

# STUDIES ON THE IMMUNOMODULATORY AND THERAPEUTIC EFFICACY OF NEEM (*AZADIRACHTA INDICA*) ON ENDOMETRITIS IN REPEAT BREEDING CROSSBRED COWS\*

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ABSTRACT

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An experiment was designed to investigate the immunomodulatory and therapeutic efficacy of neem on endometritis in repeat breeding crossbred cows. Twenty four cows were selected on the basis of history, breeding records, transrectal examination, white side test and randomly divided into 3 groups (Group A: 30 ml normal saline; Group B: 30 ml hydro-alcoholic neem bark; Group C: 30 ml hydro-acetonic neem bark). Treatments were given intrauterine beginning on the day of estrus for seven days in each group. Cervical mucus samples were collected on the day of estrus before treatment and at subsequent estrus after treatment and tested for appearance, pH, white side test and bacterial load. Blood samples were collected on the day of treatment and 24 hr after treatment and analyzed for glucose, Hb, PCV, TLC and DLC to know health status of the experimental animals. Uterine flushings were collected on the day of estrus before treatment and again on eighth day of first collection i.e. 24 hr after last treatment. These flushings were used for the estimation of total protein, immunoglobulin, TLC and polymorphonuclear cells (PMNs). At subsequent standing estrus following treatment, all cows were artificially inseminated twice using frozen semen 12 hours apart. Pregnancy was confirmed transrectally 45-60 days after insemination. Significant ( $p < 0.05$ ) decline in pH and bacterial load was observed in cervical mucus of the groups after treatment. Hemoglobin, neutrophils, lymphocytes and WBC were increased significantly ( $p < 0.05$ ) in both hydro-alcoholic and hydro-acetonic extract treated groups. Moreover, RBCs and glucose were increased in hydro-alcoholic extract treated group. Significant ( $p < 0.05$ ) rise was found in TLC, PMN and immunoglobulin concentration in both the treated groups. Although both the extracts led to improved clinical recovery and conception rates, the hydro-alcoholic extract was more effective. Based on these results, it may be concluded that hydro-alcoholic extract of the neem has a better antibacterial and immuno-modulation and can be used as a therapy for endometritis in repeat breeding crossbred cows.

**Keywords:** Crossbred cows, Endometritis, Neem (*Azadirachta indica*), Repeat breeder

## INTRODUCTION

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 to exhibit immunomodulatory, anti-

inflammatory, antifungal, antibacterial, antiviral, and antioxidant properties (Subapriya and Nagini, 2005).

Vandernat *et al.* (1986) reported that aqueous extract of the neem bark increases the production of lymphokine MIF (migration inhibitory factor), which through cellular immunity helps in skin wound healing and it possesses anti-complement activity, which correlates with its anti-inflammatory effect claimed in traditional system of medicine.

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Repeat breeding is a substantial problem in cattle breeding leading to large economic loss to the dairy producer due to more inseminations, increased calving interval and culling rates (Roberts, 1971). Incidence of repeat breeding in crossbred cows has been reported to vary from 7.4 to 21.9% (Singh *et al.*, 1983). The incidence was higher during second parity, in high milk yielders and after abnormal calving.

Bacterial infections are considered to be one of the main causes of repeat breeding in cattle (Baishya *et al.*, 1998). Therefore, the current study was designed to investigate the immunomodulatory and therapeutic efficacy of the neem on endometritis in repeat breeding crossbred cows.

