International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 2, 2018, pp.-5069-5072.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

STUDIES ON THE IMMUNOMODULATORY AND THERAPEUTIC EFFICACY OF TURMERIC (*Curcuma longa*) ON ENDOMETRITIS IN REPEAT BREEDING CROSSBRED COWS

KUMAR AMIT*[1], GUPTA H.P.[2] AND PRASAD SHIV[3]

[1]Government Veterinary Hospital, MohanChatti, 249304, Pauri Garhwal, Uttarakhand
[2,3]Department of Veterinary Gynaecology and Obstetrics, G.B. Pant University of Agriculture and Technology, Pantnagar 263145, India
*Corresponding Author: Email-amitbhojiyan@gmail.com

Received: January 14, 2018; Revised: January 25, 2018; Accepted: January 26, 2018; Published: January 30, 2018

Abstract- This research was made to investigate the turmeric for immunomodulatory and therapeutic efficacy on endometritis in repeat breeding crossbred cows. Twenty four cows were selected on the basis of history, breeding records, transrectal examination and white side test and randomly divided into 3 groups (Group A: 30 ml normal saline; Group D: 30 ml hydro-alcoholic turmeric; Group E: 30 ml hydro-acetonic turmeric). Treatments were given intrauterine beginning on the day of estrus for seven days in each group. 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 eight day of first collection i.e. 24 hr after last treatment. These flushings were used for the estimation of total protein, immunogloblobulin, TLC and polymorphonuclear cells (PMNs). 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 hydroalcoholic and hydroacetonic extract treated groups. Moreover, Hb and RBCs were increased in hydroacloholic 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 hydroalcoolic extract was more effective. Based on these results, it may be concluded that hydro-alcoholic extract of turmeric has a good antibacterial and immuno-modulation properties and can be used as a therapy for endometritis in repeat breeding crossbred cows.

Keywords- Repeat breeding, Endometritis, Turmeric, Immunomodolatory and Therapeutic.

Citation: Kumar Amit, *et al*., (2018) Studies on the Immunomodulatory and Therapeutic Efficacy of Turmeric (*Curcuma longa*) on Endometritis in Repeat Breeding Crossbred Cows. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 2, pp.-5069-5072.

Academic Editor / Reviewer: Dr Rama Shankar, Dr Rojita Mishra, B.L. Raghunandan, Dr. D. Aravind, Dr Ajay, Leena Muralidharan, Mehulkumar Laljibhai Savalia

Introduction

Turmeric is an ancient spice derived from the rhizomes of *Curcuma longa* and also known as 'Golden Spice of India. It has been used in India for medicinal purposes for centuries [1] and most biologically active agent is curcumin [2]. Turmeric is an excellent anti-inflammatory herb [3]. The anti-inflammatory action of turmeric includes lowering histamine levels and increasing the production of natural cortisone by adrenal glands. It inhibits release of the pro-inflammatory cytokine TNF-α and the gene that makes inflammatory COX-2 enzymes [1]. It acts as a scavenger of oxygen free radicals [4,5] and can protect haemoglobin from oxidation [6]. The aqueous extract of turmeric rhizomes has antibacterial effect [7]. Hydro-alcoholic and aqueous extract of turmeric showed inhibitory activity against *Staphylococcus aureus* [8]. Curcumin and other curcuminoids inhibit the growth of *Staphylococcus aureus, Salmonella paratyphi, Trichophyton gypseum* and *Mycobacterium tuberculosis* at concentration of 1 in 640000 [9]. Crude ethanol extract also possesses antifungal activity [10].

Repeat breeding is a most dangerous problem in cattle breeding. It causes heavy economic loss the dairy farmers due to more inseminations, increased calving interval and culling rates [11]. It has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities [12]. The incidence of repeat breeding in crossbred cows has been reported to about 7.4 to 21.9% [13]. The incidence of was higher during second parity, in high milk yielders and after abnormal calving [14]. Bacterial origins are considered to be most important causes of repeat breeding in cattle [15]. Bacteriological investigation of cervical mucus from cows with a history of repeat breeding indicates that the female reproductive tract has a distinct microflora, like haemolytic *E. coli*, *Staphylococcus aureus*, *Corynebacterium* spp. and *Pseudomonas aeruginosa* [16]. These organisms have been incriminated to render the female genital tract more harmful to the viability of the sperm [17] and affect the implantation of fertilized ovum to the uterus [18]. Alternatively, presence of these organisms or their metabolites in the reproductive tract for a long time causes cervicitis and endometritis [19].

