Original Research

The Effect of *In–vitro* Antibacterial Activity of Moringa & Tulsi Leaves Extract against Endometritic Repeat Breeding Cross Bred Cow

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Rec. Date:	Jun 22, 2019 12:07
Accept Date:	Dec 03, 2019 17:01
DOI	10.5455/ijlr.20190622120757

Abstract

The present study was conducted to see the effect of in–vitro antibacterial activity of Moringa & Tulsi leaves extract against endometritic repeat breeding cross bred cow at Military dairy farm, Namkum and local farmers in and around Ranchi, under supervision of Department of Veterinary Gynaecology and Obstetrics, Ranchi Veterinary College, Birsa Agricultural University, Kanke, Ranchi (Jharkhand),India. In the present study, the therapeutic efficacy of Moringa and Tulsi were assessed against endometritic repeat breeding crossbred cows. The endometritic repeat breeding animals were randomly allotted to five groups namely Groups A, B, C, D and E. Each group contained six animals. Extracts of Moringa and Tulsi were tested for In-vitro antibacterial activity against bacterial population in cervical mucus of repeat breeding crossbred cows. Estrual cervical mucus samples were collected before treatment (day of estrum) and tested for pH, white side test and bacterial load and again collected at subsequent estrus after treatment and the above-mentioned parameters were studied. Higher bacterial load was recorded prior to treatment which ranged from 212.83$\pm$*5.89 to 256.33* $\pm$ *3.07 x* 10^6*/ml in cervical mucus of endometritic repeat breeding cows. After treatment the mean bacterial load in group A, B, C, D and E recorded were* 243.5 ± 1.70 , 5.83 ± 1.14 , 11.83 ± 1.42 , 9.83 ± 0.95 , & 6.67 ± 1.09, *respectively. A marked & significant decline (p≤ 0.05) in bacterial load was recorded in all the groups from pre-treatment to post treatment except control group A. Bacterial load was significantly lowest (p≤ 0.05) in groups B followed by, group E, D, C.*

Key words: Anti-Bacterial Activity, Crossbred Cow, Moringa, Tulsi

How to cite: Kumar, S., Sinha, M., Barman, R., & Verma, R. (2019). The Effect of In-Vitro Antibacterial Activity of Moringa and Tulsi Leaves Extract against Endometritic Repeat Breeding Cross Bred Cow. International Journal of Livestock Research, 9(12), 112-123. doi: 10.5455/ijlr.20190622120757

DOI 10.5455/ijlr.20190622120757

Introduction

The reproductive efficiency of animals and productivity are complementary to each other and are essential for the expression of total production potential. There is need to optimize the reproduction so as to maintain the efficient production cycle. The biggest concern for us is to ensure and maintain the reproductive challenges of our cattle. Repeat breeding is one of the major causes (40%) of infertility in dairy cows (Bhosrekar, 1973), causing considerable economic losses to dairy farmers. Repeat breeding causes infertility in cattle that results in delayed conception and increased calving interval, loss of milk production, reduction in calf crop, increased cost of treatment and culling of useful breeding animals leading to heavy economic losses to the dairy producers. The reproductive system of animal is exposed to the external environment and thus highly prone to uterine infections like endometritis, metritis, pyometra, vaginitis and reproductive disorders like repeat breeding. Wide varieties of bacteria, both gram-positive and gram-negative, aerobes and anaerobes, have been isolated from bovine uterus post-partum. The bacterial metabolites and uterine inflammatory exudates produced from endometritic uterus alter the normal uterine environment required for fertilization, conception and birth of a live progeny. In this scenario, scrutinizing for some alternative yet effective antibacterial therapeutics like herbs, nutritional immunomodulators, bacteriophages, avian egged antibodies and others have become need of the day. Herbs being a valuable source of medication due to their important imicrobial principles, easy availability, cost effectiveness and wider therapeutic potentials may offer a potent alternative to antibiotic and hormonal therapy (Jadav and Butani, 2005).

Moringa oleifera is a woody tree mainly distributed in the tropical and subtropical regions of Asia, Africa and the Middle East provinces. It is widely used as a vegetable, functional food and medicinal plant that has rich nutritional composition with diverse pharmacological activities. Tulsi (holy basil) is a traditional plant considered as sacred by the Hindus. This religion links the plant with the Goddess figure as described in the puranas. The name Tulsi in Sanskrit means the 'incomparable one'. The Shyamatulsi or Krishna Tulsi (*Ocimum sanctum, synocimumtenuiflorum*) possess great medicinal value as mentioned in the *charak samhita,* an ancient Indian literature. It is a most common household plant in India and grows wild in tropics. Keeping in view the importance of cost effectiveness, minimum health hazards, negligible residual effects on animal produce and easy availability of the selected herbs the proposed study was planned with the effect of *in–vitro* antibacterial activity of Moringa & Tulsi leaves extract against endometritic repeat breeding cross bred cow.