### MATERIALS AND METHODS

Twenty four repeat breeding crossbred cows were selected on the basis of history of repeat breeding, transrectal examination, positive white side test and randomly divided into 3 groups (Group A: 30 ml normal saline; Group B: 30 ml hydro-alcoholic neem bark; Group C: 30 ml hydro-acetonic neem bark). Treatments were given intrauterine beginning on the day of estrus for seven days in each group. Cervical mucus samples were collected on the day of estrus before treatment and at subsequent estrus after treatment and tested for appearance, pH, white side test and bacterial load. Blood samples were collected on the day of treatment and 24 hr after treatment and analyzed for glucose, haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC) to know health status of the experimental animals. Uterine flushings were collected on the day of estrus before treatment and again on eight day of first collection i.e. 24 hr after last treatment. These flushings were used for the estimation of total protein, immunoglobulin, TLC and polymorphonuclear cells (PMNs). At subsequent standing estrus following treatment, all cows were artificially inseminated twice using deep frozen semen 12 hours apart. Pregnancy was confirmed transrectally 45-60 days after insemination. The data were analyzed statistically using Analysis of Variance (ANOVA), paired *t*-test and Chi-square test (Snedecor and Cochran, 1989).

### RESULTS AND DISCUSSION

The final per cent yield (w/w) and maximum size of zone of inhibition of hydro-alcoholic and hydro-acetonic extracts of the neem bark were 8.00% and 17mm, and 10.50% and 15 mm, respectively. The minimum inhibitory concentration (MIC) values against bacterial population in cervical mucus of repeat breeding cows suffering from endometritis were 3.00-6.50 mg/ml, and 6.00 mg/ml, respectively. The reported values of MIC for hydro-alcoholic extracts of neem are 4.50-6.50 mg/ml (Mahfuzul-Hoque *et al.*, 2007) and 2.50 mg/ml (Rathod *et al.*, 2012), while the corresponding value of hydro-acetonic extract was 6.84 mg/ml (Victor and Igeleke, 2012). The percentages of animals showing clear cervical mucus discharge before and after treatment in groups A, B, and C were 50.00% and 62.50%, 75.00% and 87.50%, and 62.50% and 75.00%, respectively. Clear estrual cervical mucus is conducive for sperm penetration and conception, whereas, turbidity retards sperm motility in estrual mucus (Dev *et al.*, 1997).

There was a significant ( $P < 0.05$ ) decline in the pH of cervical mucus before and after treatment in all the groups ( $7.77 \pm 0.22$  and  $7.68 \pm 0.16$  in Group A,  $7.93 \pm 0.14$  and  $7.37 \pm 0.12$  in Group B, and  $7.62 \pm 0.18$  and  $7.31 \pm 0.09$  in Group C, respectively). The pH of estrual cervical mucus in all the groups before treatment was alkaline (more than 7.6), thus, indicating uterine infection (Singh *et al.*, 2004). This reduction in pH may be due to decline in bacterial load and inflammatory process in uterus after treatment (Shaktawat, 2005).

The treatment with hydro-alcoholic and hydro-acetonic extracts of the neem led to a significant ( $P < 0.05$ ) reduction in the proportion of white side positive animals from 100% to 25% and 50%, respectively as compared to the control (87.5%). This result showed that the neem is antibacterial and immunomodulatory in nature and thus, reduced bacterial load and subsequently inflammation process (Owis *et al.*, 2005).

A significant decline ( $p < 0.05$ ) in bacterial load was observed in all the groups from pre treatment to post treatment ( $301.29 \pm 0.92$  and  $282.38 \pm 1.03$  in Group

A,  $272.18 \pm 1.55$  and  $1.02 \pm 0.02$  in Group B, and  $270.65 \pm 0.83$  and  $1.29 \pm 0.11$  in Group C). After treatment, bacterial loads in Groups B and C were significantly lower ( $p < 0.05$ ) as compared to control group A. In comparison to the present study on bacterial load, a higher range was reported by Singh *et al.* (1993) with a range of  $45.8 \times 10^6$  to  $47.28 \times 10^7$  per ml of cervical mucus in endometritic cases. However, Goswami *et al.* (1992) reported a lower range of bacterial load of  $35.05 \times 10^3$  per ml in cervical mucus of repeat breeding cows. The severe reduction in bacterial load observed after treatment with the neem indicates its potent antibacterial action while the mild reduction in the control group could be attributed to natural uterine defense mechanisms.