In the light of the above reviewed literature, the current study was designed to investigate the immunomodulatory and therapeutic efficacy of turmeric on endometritis in repeat breeding crossbred cows.

Materials and Methods

Twenty-four repeat breeding crossbred cows were selected on the basis of history, breeding records, transrectal examination and white side test and randomly divided into 3 groups (Group A: 30 ml normal saline; Group D: 30 ml hydro-alcoholic turmeric; Group E: 30 ml hydro-acetonic turmeric). Treatments were given intrauterine beginning on the day of estrus for seven days in each group. On the day of estrus before treatment the Cervical mucus samples were collected and after treatment at subsequent estrus 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 [20].

Results and Discussion

The final per cent yield (w/w) and maximum sizes of zone of inhibition of hydro-hydroalcoholic and hydroacetonic extracts of turmeric were 25.0% and 14 mm, and 12.0% and 13 mm, respectively. The minimum inhibitory concentration (MIC) values against bacterial population in cervical mucus of repeat breeding cows suffering from endometritis were 8.00-12.00 mg/ ml, and 5.00 mg/ ml, respectively. The MIC of hydroalcoholic extract of turmeric reported as 4-20 mg/ ml and 19 mg/ ml, respectively [8,21]. This range/ variation in MIC value suggests that the bacterial population in cervical mucus of repeat breeding cows does not remain constant and mixed bacterial flora exist in the mucus. The percentages of animals showing clear cervical mucus discharge before and after treatment in groups A, D, and E were 50.0% and 62.5%, 50.0% and 75.0%, and 62.5% and 75%, respectively. Clear estrual cervical mucus is conducive for sperm penetration and conception, whereas, turbidity retards sperm motility in estrual mucus [22]. There was a significant (P<0.05) decline in the pH of cervical mucus from before to after treatment in all the groups (7.77±0.226 and 7.68±0.161 in Group A, 7.56±0.175 and 7.12±0.125 in Group B, and 7.81±0.187 and 7.75±0.133 in Group C, respectively) except control Group A. The pH of estrual cervical mucus in all the groups before treatment was alkaline (more than 7.6), thus, indicating uterine infection [23-25]. This increase in pH may be caused due to metabolites of bacteria and inflammatory exudates in estrual cervical mucus [26]. In the present study a significant decline in pH was observed in treatment groups after treatment. This reduction in pH may be due to decline in bacterial load and inflammatory process in uterus after treatment [27]. All the animals tested positive on White side test but treatment with hydroalcoholic and hydroacetonic extracts of turmeric led to a significant (P<0.05) reduction in the proportion of positive animals (37.5% and 50%, respectively) compared to the control (87.5%). Positive reaction to white side test could be explained on the basis of number of leukocytes present in the uterine discharge [28]. The normal discharge has less number of leukocytes to cause any change of colour, whereas in clinical and subclinical cases of endometritis, discharge contains increased number of leukocytes causing a color reaction [29]. The absence of color development to white side test in higher number of cows treated with the herbal extracts revealed their efficacy for combating infection. This result showed that turmericis antibacterial and immunomodulatory in nature and thus, reduced bacterial load and subsequently inflammation process [30, 31]. A significant decline (p< 0.05) in bacterial load (× 10^4/ml) was observed in all the groups from pre treatment to post treatment (301.29±0.920 and 282.38±1.030 in Group A, 265.40±0.453 and 1.06±0.035 in Group B, and 279.82±0.636 and 2.93±0.206 in Group C). After treatment, bacterial loads in Groups B and C were significantly lower (p< 0.05) as compared to control group A. a higher range of the bacterial load was reported [32], who observed a range of 45.8 x 10^6 to 47.28 x 10^7 per ml of cervical mucus in endometritic cases. In contrast to this, A lower range of bacterial load of 35.05 x 10^3 per ml in cervical mucus of repeat breeding cows [33]. The severe reduction in bacterial load after treatment with turmeric indicates its potent antibacterial action while the mild reduction in the control group could be attributed to natural uterine defense mechanisms.

The mean values of different blood and uterine flushing parameters in the control group and hydroalcoholic and hydroacetonic extract of turmeric treated groups are shown in [Table-1] and [Table-2], respectively.

