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## Therapeutic efficacy of ethanolic herbal extracts and ciprofloxacin in metritic crossbred cows

**SS Mahour, SP Nema, Neetu Rajput, Daljeet Chhabra, SK Karmore, N Kurechiya and R Sikrodiya**

**Abstract**

The aim of this study was to evaluate the therapeutic efficacy of the ethanolic extracts of Neem, Garlic, Tulsi, and Turmeric compared with an antibiotic Ciprofloxacin in Metritis affected crossbred cows. The present study was conducted on 30 crossbred cows having the history of abnormal puerperium. The cows showing the presence of pus plaques in the cervico-vaginal discharge, 30 days postpartum were included in this study. All such cows were divided into five therapeutic groups A1 (Neem), A2 (Garlic), A3 (Tulsi) A4 (Turmeric) and A5 (Ciprofloxacin). The therapeutic efficacy was evaluated in terms of conception rate. The cows that conceived after treatment with Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin up to 3<sup>rd</sup> insemination were 50.00, 33.33, 33.33, 50.00 and 83.33 percent, respectively.

**Keywords:** Crossbred cows, conception rate, ethanolic extract, herbal plants, metritis

**Introduction**

Postpartum uterine inflammatory diseases such as metritis can affect the fertility of dairy cows to a varying degree by disturbing the molecular milieu of the uterus. 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 made it unethical and uneconomical (Hussain and Daniel, 1992) [1] Now a day, the major problem faced by the world is the multiple drug resistance pathogens (MDR) because of the indiscriminate use of commercial antimicrobial drugs.

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) [2] Antimicrobials of plant origin have enormous therapeutic potential (Cunha, 2001) [3] They are effective in the treatment of infectious diseases while simultaneously minimizing many of the side effects that are often associated with synthetic antimicrobials or antibiotics. There are many published reports on the 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) [4]

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) [5]. It has been demonstrated that many other herbal medicine 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) [6, 7].

**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 presence of pus plaques in the cervico-vaginal discharge, 30 days postpartum were considered as metritic cows and included in this study.

All such cows were divided into five therapeutic groups A1 (Neem), A2 (Garlic), A3 (Tulsi), A4 (Turmeric) and A5 (Ciprofloxacin), consist in 6 animals in each group.

### 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 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 thus to facilitate it 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) [8-11]. Each cow of the therapeutic groups A1, A2, A3, A4 was treated thrice at 24 hrs. interval with intrauterine ethanolic extract of Neem, Garlic, Tulsi and Turmeric extract dissolved in 30 ml normal saline @ dose rate of 7.5 mg/ml, 20 mg/ml, 65 mg/ml and 20 mg/ml respectively. The cows of therapeutic group A5 were treated with intrauterine Ciprofloxacin dissolved in 30 ml distilled water @ dose rate of 4mg/kg body weight thrice at 24 hrs. interval.

Artificial insemination was performed at the next 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 metritis affected cows that conceived after treatment with Neem, Garlic, Tulsi, Turmeric extract and Ciprofloxacin are presented in the table 1. The conception rate in each treatment group A1, A2, A3, A4 and A5 cows after 1<sup>st</sup> insemination was 16.66, 0.00, 0.00, 33.33 and 50.00 percent, respectively. Further, the conception rate after 2<sup>nd</sup> and on 3<sup>rd</sup> insemination in cows had a further increase of another 16.66 percent in all the treatment groups except in Turmeric treatment group cows where on 3<sup>rd</sup> insemination no increase in conception was observed. The overall conception rate in metritic group cows treated with Neem, Garlic, Tulsi, Turmeric ethanolic extract and Ciprofloxacin were 50.00, 33.33, 33.33, 50.00 and 83.33 percent respectively upto 3<sup>rd</sup> insemination.

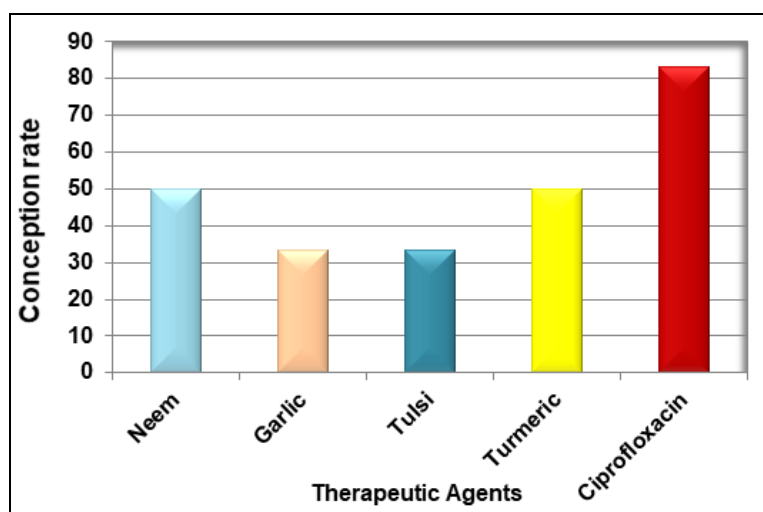
Chi - square analysis using online software WASP 1 (Web Agri Stat Package) of overall conception rate in metritis affected cows after treatment revealed significantly greater values ( $p < 0.05$ ) in A5 group cows as compared to other four herbal extract treated group A1, A2, A3 and A4 cows. The difference between group A1, and A4, also in group A2 and A3 was non-significant.