A significant increase ( $P < 0.05$ ) in the mean values of Hb, neutrophils, lymphocytes and WBC before and after treatment was observed in both hydro-alcoholic and hydro-acetonic extract treated animals. Moreover, an increase ( $P < 0.05$ ) in RBC and glucose was noticed in the hydro-alcoholic group. In contrast, a decrease ( $P < 0.05$ ) in Hb and WBC was recorded in the control group after treatment. The mean Hb and PCV values before treatment in the repeat breeding cows selected for the study were lower than the values found in normal cyclic fertile cows. This finding is in agreement with Sharma *et al.* (1983). Similarly, Roberts (1971) reported that anemia (reduced Hb) in repeat breeding cows may be associated with reproductive disorders. Thrall (2004) reported that in inflammatory disease, erythropoietin is diminished presumably because of inflammatory cytokines leading to lowered erythropoiesis and ultimately lowered PCV in blood. The neem has been found to increase Hb content in cattle (Amin *et al.*, 2008). The significant increase in neutrophil, lymphocyte and WBC in the neem treated groups suggests an effective immunomodulatory role of the neem. In hydro-alcoholic extract treated groups; blood glucose values became

normal indicating that the treatment improved the general body condition also.

Uterine flushing parameters (TLC, PMNs, Protein and Total Immunoglobulin) increased after treatment with both hydro-alcoholic and hydro-acetonic extracts of neem indicating a positive effect on the uterine defense mechanism. This increased intrauterine populations and oxidative burst activity of neutrophils favours the spontaneous resolution of uterine infection (Mateus *et al.*, 2002). Neutrophils are known to play a primary role in the defense of the uterus against infection. Influx of neutrophils into the uterus is thought to be mediated by chemoattractants, chemokines and adhesion molecules, such as  $\alpha 2$ -integrin and I-selectin (Tizard, 2000). Several workers have demonstrated a marked increase in protein concentration and PMNs infiltration in uterine flushings following uterine contamination (Strezemienski and Kenney, 1984). It is reasonable to assume that this response may play an important role in controlling bacterial infection introduced at coitus or parturition.

The cows were considered to have recovered from endometritis at subsequent estrus after treatment on the basis of appearance of clean estrual cervical mucus, reduction in bacterial load and negative white side test. The clinical recovery and conception rates in Groups A, B, and C were 25.00% and 0.00%, 87.50 and 62.50%, and 75.00% and 50.00%, respectively. Therefore, hydro-alcoholic extract of the neem yielded the best results in terms of clinical recovery and conception rates. Therefore, it may be concluded that the hydro-alcoholic and hydro-acetonic extracts of the neem have potent immuno-modulatory and therapeutic efficacy on endometritis in repeat breeding crossbred cows. However, hydro-alcoholic extract gave better results in terms of clinical recovery and conception rates.