Table-1 *Blood parameters of repeat breeding crossbred cows treated intrauterine with hydroalcoholic and hydroacetonic extracts of turmeric (control group was treated with normal saline)*

Parameter	Group A; Control		Group B; Turmeric (Hydroalcoholic)		Group C; Turmeric (Hydroacetonic)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Hb (g/dl)	8.25±0.105	6.98±0.121^{a}	8.47±0.177^{A}	13.80±0.188^{Bb}	8.97±0.095^{A}	12.87±0.155^{Bb}
PCV (%)	27.50±0.944	26.12±0.811^{b}	26.50±1.21^{A}	29.50±0.906^{Ba}	26.62±1.30^{A}	34.25±1.03^{Ba}
RBC (10^6/cumm)	4.72±0.243	4.00±0.245^{a}	4.62±0.155	7.24±0.190^{b}	4.16±0.180	6.17±0.269^{c}
Neutrophil (%)	43.75±2.366	37.62±2.389^{a}	24.37±0.595^{A}	29.62±0.532^{Bb}	23.50±0.906^{A}	30.37±0.800^{Bb}
Lymphocyte (%)	34.87±1.301	30.87±1.619^{a}	38.50±1.133^{A}	50.25±1.221^{Bb}	40.37±0.962^{A}	52.75±1.250^{Bb}
WBC (10^3/ml)	7.45±0.225^{B}	6.78±0.133^{Aa}	8.08±0.271^{A}	10.27±0.434^{Bb}	8.57±0.196^{A}	10.68±0.350^{Bb}
Glucose (mg/dl)	51.23±0.545^{A}	53.25±0.403^{Ba}	55.46±1.48^{A}	70.02±0.824^{Bb}	51.83±0.678^{A}	70.41±0.699^{Bb}

Table-2 *Uterine flushing parameters of repeat breeding crossbred cows treated intrauterine with hydroalcoholic and hydroacetonic extracts of turmeric (control group was treated with normal saline)*

Parameter	Group A; Control		Group B; Turmeric (Hydroalcoholic)		Group C; Turmeric (Hydroacetonic)	
	Before treatment	After treatment	Before treatment	After Treatment	Before treatment	After treatment
TLC (10^6/ml)	0.422±0.037^{A}	0.574±0.024^{Ba}	0.678±0.050^{A}	4.25±0.171^{Bb}	0.483±0.018^{A}	5.18±0.188^{Bc}
PMN (%)	29.87±0.440^{B}	27.12±0.295^{Aa}	29.12±0.440^{A}	56.25±1.35^{Bb}	28.25±0.725^{A}	51.00±0.845^{Bc}
Protein (mg/dl)	159.99±3.518	164.34±5.724^{a}	159.14±1.596^{A}	179.79±1.195^{Bb}	156.51±0.909^{A}	173.50±1.841^{Bb}
Total IG (mg/dl)	38.78±0.584^{A}	42.05±0.394^{Ba}	39.03±0.527^{A}	54.91±1.110^{Bb}	39.91±0.566^{A}	51.94±1.385^{Bc}

Different capital letters (A,B) indicate significant (P<0.05) difference between before and after treatment values within a group whereas, different small letters (a,b,c) indicate significant difference between groups in before treatment and after treatment values, respectively.

The significant increase (p< 0.05) in the mean Hb and PCV values was observed from pre treatment to post treatment in treated groups except control group. After treatment, the mean Hb and PCV values in hydroalcoholic and hydroacetonic group were significantly higher (p< 0.05) than the mean value in control group. There was a significant increase (p< 0.05) in the mean RBC and WBC count from pre to post treatment. Between the groups, after treatment mean values in hydroalcoholic and hydroacetonic group were significantly higher (p< 0.05) compared to the control group. A significant increase (p< 0.05) in the mean neutrophil, lymphocyte count and blood glucose were observed from pre to post treatment. In contrast, a significant decrease (p< 0.05) was observed in control group. Significant increase in haemoglobin was found in treated groups indicating efficacy of treatment in improvement of general body condition. Anaemia (reduced Hb) in repeat breeding cows may be associated with reproductive disorders reported [34] and also found that above blood parameter increased after treatment with turmeric [35]. In inflammatory disease, erythropoietin is diminished presumably because of inflammatory cytokines leading to lowered erythropoiesis [36]. Lymphocytes play a very important role in immunity. B-lymphocytes produce antibodies, mainly IgG [37]. Lymphocytes play a critical role in humoral antibody response as well as in cell mediated immunity [38]. Mean blood glucose value in selected cows before treatment in the present study was lower than the value found in normal cyclic fertile cows [39].