Materials and Methods

The present study was conducted to see the effect of *in–vitro* antibacterial activity of Moringa & Tulsi leaves extract against endometritic repeat breeding cross bred cow at Military Dairy Farm, Namkum and

DOI 10.5455/ijlr.20190622120757

local farmers in and around Ranchi, under supervision of Department of Veterinary Gynaecology and Obstetrics, Ranchi Veterinary College**,** Birsa Agricultural University, Kanke, Ranchi (Jharkhand), India.

In the present study, the therapeutic efficacies of Moringa and Tulsi were assessed against endometritic repeat breeding crossbred cows. The endometritic repeat breeding animals were randomly allotted to five groups namely Groups A, B, C, D and E. Each group contained six animals. Extracts of Moringa and Tulsi were tested for *In-vitro* antibacterial activity against bacterial population in cervical mucus of repeat breeding in crossbred cows. Estrual cervical mucus samples were collected before treatment (day of estrum) and tested for pH, white side test and bacterial load and again collected at subsequent estrus after treatment and the above-mentioned parameters were studied.

Regimen of Treatment of Endometritic Repeat Breeding Crossbred Cows

Groups	Drugs to be used	Dose and Duration	Route of Administration
A (Control)	N. S	20 ml OD for 5 days	I/U
B	Cephapirin	500 mg cephapirin excipient to 19 gm suspension single dose	I/U
C	Diluted Tulsi extract in N.S	20 ml(6%w/v), OD for 5 days	I/U
D	Diluted Moringa extract in N.S	20ml(6%W/V), OD for 5 days	I/U
E	Diluted Moringa+ Tulsi extract in N.S	20 ml(6%w/v), OD for 5 days	I/U

Physio-Chemical and Microbial Studies on the Cervical Mucus

Collection of Estrual Cervical Mucus

Cervical mucus was collected twice, one before starting of treatment at the time of estrus and second time at the subsequent estrus following treatment by recto vaginal technique following the procedure with slight modification by Sharma (1981).

Determination of Bacterial Load

Bacterial load was estimated in estrual cervical mucus of all the cows collected at pre-treatment and post treatment subsequent estrus. Bacterial load in mucolysed cervical mucus was estimated by standard plate count method.

Calculation

Bacterial count (colony forming unit) per ml = (Average no. of colonies x Reciprocal of dilution factor x 0.1) ×10.

Studies on Uterine Flushing

Collection of Uterine Flushing

 DOI 10.5455/ijlr.20190622120757

Uterine flushing was collected twice i.e. one before giving the treatment at 12 hours after onset of estrus and second 24 hours after treatment following the procedure of Anderson *et al*. (1985). The collection technique involves aspiration of uterine flushing by Foley's catheter using the lavage.

Analysis of Uterine Flushing: Total Leukocyte Count (TLC)

Haemocytometer method was used for determination of TLC in uterine flushing (similar to blood).

Total Protein Estimation

Total protein concentration in the cell free uterine flushing was estimated with folin phenol reagent method using bovine serum albumin (BSA) as a standard.

Artificial Insemination and Pregnancy Diagnosis

Cows during subsequent standing estrus following treatment were inseminated twice, 12 hours apart. Cows which returned to heat after first insemination were again inseminated at second and third subsequent estrus after treatment, Pregnancy was confirmed per-rectally 45-60 days after insemination &Conception rate was calculated as below-

$$Conception\ rate = \frac{No.\ of\ cow\ conceived}{No.\ of\ cow\ inseminated} \times 100$$

Statistical Analysis

The data were analyzed statistically using Analysis of Variance (ANOVA) and Chi-square test as per the method described by Snedecor and Cochran, 2004.

Results and Discussion

The present study was conducted to see theeffect of *In–vitro* antibacterial activity of Moringa & Tulsi leaves extract against endometritic repeat breeding in cross bred cow at Military dairy farm, Namkum and local farmers in and around Ranchi, under supervision of Department of Veterinary Gynaecology and Obstetrics, Ranchi Veterinary College**,** Birsa Agricultural University, Kanke, Ranchi (Jharkhand). *In-vitro* antibacterial activity of herbal extracts either alone or in combination were determined by Disc diffusion method against mixed bacterial population in cervical mucus of repeat breeding cross bred cows.