**Table 1:** Conception rate after treatment with herbal extract and Ciprofloxacin in metritis affected cows

Treatment groups (n=6)	Conception rate (%)			
	1 <sup>st</sup> Insemination	2 <sup>nd</sup> Insemination	3 <sup>rd</sup> Insemination	Overall
A1 (Neem)	16.66 (1)	16.66 (1)	16.66 (1)	50.00 (3) <sup>b</sup>
A2 (Garlic)	0.00 (0)	16.66 (1)	16.66 (1)	33.33 (2) <sup>ab</sup>
A3 (Tulsi)	0.00 (0)	16.66 (1)	16.66 (1)	33.33 (2) <sup>ab</sup>
A4 (Turmeric)	33.33 (2)	16.66 (1)	0.00 (0)	50.00 (3) <sup>b</sup>
A5 (Ciprofloxacin)	50.00 (3)	16.66 (1)	16.66 (1)	83.33 (5) <sup>a</sup>

Values bearing different superscripts (a, b) vary significantly ( $p < 0.05$ ) between groups.

Values in parenthesis indicated number of animals



**Fig 1:** Overall conception rate in metritic cows after treatment

The results of conception rate in metritis affected cows treated with intra uterine herbal extracts and Ciprofloxacin showed that Ciprofloxacin group cows had maximum overall conception rate followed by Turmeric, Neem, Garlic and Tulsi extract group treated cows. The conception rate on 1<sup>st</sup> insemination was higher with Ciprofloxacin treated animals than other herbal groups which might be due to Ciprofloxacin has greater antibacterial property than all the herbal extracts. It may be due to these herbal extracts may need more duration to ameliorate the uterine inflammatory condition through associated effect such as immuno-modulatory and anti-inflammatory properties apart from antimicrobial potential of these herbal extracts.

Sarkar *et al.* (2006) [12] reported that treatment of endometritic cows with the Garlic extract enhances the immune system by stimulating release of cytokines such as IL-2, IFN- $\alpha$ , IFN- $\gamma$  and increase the natural killer activity and phagocytic activity of peritoneal macrophages. Similarly Bhatia *et al.* (2013) [13] reported that methanolic extracts and aqueous suspension of leaves and seed oil of Tulsi plant can induce cytokine secretion. The oil and linolenic acid of Tulsi possess good analgesic, antipyretic as well as anti-inflammatory activities. Jagetia and Aggrawal (2007) [14] also reported that curcumin to be a potent immunomodulatory agent that can modulate the activation of T cell, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells rather than having more direct antimicrobial property.

The findings in the present study with reference to Neem bark were similar to Brahmanand (2017) [15] who reported 50% conception rate in repeat breeder cows suffering from endometritis with the use of Neem bark extract intrauterine thrice at 24 hrs. interval.

The overall conception rate in the present study were slightly lower (33.33%) after treatment with Garlic and similar (50.00%) after treatment with Turmeric ethanolic extract when compared to the findings of Kumar (2016) [16] studies on immunomodulatory and therapeutic efficacy of garlic (*allium sativum*) and turmeric (*curcuma longa*) in endometritic repeat breeding crossbred cows and found 83.33% recovery rate and 50% conception rate with intrauterine administration of garlic and turmeric (*curcuma longa*) extracts. In the present study conception rate in Turmeric extract treated animals are also in close accordance with Kumar (2019) [17] who reported 50% conception rate in metritic cow treated with Turmeric ethanolic extract. Treatment with ethanolic extract of Tulsi also has given lower conception rate in the present study.

The findings of ciprofloxacin in the present study were in close accordance with Bhardwaz (2018) [18], reported 90% recovery rate with 70% conception rate in infectious repeat breeder cows after intra uterine administration of ciprofloxacin.

Different parts of herbal plants possessing different active ingredient and accordingly medicinal value. Bindrawan *et al.* (2002) [19] used crude neem oil (40 ml thrice at 24 hrs. interval) and recorded 88% recovery in endometritic cattle. Similarly, Rathod *et al.* (2012) [20] reported that neem bark has more significant antibacterial activity than neem leaves and tulsi leaves.

The process of extraction of active ingredient and dissolution capacity of active ingredient in different solvent also affect the quality and therapeutic efficacy of active ingredient present in the herbal plants. Kumar *et al.* (2013) [21] reported that hydro-alcoholic extract of the neem has a better

antibacterial and immunomodulation and can be used as a therapy for endometritis in repeat breeding crossbred cows. Crude extract of garlic has been found to possess marked antibacterial, property against a wide variety of bacteria found in genital tract of repeat breeder cows (Arunachalam, 1980) [22].