Uterine flushing parameters (TLC, PMNs and total immunoglobulin) increased

after treatment with both hydroalcoholic and hydroacetonic extracts of turmeric 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 [40]. Neutrophils are known to play a primary role in the defense of the uterus against infection. Increase numbers of neutrophils in to uterus by chemoattractants, chemokines and adhesion molecules, like β2-integrin and l-selectin [41]. Numbers of researchers have noticed increase in protein concentration and PMNs infiltration in uterine flushings following uterine contamination [2, 42]. 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 clean appearance of estrus cervical mucus, reduction in bacterial load and negative white side test. The clinical recovery and conception rates in Groups A, B, and C were 25.0% and 0.0%, 75.0% and 50.0%, and 62.5% and 37.5%, respectively. Therefore, hydroalcoholic extract of turmeric yielded best results in terms of clinical recovery and conception rates.

Conclusion

The hydroalcoholic and hydroacetonic extracts of turmeric have potent immunomodulatory and therapeutic efficacy on endometritis in repeat breeding crossbred cows. Hydroalcoholic extract gave best results in terms of clinical recovery and conception rates.

Application of research:

1. In-vitro antibacterial activity of turmericextract against mixed micro flora of endometritis.
2. Minimum inhibitory concentration (MIC) of turmeric herbal extracts against mixed micro flora in cervical mucus of repeat breeding crossbred cows.
3. Comparative immunomodulatory property and therapeutic efficacy of turmeric in repeat breeding crossbred cows in relation to traditional antibiotic.

Research Category: Repeat breeding, Endometritis, Animal Reproduction

Abbreviations:

%	:	Percent
hr	:	Hour
mg	:	Milligram
ml	:	Milliter
Hb	:	Haemoglobin
w/w	:	Weight/Weight
PCV	:	Packed Cell Volume
TLC	:	Total Leukocyte Count
DLC	:	Differential Leukocyte Count
pH	:	Potential of hydrogen
PMNs	:	Polymorphonuclear Cells
WBC	:	White Blood Cell
RBCs	:	Red Blood Cells
TNF-α	:	Tumor Necrosis Factor Alpha
COX-2	:	Cyclooxygenase-2
ANOVA	:	Analysis of Variance
MIC	:	Minimum Inhibitory Concentration
mm	:	Millimeters
dl	:	Deciliter
IgG	:	Immunoglobulin G

Acknowledgment / Funding:

We are extending our gratitude to G.B. Pant University of Agriculture and Technology, Pantnagar-263145 Uttarakhand, India for permitting us to conduct this research study and to provide essential facilities. We would like to thank participants; without their cooperation, the study would not have been possible.

Research Guide: Dr HP Gupta, Professor, Department of Veterinary Gynaecology and Obstetrics, G.B.Pant University of agriculture and Technology, Pantnagar