A total of thirty endometritic repeat breeding cross bred cows were selected on the basis of history and breeding records, per-rectal examination and white side test of cervical mucus. These selected animals were randomly allotted to five groups (A, B, C, D & E) containing six animals in each group. Cows of group B received 500mg cephapirin, excipient to 19 gram suspension, single dose, group C received 20ml of Tulsi extracts, group D received 20 ml of Moringa extracts, group E received 20 ml of Moringa+Tulsi extracts in equal proportion. The cows of group A were administered with 20 ml normal saline and served as control.

 DOI 10.5455/ijlr.20190622120757

All the herbal extracts, antibiotic and normal saline were administered through intra- uterine route (I/U) and started from day of estrus to five consecutive days.

Cervical mucus samples of repeat breeding cross bred cows were collected aseptically on the day of estrus before treatment and tested for pH, white side test and bacterial load for initial diagnosis. They were also collected at subsequent estrus after treatment and the same parameters were studied for assessment of clinical recovery. Uterine flushing was also collected twice, first at 0 hour (day of estrum before treatment) and second at 24 hours after the treatment. These flushing's were used for estimation of total leukocytes, polymorphonuclear neutrophil, total protein and total immunoglobulin concentration. All the animals were inseminated using frozen semen at subsequent estrum post treatment. Pregnancy was confirmed at 45-60 days of insemination by trans rectal examination. The data so generated were analyzed statistically and tabulated.

In-vitro Antibacterial Activities of Herbal Extracts and Antibiotic

In-vitro antibacterial activities of herbal extracts (at 6%w/v) were tested and compared with antibacterial activity of antibiotic (Cephapirin) before the start of the experiment. Results showed that hydro-alcholic extract of Moringa was better antibacterial in nature whereas Tulsi extract also had good antibacterial activity. The average size of zone of inhibition of Tulsi, Moringa and Tulsi+Moringa were 12.25±1.01 mm, 15.66±0.62 mm and 17.75±0.62 mm, respectively. This finding is in agreement with the finding of Rahman *et al*. (2009), Bukar *et al*. (2010) and Onuoha and Alisa (2013).Studies have shown that the antimicrobial potential of *M. oleifera* leaf extract may be attributed to the presence of an array of phytochemicals such as alkaloids, tannins, flavonoids, glycerides, steroids, terpenoidssaponins and anthraquinone in its leaves (Sinha, 2012).This would indicate that the antibacterial activities observed in the present study could be attributed to such compounds. The presence of alkaloids such as moringine, moringinine and spirachin, as well as pterygospermin have been shown to demonstrate antibacterial activity. The presence of a short polypeptide named 4-(alpha-L- rhamnosyloxy) benzyl isothiocynate was investigated by Guevara *et al*. (1999) in moringa leaves and they argued that the peptide might act directly on microbes and result in growth inhibition by disrupting cell membrane synthesis, a mechanism of action similar to Beta Lactam and Cephalosporin antibiotics, or inhibition of the synthesis of essential enzymes (Suarez *et al*., 2003 and Bukar *et al*., 2010). Flavonoids are strong antibacterial substances in–vitro against a large number of microorganism by inhibition of the membrane bound enzymes (Pretorious, 2003). Hydroalcholic extract of Tulsi was also found to have antibacterial activity as it produced zone of inhibition against most of the bacteria present in the cervical mucus of endometritic repeat breeding cows. This finding is in accordance with the findings of Prakash and Gupta (2005); Goyal *et al.* (2011); Mishra and Mishra (2011); Jothi

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Karumari *et al.* (2014) and Eswer *et al*. (2016). Moringa and tulsi in combination gave synergistic effect with maximum size of zone of inhibition. This combination was almost equivalent to cephapirin.

