle.

### Conception Rate

In the present study, the cows that conceived after treatment with Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin were presented in the Table 2, Fig. 2. The conception rate on 1<sup>st</sup> insemination was 16.66, 0.00, 16.66, 33.33 and 66.66 per cent. The conception rate on 2<sup>nd</sup> insemination had a further hike of 16.66 per cent in Neem, Garlic Tulsi and Ciprofloxacin treated animals while it was 33.33 per cent hike with Turmeric treated cows. The conception rate hike in cows at 3<sup>rd</sup> insemination was 16.66 per cent in all the herbal extract treatment group cows. The overall conception rate in subclinical endometritic group cows treated with ethanolic extract of Neem, Garlic, Tulsi & Turmeric and Ciprofloxacin was 50.00, 33.33, 50.00, 83.33 and 83.33 per cent, respectively up to 3<sup>rd</sup> insemination. The statistical analysis of variance using Chi - square analysis by online software WASP 1 (Web Agri Stat Package) revealed that overall conception rate in subclinical endometritis cows after treatment was significantly higher ( $p < 0.05$ ) in C5 and C4 group cows followed by C1 and C3 group cows. Significantly lowest conception rate

after treatment was observed in group C2 cows. However, difference between C5 and C4 group cows was non-significant. The difference between group C1 and C3 was also non-significant.

**Table 2:** Overall conception rate in subclinical endometritic cows treated with different medications

Treatment groups (n=6)	Conception rate (%)			
	1 <sup>st</sup> Insemination	2 <sup>nd</sup> Insemination	3 <sup>rd</sup> Insemination	Overall
C1 (Neem)	16.66 (1)	16.66 (1)	16.66 (1)	50.00 (3)
C2 (Garlic)	0.00 (0)	16.66 (1)	16.66 (1)	33.33 (2)
C3 (Tulsi)	16.66 (1)	16.66 (1)	16.66 (1)	50.00 (3)
C4 (Turmeric)	33.33 (2)	33.33 (2)	16.66 (1)	83.33 (5)
C5 (Ciprofloxacin)	66.66 (4)	16.66 (1)	0.00 (0)	83.33 (5)



**Figure 2:** Overall conception rate in subclinical endometritic cows treated with different medications

The findings on overall conception rate in the present study are in close accordance with Bhardwaz (2018) who reported 50 per cent conception rate in cows treated with Neem extract, 60 per cent conception rate in infectious repeat breeder cows treated with Garlic extract and 70 per cent conception rate in cows treated with Ciprofloxacin. Screening of available literature revealed no record on conception rate in subclinical endometritic cows treated with Tulsi and Turmeric however, reports of various workers showed that Tulsi and Turmeric has antibacterial, anti-inflammatory and immunomodulatory property.

Among herbal therapeutic agents used in this study Turmeric extract has given results with good success in terms of conception rate probably due to potent immunomodulatory property of turmeric. Jagetia and Aggrawal (2007) also reported that curcumin has shown to be a potent immunomodulatory agent that can modulate the activation of T cell, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells. Curcumin can also down regulate the expression of various proinflammatory cytokines including TNF, 1L-1, 1L-2, 1L-6, 1L-8, 1L-12 and chemokines, most likely through inactivation of the transcription factor NF- Kappa B.)

Thus, turmeric results by improving uterine atmosphere to improve conception are comparable with ciprofloxacin.

## Conclusion

After treatment with herbal extract of Neem, Garlic, Tulsi and Turmeric maximum number of cervical mucus samples showed absence of color reaction. This showed that these herbal extracts possess antibacterial and immunomodulatory property, causes reduction in bacterial load and further reduction in inflammation of endometrium, depending on concentration of therapeutic agent, frequency of administration and duration of therapy. In the present study it has been also observed that conception rate in subclinical endometritic crossbred cows shown

by therapeutic agents of plant origin were increased with number of inseminations that indicates therapeutic agents of plant origin in this study needs more number of inseminations to show their efficacy that might be due to higher immunomodulatory property of herbal therapeutic agents than the antimicrobial property. Among all therapeutic agents used in the present study, an antibiotic ciprofloxacin exhibited highest therapeutic efficacy 83.33% (Conception rate) for the treatment of subclinical endometritis in crossbred cows. Similar results were also given by ethanolic extract of turmeric.

## Conflict of Interests

There is no conflict of interest.

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