## REFERENCES

- Amin, M.R., Mostofa, M., Awal, M.A. and Hossain, A. (2008). Effects of neem (*Azadirachta indica*) leaves against gastrointestinal nematodes in cattle. *J. Bangladesh Agric. Univ.*, **6**(1): 87-99.
- Baisya, S.K., Das, K.K., Rahman, H. and Borgohain, B.N. (1998). Antibiogram of bacterial isolates from uterine discharge of repeat breeding cattle. *Indian J. Comp. Microb. Immunol.*, **19**(2): 130-131.
- Bandyopadhyay, U., Biswas, K., Chatterjee, R., Bandyopadhyay, D., Chattopadhyay, I., Ganguly, C.K., Chakraborty, T., Bhattacharya, K. and Banerjee, R.K. (2002). 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 Sci.*, **71**: 2845-2865.
- Dev, S., Pangawkar, G.R., Sharma, R.K. and Verma, H.K. (1997). Sperm penetration in relation to physical characteristics of buffalo estrual mucus. *Int. J. Anim. Sci.*, **12**: 89-91.
- Goswami, I.C., Kher, H.N., Jhala, M.K. and Derashri, H.J. (1992). Quantative and qualitative studies on the genital bacteria of buffaloes. *Indian J. Anim. Reprod.*, **13**(12): 180-182.
- Mahfuzul-Hoque, M.D., Bari, M.L., Inatsu, Y., Juneja, V.K. and Kawamoto, S. (2007). Antibacterial activity of Guava (*Psidium guajava* L.) and Neem (*Azadirachta indica* A. Juss) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathog. Dis.*, **4**(4): 481-488.
- Mateus, L., Lopes da Costa, L., Carvalho, H., Serra, P., and Silva, J.R. (2002). Blood and intrauterine leucocyte profile and function in dairy cows that spontaneously recovered from post-partum endometritis. *Reprod. Domestic Anim.*, **37**: 176-180.
- Owis, M., Sharad, K.S., Shehbaz, A. and Saleemuddin, M. (2005). Antibacterial efficacy of *Withania somnifera* ashwagandha an indigenous medicinal plant against experimental murine salmonellosis. *Phytomed.*, **12**: 229-35.
- Rathod, G.P., Kotecha, B.M., Sharma, R., Amin, H. and Prajapati, P.K. (2012). *In vitro* Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica* L.) and Tulsi (*Ocimum sanctum* L.). *Int. J. Pharmaceutical Biol. Arch.*, **3**(3): 582-586.
- Roberts, S.J. (1971). *Veterinary Obstetrics and Genital Diseases*. 2<sup>nd</sup> ed. CBS Publishers and Distributors, New Delhi. pp.776.
- Shaktawat, J.S. 2005. Therapeutic use of E. coli lipopolysaccharide in endometritis in crossbred cattle. M.V.Sc. Thesis, G. B. Pant University of Agriculture and Technology, Pantnagar.
- Sharma, M.C., Shankar, U., Gupta, Q.P. and Verma, R.P. (1983). Haematological studies in normal cyclic, anoestrous and repeat breeding crossbred cows. *Indian Vet. Med. J.*, **7**: 153-155.
- Singh, P., Singh J., Sharma N.C., Dhalival, G.S. and Kumar A. (2004). Effect of post A.I. immunotherapy on dynamics of uterine flora and conception in subclinical endometritis cows. *Indian J. Anim. Sci.*, **74**(7): 706-709.
- Singh, R.B., Sharma, R.D. and Singh, G.B. (1983). Incidence of repeat breeding in cows and buffaloes. *Indian J. Dairy Sci.*, **36**: 314-315.
- Singh, V.I., Singh, G., Dwivedi, P.N. and Sharma, R.D. (1993). In vitro evaluation of drug sensitivity patterns of bacterial isolates in repeat breeder cows. *Indian J. Anim. Sci.*, **63**(4): 425-426.
- Snedecor, G. W. and Cochran, W. G. (1989). *Statistical Methods*. 8<sup>th</sup> ed. Iowa, The Iowa State University Press. pp. 503.
- Strezemienski, P.J. and Kenney, R.M. (1984). Effect of stage of cycle, sampling frequency and recovery and recovery of microorganisms on total protein content of mare uterine flushings. *J. Reprod. Fert.*, **70**: 327-332.

Subapriya, R. and Nagini, S. (2005). Medicinal properties of neem leaves: a review. *Curr. Med. Chem. Anticancer Agents*, **5**(2): 149-6.

Thrall, M.A. (2004). *Veterinary Haematology and Clinical Chemistry*. Lippincott Williams and Wilkins, Philadelphia, pp. 71, 84-85, 147, 148.

Tizard, I. R., (2000). Innate immunity: Inflammation. In: *Veterinary Immunology*, 6<sup>th</sup> edn, ed. I. R. Tizard, W. B. Saunders Company, Philadelphia, PA, pp. 36-46.

Vandernat, J.M., Beijnen, J.H., Van-Dijk, H., Venderberg, W.J.M. and Labule, R.P. (1986). Suppression of migration of inhibition factor (MIF) produced by mitomycines in vitro. *Immunol.*, **172**: 120-127.

Victor, I.U. and Igeleke, C.L. (2012). Antimicrobial properties of the extracts of locally sold garlic and neem leaf in Benin City, Nigeria. *Int. J. Biosci.*, **2**(4): 21-27.