Physico-Chemical Studies on Estrual Cervical Mucus

Physio-chemical studies on estrual cervical mucus showed following results.

pH of Cervical Mucus

The mean pH values of estrual cervical mucus collected from endometritic repeat breeding cross bred cows pre and post treatment in all the five groups. The mean pH values of estrual mucus before the treatment were 8.16±0.211, 8.41±0.154,7.83±0.333,8.08±0.239 & 8.25±0.214 in group A-E respectively. The mean pH value after treatment were 7.91±0.154, 7.16±0.167, 7.30±0.03, 7.28±0.183, 7.22±0.112 respectively. The mean pH value showed a significant (P≤0.05) decline towards normal in all the groups except control group A from pre-treatment to post-treatment. The pre-treatment pH of the cervical mucus from Groups A-E showed non-significant difference among themselves Similarly, the post-treatment pH of estrual mucus from the Groups A-E also showed non-significant difference among themselves except control group A. (Table 1). Number of workers found that higher pH of cervical mucus towards alkaline side is associated with uterine infection (Sathesh Kumar and Punniamurthy, 2007; Dodamani *et al*., 2010 and Sarma *et al*., 2010). After treatment with herbal extract of moringa, tulsi, Moringa+Tulsi and cephapirine, the reduction in pH in different groups was observed which ranged between 7.16 to 7.30.

Table 1: pH (Mean ± SE) in cervical mucus of endometritic repeat breeding cross bred cows of different groups before and after treatment

Groups	Pre treatment	Post treatment	t value
A	8.16±0.211	7.91^{a}±0.154	0.95^{NS}
B	8.41±0.154	7.16^{b}±0.167	5.51**
C	7.83±0.333	7.30^{b}±0.03	2.38*
D	8.08±0.239	7.28^{b}±0.183	2.65*
E	8.25±0.214	7.22^{b}±0.112	3.38**

*Means bearing different superscripts (within the column) varies significantly between the groups; **p≤0.01, *p≤0.05 between pre and post treatment.*

Reduction in pH after treatment might be due to the decline in bacterial load and inflammatory process in uterus which is attributed due to antibacterial property of herbal extract of Moringa (Rahman *et al*., 2009; Bukar *et al*., 2010 and Onuoha and Alisa, 2013) and Tulsi (Prakash and Gupta, 2005; Goyal *et al*., 2011; Jothi Karumari *et al*., 2014 and Eswer *et al*. (2016). In control group there was also reduction in pH which might be due to natural uterine defense mechanism. This finding was in close approximation with the finding of Sharma *et al.* (2010), Rahi *et al.* (2013) and Kumar *et al*. (2017).

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White Side Test

White side test was performed on the estrual cervical mucus collected from all the 30 animals, before and after treatment. All the cows were positive for white side test before treatment. However, after treatment, 83.33 % cows in group A, 33.3% in group C,16.66% cows in groups B, D & E were found positive to white side test (Table 2).

Chi-square analysis of the percentage of white side positive samples revealed that the effect of treatment in groups B, D & E were different from the groups A & C. Although the overall chi square value was non-significant but minimum white side positive cases were obtained in group B, D & E followed by group C & group A. The positive result of white side test could be explained on the basis of number of leucocytes & cellular debris present in the cervical mucus. The normal cervical mucus has less number of leucocytes which doesn't cause any change of colour. Whereas, in clinical or sub clinical endometritis the cervical mucus contains increased number of leucocytes which causes colour reaction (Deori, 2002). The reason for colour reaction might be due to ribonucleic acid present inside the nucleus of white blood cells which reacts with 5% NaoH to produce Yellow colour (Gupta *et al.*, 2011).

Table 2: White side test on estrual cervical mucus in endometritic repeat breeding crossbred cows of different groups before and after treatment

Groups	Positive for white side test before treatment	Positive for white side test after treatment
A	100%	83.33%
B	100%	16.60%
C	100%	33.30%
D	100%	16.60%
E	100%	16.60%

Chi Square (at P= 0.025) =3.7^{NS}

After treatment with herbal extract of Moringa, Tulsi alone or in combination maximum no of cervical mucus samples showed absence of colour reaction. This showed the antibacterial property of these herbal extracts which caused reduction in bacterial load and inflammatory processes (Bukar *et al.*, 2010; Onuoha and Alisa, 2013; Goyal *et al.*, 2011; Jothi Karumari *et al.*, 2014 and Eswer *et al*. (2016).

Bacterial Load

Higher bacterial load was observed prior to treatment in all the groups which ranged from 212.83 ± 5.89 to $256.33 \pm 3.07 \times 10^6$/ml. The present findings were in accordance with the finding of Singh *et al*. (1993), Mahto *et al.* (2012). However, a higher range of bacterial load was reported by Sarma *et al.* (2010) who observed the bacterial load in the range of 264.70 $\pm$17.23 x 10^6/ ml to 322.0 ±10.85 x 10^6/ml of uterine discharge in endometritis cases. In contrast to this a lower range of bacterial load in the range between

 DOI 10.5455/ijlr.20190622120757

72.84 ± 0.97 & 82.93 ±0.91 x 10^6/ml of cervical mucus in cows affected with endomteritis was reported by Palanisamy *et al*. (2015).