Research project name or number: M.V.Sc. Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Rathaur P., Raja W., Ramteke P.W. and John S.A. (2012) *IJPSR,* 3(7), 1987-1994.
[2] Elgendy M.Y., Hakim A.S., Ibrahim T.B., Soliman W.S. and Ali S.E. (2016) *Journal of Fisheries and Aquatic Science*, 11, 206-215.
[3] Surh Y.J., Chun K.S., Han H.H., Keum S.S., Park Y.S. and Lee S.S. (2001) *Mutation Research*, 48, 243–268.
[4] Subramanian M., Sreejayan Rao, M.N.A., Devasagayam T.P.A. and Singh B.B. (1994) *Mutation Research,* 311, 249–255.
[5] Ruby A.J., Kuttan G., Dinesh B.K., Rajasekharan K.N. and Kuttan R. (1995) *Cancer Letters,* 94, 79–83.
[6] Unnikrishnan M.K. and Rao M.N. (1995) *Pharmazie*, 50, 490–492.
[7] Kumar S., Narain U., Tripathi S. and Misra K. (2001) *Bioconjugate Chem*istry, 12, 464-469.
[8] Hedge M.N., Shetty S., Yelapure M. and Patil A. (2012) *IOSR Journal Pharmacy*, 2(2), 192-198.
[9] Khanna N.M. (1999) *Current Science,* 76(10), 1352-1356.
[10] Wuthi-Udomler M., Grisanapan W., Luanratana O. and Caichompoo W. (2000) *Southeast Asian* Journalof *Tropical Medicine and Public Health*, 31, 178–182.
[11] Lafi S.Q. and Kaneene J.B. (1992) *Preventive Veterinary Medi*cine, 14, 87-98.
[12] Zemjanis R. (1980) Repeat Breeding or conception failure in cattle. In: D.A. Morrow (Editor) *Current Therapy in Theriogenology*, W.B. Saunders, Philadelphia, PA. 205-213.
[13] Singh R.B., Sharma R.D. and Singh G.B. (1983) *Indian Journal of Dairy Science,* 36, 314-315.
[14] Martinez J. and Thibier M. (1984) *Theriogenology,* 21, 569-581.
[15] Baisya S.K., Das K.K., Rahman H. and Borgohain B.N. (1998) *Indian Journal Comp. Microbiology Immunology*, 19(2), 130-131.
[16] Krishnamurthy G.C., Navjaiah R.D. and Krishnamurthy B.S. (1974) *Indian Journal Veterinary Science,* 51, 264-267.
[17] Plazas M.L. (1955) *Veterinary Bulletine,* 25, 2749.
[18] Raghavan R., Nilakantan P.R. and Uppal P.K. (1971) *Indian Veterinary Journal,* 48(8), 779-783.
[19] Farrelly B.T. and Mullaney P.E. (1964) *Iris Veterinary Journal,* 18, 201.
[20] Snedecor G.W. and Cochran W.G. (1989) *Statistical Methods, Eighth Edition, Iowa State University Press.*
[21] Naz S., Jabeen S., Ilyas S., Manzoor F., Aslam F. and Ali A. (2010) *Pakistan Journal of Botany,* 42(1), 455-462.
[22] Dev S., Pangawkar G.R., Sharma R.K. and Verma H.K. (1997) *International Journal Animal Science*, 12, 89-91.
[23] Wani G.M., Tripathi S. and Saxena V.B. (1981) *Indian Journal of Animal Reproduction,* 1(1), 76-79.
[24] Gupta R.C., Sinha A.K. and Krishnaswami A. (1983) *Theriogenology,* 20(5), 559-564.
[25] Singh P., Singh J., Sharma N.C., Dhalival G.S. and Kumar A. (2004) *Indian*

Journal of Animal Science, 74(7), 706-709.
[26] Salphale G.B., Kadu M.M., Fasihddin M. and Kadu M.S. (1993) *Indian Journal of Animal Reproduction,* 14, 77-78.
[27] 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.*
[28] Popov Y.N. (1969) *Veterinarya Moscow*, 4, 85-87.
[29] Pateria A.K. and Rawal C.V.S. (1990) *Indian Journal of Animal Reproduction,* 11, 142-144.
[30] Owis M., Sharad K.S., Shehbaz A. and Saleemuddin M. (2005) *Phytomedicine*, 12, 229-35.
[31] Mustafa R. and Blumenthal E. (2017) *Journal of Immunoassay & Immunochemistry*, 38(2),140-146.
[32] Singh V.I., Singh G., Dwivedi P.N. and Sharma R.D. (1993) *Indian Journal of* Animal *Science,* 63(4), 425-426.
[33] Goswami I.C., Kher H.N., Jhala M.K. and Derashri H.J. (1992) *Indian Journal Animal Reproduction,* 13(12), 180-182.
[34] Roberts S.J. (1971) *Veterinary Obstetrics and Genital Diseases. 2nd ed. New Delhi, CBS Publishers and Distributors*, 776.
[35] Amin M.R., Mostofa M., Islam M.N. and Asgar M.A. (2010) *Journal Bangladesh Agriculture University,* 8(2), 259–263.
[36] Thrall M.A. (2004) *Veterinary Haematology and Clinical Chemistry. Published by Lippincott Williams and Wilkins, Philadelphia*, pp, 71, 84-85, 147, 148.
[37] Yan H., Yuan Y., Zheng X., Zhang K., Chen S. and Zhiyun D. (2015) *Molecules*, 20, 9183-9213.
[38] Swenson M.J. and Reece W.O. (1996) *Duke's physiology of domestic animals. 11th ed. Panima publishing corporation. New Delhi and Bangalore*.
[39] Ramakrishna K.V. (1996) *Indian Journal of Animal Reproduction,* 17(1), 30-32.
[40] Mateus L., Lopes da Costa L., Carvalho H., Serra P. and Silva J.R. (2002) *Reproduction Domestic Anim*al, 37, 176–180.
[41] Tizard I. R., (2000) *Innate immunity: Inflam-mation. In: Veterinary Immunology, 6th edn, ed. I. R. Tizard, W. B. Saunders Company, Philadelphia, PA,* pp. 36–46.
[42] Strezemienski P.J. and Kenney R.M. (1984) *Journal of Reproduction and Fertility,* 70, 327-332.