### Conclusion

In the present study it has been observed that conception rate in metritic 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 comparatively 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 metritis in crossbred cows.

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### References

- Hussain AM, Daniel RCW. Effects of intrauterine infusion of E coli endotoxin in normal cows and in cows with endometritis induced by experimental infection with streptococcus agalactiae. *Theriogenology*. 1992; 37:791-810.
- Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology*. 2005; 29:41-47
- Cunha BA. Antibiotics side effects. *Medical Clinics of North America*. 2001; 85:149-185.
- Evans WC. *Trease and Evan's Pharmacognosy*. 5th ed., Haarcourt Brace and Company. 2002, 336.
- Bandyopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly CK, *et al.* Gastroprotective effect of Neem (*Azadirachta indica*) bark extract: Possible involvement of H<sup>+</sup>-K<sup>+</sup>-ATPase inhibition and scavenging of hydroxyl radical. *Life Science*. 2002; 71:2845-2865.
- Ghazanfari T, Hassan ZN, Ebrahimi M. Immunomodulatory activity of a protein isolated from garlic extract on delayed type of hypersensitivity. *International Immunopharmacology*. 2002; 2(11):1541-9.
- Subapriya R, Nagini S. Medicinal properties of Neem leaves: a review. *Current Medicinal Chemistry-Anticancer Agents*. 2005; 5(2):146-149.
- Nayak A, Nayak RN, Soumya GB, Bhat K, Kudalkar M. Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extract of neem (*azadirachta indica*) an *in vitro* study. *International Journal of Research in Ayurveda & Pharmacy*. 2011; 2(1):230-235.
- Srinivasan Durairaj, Sangeetha Srinivasan P, Lakshmanaperumalsamy. *In vitro* antibacterial activity and stability of garlic extract at different ph and temperature. *Electronic Journal of Biology*. 2009; 5(1):5-10
- Bhatt MK, Shankar MB, Saluja AK, Dholwani KK, Captain AD. Evaluation of anti-microbial activity of *Ocimum sanctum* methanolic extract. *Journal of*

- Pharmaceutical and Scientific Innovation. 2012; 1(4):39-41.
11. Nkambule TP. Antimicrobial properties of selected Asian herbs. M Sc. thesis (graduate school) university of Florida, 2008.
  12. Sarkar P, Kumar H, Rawat M, Varshney VP, Goswami TK *et al.* Effect of administration of garlic extract and PGF $2\alpha$  on hormonal changes and recovery in endometritic cows. Asian-Australasian Journal of Animal Science. 2006; 19(7):964-969.
  13. Bhatia AK, Kumar A, Goel A, Rahal A. Immunomodulatory activity of hot aqueous extract of *Ocimum sanctum* leaves. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 2013; 34:33-37.
  14. Jagetia GC, Aggarwal BB. "Spacing up" of the immune system by curcumin. Journal of Clinical Immunology. 2007; 27(1):19-35.
  15. Brahmanand. Therapeutic management of repeat breeding due to sub-clinical endometritis in cattle using herbal plants. M.V.Sc. thesis (Veterinary Gynaecology and Obstetrics) Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.), 2017.
  16. Kumar R. Studies on the immunomodulatory & therapeutic efficacy of ashwagandha (*Withania somnifera*), garlic (*Allium sativum*) & turmeric (*Curcuma longa*) on endometritic repeat breeding crossbred cows. Ph. D. thesis, (Veterinary Gynaecology & Obstetrics) Birsa Agricultural University, Kanke, Ranchi, Jharkhand, 2016.
  17. Kumar R. Comparative efficacy of intrauterine turmeric extract and ceftiofur sodium alone and in combination with micronutrients supplementation on fertility augmentation in postpartum metritic crossbred cows. M.V. Sc. Thesis (Veterinary Gynaecology and Obstetrics) Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.), 2019.
  18. Bhardwaz A. Fertility augmentation by hormones and immunomodulators in repeat breeder crossbred cows. Ph. D. thesis (Veterinary Gynaecology & Obstetrics) Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.), 2018.
  19. Bindrawan RM, Yadav MC, Kumar H, Shrivastava SK. Administration of Neem oil and colostral whey reduces bacterial load in endometritis in cattle. 10<sup>th</sup> International Congress of Asian- Australasian Association of Animal Production Societies, 23-27, September New Delhi, India, 2002, 248.
  20. Rathod GP, Kotecha MB, Sharma R, Amin H, Prajapati PK. *In vitro* antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica* L.) and Tulsi (*Ocimum sanctum* L.). International Journal of Pharmaceutical and Biological Archives. 2012; 3(3):582-586.
  21. Kumar A. Evaluation of immunomodulatory and therapeutic efficacy of turmeric (*Curcuma longa*) Neem (*Azadirachta indica*) and Garlic (*Allium sativum*) on endometritis in repeat breeding crossbred cows. M.V.Sc. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar, 2013.
  22. Arunachalam K. Antimicrobial activity of Garlic, Onion and Honey. Geobias. 1980; 7:46-47.