A marked reduction in bacterial load was observed in all the groups from pre-treatment to post treatment. Bacterial load was lowest in cephapirin treated group B followed by herbal extract group E, D, C & A. The result of the *In-vitro* antibacterial activities of herbal extracts showed that hydroalcoholic extract of Moringa was better antibacterial in nature where as Tulsi extract also has good antibacterial activity. These extracts reduced bacterial load by direct action on bacteria present in uterus and/ or increasing neutrophils count in the uterine lumen. Due to increased neutrophil count, rate of phagocytosis might have increased resulting in reduction in viable bacterial count (Sarkar *et al*., 2006). The immunotherapeutic properties of Moringa & Tulsi also helps in reduction of bacterial load through nonspecific host responses parameters like morphological alteration, phagocytosis and intra cellular killing capacity of macrophages (Gupta *et al*., 2010; Kumar *et al*., 2013; Akhouri *et al*., 2014). Reduction in bacterial load in control group may be due to natural defense mechanism as well as uterine flushing might have also reduced bacterial load.

Study on Uterine Flushing

Studies on uterine flushing revealed following results-

Total Leukocyte Count, 10^6/ml

The mean TLC values in uterine flushing of endometritic repeat breeding cross bred cows have been presented in Table 3. Before treatment mean TLC values of all groups (A-E) were, 0.44 ± 0.01 , 0.53 ± 0.02 , 0.49 ± 0.01 , 0.46 ± 0.01 & 0.51 ± 0.01 respectively. After treatment the mean TLC values recorded were 0.49 ± 0.02 , 0.18 ± 0.02 , 3.02 ± 0.14 , 3.23 ± 0.05 & 3.51 ± 0.03 in groups A, B, C, D & E respectively.

The mean TLC values revealed a significant increase ($P \leq 0.05$) in all the herbal extract treated groups (C-E) from pre-treatment to post-treatment while it significantly decreased in antibiotic treated group B. The pre-treatment mean TLC values of the groups A-E showed non-significant difference among themselves except group A & B. The post-treatment mean TLC values of the groups A-E showed significant difference ($P \leq 0.05$) among themselves except group C & D. Mean TLC values of the herbal extract treated groups C-E were significantly higher ($P \leq 0.05$) as compared to control group A. On contrary the mean TLC value in antibiotic treated group B was significantly lower ($P \leq 0.05$) than group A (Table 3). This increase in intrauterine population and oxidative burst activity of neutrophils favours the spontaneous resolution of uterine infection (Mateus *et al*., 2002).

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Table 3: TLC (10^6/ml) in uterine flushing of endometritic repeat breeding cross bred cows of different groups before and after treatment

Groups	Pre treatment	Post treatment	t value
A	0.44a±0.01	0.49^{b}±0.02	1.45^{NS}
B	0.53c±0.02	0.18^{a}±0.02	8.90**
C	0.49abc±0.01	3.02^{c}±0.14	17.58**
D	0.46ab±0.01	3.23^{c}±0.05	48.43**
E	0.51bc±0.01	3.51^{d}±0.03	74.07**

*Means bearing different superscripts varies significantly (within column) between the groups. **P≤0.01 between pre and post treatment*

On contrary a significant decline in mean TLC and PMNs value in antibiotic treated group B than control group A was observed in the present study. Antibiotic lowers uterine defence mechanism by inhibition or destruction of phagocytic activity of PMNs. Infusion of antibiotic might have killed bacteria effectively with reduction in bacterial population, inflammatory response would have declined resulting in simultaneous reduction in PMNs flow towards uterine lumen and decline in TLC (Shaktawat, 2005).

Total Protein Concentration (mg/dl) in Uterine Flushing

Before treatment the mean total protein in the uterine flushing of endometritic repeat breeding, cows ranged between 150.78 ± 5.35 to 158.13 ± 2.77. The mean total protein values showed a significant (P≤0.05) increase in all the herbal extract treated groups(C-E) from pre to post treatment while it significantly decreased in antibiotic treated group (B). The mean total protein values of the herbal extract treated group C-E were significantly higher as compared to control group A which might be due to stimulation of uterine immune responses after herbal extract treatment. The present findings are in accordance with the findings of Kumar *et al*. (2011). Kumar *et al*. (2013a) reported that uterine flushing parameter such as TLC, PMNs Protein and total immunoglobulins increased after treatment with hydro-alcoholic and hydro-acetonic extract of neem indicating a positive effect on the uterine defense mechanism. The influx of total protein into uterus following infection or inflammation might be due to transduction of inflammatory exudates resulting from inflammatory response to cytokines. The increase in total protein might also be due to increased level of secretory protein, cellular debris and tissue damage. The herbal extract which acts as a chemo attractant might have produced vasodilation and extravasation of fluid, protein and serum to give higher protein value in uterine flushing at post treatment estimation as has also been suggested.

A significant decline (P≤0.05) in mean total protein concentration was observed after antibiotic treatment, this might be due to effective killing of bacteria as a result inflammatory response in uterus might have declined and so the protein influx towards the uterine lumen had reduced (Prasad, 2006). In control group after infusion of NS, non-significant rise in mean total protein concentration was achieved, this might be due to natural uterine defense mechanism.

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Assessment of Clinical Recovery and Conception Rate (%)

The efficacy of treatment by herbal extracts & antibiotic were assessed on the basis of recovery rate and subsequent conception following insemination. Table 4 revealed that after treatment by Moringa and Tulsi extract alone or in combination in different groups, maximum recovery and conception rate as 83.33% & 66.67% respectively were achieved in combined herbal extract treated group E and Moringa treated group D, followed by 66.67% & 50% in Tulsi treated group C & lowest in control group A (16.66 & 0%). Better recovery & conception rate observed in combined herbal extract treated group might be due to their synergistic effect, combined antibacterial & good immunomodulatory properties. Higher recovery rate in Moringa & Tulsi treated groups C & D might be due to significant reduction in bacterial load (Rahman *et al*., 2009; Bukar *et al*., 2010 and Onuoha and Alisa,2013; Prakash and Gupta, 2005; Goyal *et al.*, 2011; Jothi Karumari *et al*., 2014 and Eswer *et al*., 2016).

Table 4: Recovery rate (%) and conception rate (%) in different groups of endometritic repeat breeding crossbred cows after treatment

Groups	Recovery Rate	Conception Rate
A	16.66%	0%[a]
B	83.33%	83. 33%[b]
C	66.67%	50%[ab]
D	83.33%	66.67%[b]
E	83.33%	66.67%[b]
Chi square (at p=0.025)	9.00[NS]	13.93*

Means bearing different superscripts varies significantly (within column) between the groups

In the present study highest recovery and conception rate (83.33%) was recorded in antibiotic (cephapirine) treated group B. This might be due to efficient reduction in bacterial population as compared to herbal extracts groups. This finding is in close approximation with the finding of Ahmadi *et al*., 2005.

Conclusion

On the basis of the result obtained during the present study, it is concluded that the hydroalcholic extract of Tulsi & Moringa in combination (6%w/v) may be recommended as alternative treatment for endometritic repeat breeding cross bred cows over conventional antibiotic therapy.

Acknowledgement

Authors are thankful to R.V.C., Birsa Agricultural University, Ranchi, Jharkhand, India

References

1. Anderson, K.L., Hemeida, N.A., Farik, A., Whitemore, H.L. and Gustafsson, B.K. (1985). Collection and phagocytic evaluation of uterine neutrophillic leukocytes. *Theriogenology*, 24 (3): 305-315.
2. Ahmadi, M.R., Nazifi, S. and Ghaisari, H.R.(2005). The Effect of Intrauterine Cephapirin on Treatment of Endometritis in Commercial Dairy Cattle. *Arch. Razi Ins.59:35-45*

DOI 10.5455/ijlr.20190622120757

3. Bhosrekar, M.(1973). Investigation into the incidence and causes of repeat breeding in dairy cattle at National Dairy Research Institute, Karnal Haryana. *Indian Vet. J.*, 50: 418-429.
4. Bukar, A., Uba, A. and Oyeyi, T.I. (2010). Antimicrobial profile of *Moringaoleifera*extracts against some food-borne microorganisms. *BayeroJ.Pure and Appl. Sci.*, 3(1): 43-48.
5. Deori, S. (2002). Use of bacterial modulins in treatment of endometritis in cows. M.V.Sc. Thesis, IVRI, Izatnagar
6. Dodamani, M.S., Mohteshamuddin, K., Awati, S.D., Tandle, M.K. and Honnappagol, S.S.(2010). Evaluation of Pre and Post Artificial Insemination effect of GnRH Hormone on conception of repeat breeder Deoni Cows. *Vet. World*.,3(5): 209-211.
7. Eswar, P., Devaraj, C.G., Agarwal,.P.(2016). Anti-microbial Activity of Tulsi {*Ocimum Sanctum (Linn.)*} Extract on a Periodontal Pathogen in Human Dental Plaque: An Invitro Study. *J. Clinic. Dia. Res*., 10(3): ZC53-ZC56.
8. Goyal, P., and Kaushik, P., (2011). In vitro Evaluation of Antibacterial Activity of Various Crude Leaf Extracts of Indian Sacred Plant, *Ocimum sanctum* L. *British Microbiol.Res.J*.,1(3): 70-78.
9. 19. Guevara, A.P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuku, M., Ito, Y., Tokuda, H. and Nishino, H.(1999). An antitumour promoter from *Moringaoleifera*Lam. *Mutation Res.*, 440: 181-188
10. Gupta, A., Gautam, M.K., Singh, R.K., Kumar, M.K., Rao, C.V., Goel, R.K. and Anupurba, S.(2010). Immunomodulatory effect of *Moringaoleifera*Lam extract on cyclophosphamide induced toxicity in mice. *Indian J.Exp. Biol.*, 48: 1157-1160.
11. Gupta, J.P., Shyma, K.P., Mohandas, A.C. and Sneharaj, R.K. (2011). White side test to study subclinical endometritis in crossbred cattles. *Bhartiya Krishi Anushandhan Patrika*. 26 (3&4): 123-125.
12. Jadav, A. N. and Bhutani, K.K.(2005). Ayurveda and gynaecoogical disorders. *J. Ethnopharmacol*., 97: 151-159.
13. JothiKarumari, R., Vijayalakshmi, K., Tamilarasi, L and Ezhilarasi, B.(2014).Antibacterial Activity of Leaf Extracts of *Aloe Vera*, *Ocimum Sanctum* and *Sesbania Grandiflora* against the Gram Positive Bacteria. *Asian J.Biomedical and Pharmaceu. Sci*., 4(35):60-63
14. Kumar, A.(2013). Evaluation of immunomodulatory and therapeutic efficacy of turmeric*(curcuma longa)*,neem(*Azadirachtaindica*) and garlic(*Allium sativum*) on endometritis in repeat breeding cross breed cows.M.V.Sc. Thesis,G.B Pant University of Agriculture and Technology, Pantnagar.
15. Kumar, A., Gupta, H.P. and Prasad, S.(2013a) Studies on the immunomodulatory and therapeutic efficacy of Neem *(Azadirachtaindica)* on endometritis in repeat breeding cross bred cows *Indian J. Anim. Reprod.*, 34(2): 1-5.
16. Kumar,R., Sinha,M.P., Roy,B.K.,Prasad, A. And Ishwar, A.K.(2017). Effect of herbal extract therapy on physiochemical and microbiological studies of estrual cervical mucus of endometritic repeat breeding cross bred cows. *Progressive Res- An Int. J.*,12(1):45-47
17. Kumar,S., Gupta, H.P., Prasad, S., and Singh, B.(2011). Immunomodulatory effects of garlic and tulsi in repeat breeding cross bred cows. *Indian J .Anim.Reprod*.,32 (1).
18. Mahto, D., Sinha, M.P., Singh, B. and Jha, D.K.(2012). Effect of systemic and intrauterine administration of drug on endometritis in cow. *Indian J. Anim. Reprod.*, 33 (1). 51-53.
19. Mateus, L., Costa, L.L., DaCarvalho, H., Serra, P. and Sivav, J.R.(2002). Blood and intrauterine leucocyte profie and function in dairy cows that spontaneously recovered from post- partum endometritis. *Reprod. Domest. Anim*., 37: 176-180.
20. Mishra, P. and Mishra,S.(2011). Study of Antibacterial Activity of Ocimum Sanctum Extract Against Gram Positive and Gram Negative Bacteria.*American J. Food Tech.*,6(4) :336-341.
21. Nair, S. and Kharche, K.G.(1988). Study of calcium, chloride and magnesium in cervicovaginal mucus of normal and repeat breeder crossbred cows. *Indian J. Dairy Sci.*, 41: 491-492.
22. Onuoha, S.C. and Alisa, C.O.(2013). Antimicrobial potential of leaf juice and extracts of *Moringaoleifer a*lam against some human pathogenic bacteria. *J. Pharmacy Biol. Sci.*, 5(4): 37-42.

DOI 10.5455/ijlr.20190622120757

23. Palanisamy, M., Ezakial, N, R., Selvaraju, M., Krishnakumar, K., Malmarugan, S., Balasubramaniam, G, A., and Manokaran, S.(2015). Effect of intrauterine infusion of immunomodulators on cytology of uterus in endometritis affected cows. *Indian Vet. J*., 92(12): 31-34.
24. Prakash, P. and Gupta, N.(2005).Therapeutic uses of *Ocimum sanctum* Linn with a note on Eugenol and its pharmacological action: A Short Review. *Indian J. Physiol. Pharmacol*., 49(2):125-131.
25. Prakash, P. and Gupta, N.(2005).Therapeutic uses of ocimum sanctum Linn with a note on Eugenol and its pharmacological action: A Short Review. *Indian J. Physiol. Pharmacol*., 49(2):125-131.
26. Prasad, J. K. (2006). Effect of immunomodulators endometritis in crossbred cows. Ph.D. Thesis, G. B. Pant University of Agriculture and Technology, Pantnagar.
27. Pretorious, J. C. (2003). Antiinfective agents, *Current Medicinal Chem*.,2:335-353.
28. Rahi, S., Gupta, H.P., Prasad, S. and Baithalu, R.K.(2013). Phytotherapy for endometritis and subsequent conception rate in repeat breeding crossbred cows. *Indian J. Anim. Reprod.*, 34 (1): 9-12.
29. Rahman, M.M., Sheikh, M.M.I., Sharmin, S.A., Islam, M.S., Rahman, M.A., Rahman, M.M. and Alam, M.F.(2009). Antibacterial activity of leaf juice and extracts of *Moringaoleifera*Lam against some human pathogenic bacteria. *Chiang Mai Uni. J. Nat. Sci.*, 8(2): 219-227.
30. Sarkar, P.,Kumar, H., Rawat, M., Varshney, V.P., Goswarni, T.K., Yadav, M.C. and Srivastava, S.K.(2006). Effect of administration of garlic extract and PGF2α on hormonal change and recovery in endometritis cow. *Asian Australian J. Anim. Sci.*, 19(7): 964-969.
31. Sarma, O.K., Singh, B., Singh, M.P., Tiwary, B.K. and Sinha, M. P. (2010). Efficacy of immunomodulators for treatment of endometritis in cows. *Indian J. Anim. Repord*.,31: 59.
32. Sathesh Kumar, S. and Punniamurthy, N. (2007). Subclinical uterine infections in repeat breeder cows. *Indian Vet. J*., 84: 654-655.
33. Shaktawat, J. S. (2005). Therapeutic use of E. coli lipopolysaccharide in endometritis in cross bred cattle. M.V.Sc. Thesis, G. B. Pant University of Agriculture and Technology, Pantnagar.
34. Sharma, R. N. (1981). Studies on some aspects of repeat breeding in cross bred cattle. M.V.Sc. Thesis, submitted to BAU, Kanke, Ranchi
35. Singh, V.I., Singh, G., Dwivedi, P.N. and Sharma, R. D. (1993). *In- vitro* valuation of drug sensitivity patterns of bacterial isolates in repeat breeder cows. *Indian J. Anim. Sci.*, 63(4): 425-426.
36. Sinha,S.N.(2012).Phytochemical analysis and antibacterial potential of moringaoleifera lam *Int. J.Sci. Inno. Disc*.,2(4):401-407.
37. Snedecor, G.W. and Cochran, W.G.(2004). Statistical method 8th edn. Oxford and IBH Publishing Co.,Kolkata.
38. Suarez, M., Entenza, J.M., Doerries, C., Meyer, E., Bourquin, L., Sutherland, J., Marison, I., Moreillon, P. and Mermod, N. (2003). Expression of a plant-derived peptide harbouring water cleaning and antimicrobial activities. *Biotech. And Bioeng.*, 81: 13-20.
39. Sharma, S., Sharma, H., Dhami, A.J. and Bhong, C. D. (2010). Physico-microbial properties of cervico-vaginal mucus and its antibiotic sensitivity pattern in repeat breeding buffaloes. *Indian J. Anim. Reprod.*, 29: 19-26.

DOI 10.5455/ijlr.20190622120757