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Effect of *Tinospora cordifolia* and autologous blood plasma on activity of certain enzymes in genital secretion of post partum buffaloes

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Various enzymes such as alkaline phosphatase, lysozyme, cholinesterase, and transaminases are used as markers of uterine pathology in different species (Wittkowski *et al.*1989, Katila *et al.*1990). Enzyme, guaicol peroxidase, present exclusively in endocervix is also related to infection (Srivastava *et al.* 2000). The concentration of all the above mentioned enzymes increases during genital tract infections. Such information in buffaloes, especially in reference to immuno-modulation activity of *Tinospora cordifolia* and autologous blood plasma against genital tract infections is lacking and was therefore investigated.

Experimental animals: The study was carried out on 24 Murrah buffaloes, maintained at Livestock Production Managment Farm, Indian Veterinary Research Institute, Izatnagar. All the animals were maintained in one-fourth covered shed under loose housing system. They were fed with balanced ration (green fodder and wheat *bhoosa* mixed with concentrate) and given free supply of drinking water. All the buffaloes were in age group of 6–8 years, belonged to second to fourth lactation with body weight ranging from 400– 550 kg.

Calved buffaloes at 40 days or more postpartum were selected for the present study. Genitalia of these buffaloes were examined per-rectally for the presence of infection and divided randomly into 4 groups. Some animals not showing infection clinically but their genital secretions collected after flushing were positive for infection.

Experimental protocol: Amongst 24 selected buffaloes, 18 buffaloes had either clinical genital infection as assessed by visual or by the White Side Test. These buffaloes were divided into 3 groups 1, 2, 3 comprising 6 animals each, whereas the group 4 comprised normal animals having low bacterial load and clear genital discharge.

Buffaloes in group 1 were treated with intrauterine infusion of 3000 mg (total dose) of aqueous extract of *T. cordifolia* (suspension of immumod tablet, 50 ml)), buffaloes in group 2 were given 150 ml of autologous blood plasma whereas group 3 received 150 ml phosphate buffered saline (pH 7.2) intrauterine. Uterine infusates in all the 3 groups were divided in 3 equal parts. The uterine infusions were continued for 3 consecutive days starting from day 1 of estrus. Group 1 and group 2 were treatment groups whereas group 3 and group 4 served as control, respectively.

Collection of uterine secretions and CVM: The uterine secretion was collected from animals of group 1–3, at pre-treatment and post-treatment estruses, whereas only at pretreatment estrus from animals of group 4. Uterine secretion was collected aseptically by flushing using disposable embryo transfer catheter attached to a disposable 50 ml sterile syringe that was utilized to inject 30 ml of sterile phosphate buffer saline (pH 7.2). The flushing fluid was allowed to mix with uterine secretions by gentle massaging of uterus per rectal. The uterine contents were then aspirated completely in aseptic manner into a sterilized vial. The contents were centrifuged at 3000 rpm for15 min. The supernatant was collected and stored –20°C for estimation of various enzymes.

CVM was also collected aseptically from all buffaloes in estrus before treatment and on subsequent estrus after treatment in groups 1–3 and at pretreatment estrus in group 4 as per Nair and Kharche (1988). The collected mucus was stored at –20°C till enzyme estimation. Another part of mucus was mucolysed and used for determination of bacterial load by standard plate count method (Cowan 1974).

Estimation of enzyme activity: The stored uterine fluid and CVM were thawed just before estimation of enzyme activity. Alkaline phosphatase (ALP) was estimated as per King and Armstrong (2000) and myeloperoxidase (MPO) as per Bertz and Baggioline (1974). Lysozyme (LZM) and guaicol peroxidase (GPO) was estimated as per Saint Blancard *et al.* (1970) and Tsibris *et al.* (1986) respectively. ALP, MPO and LZM were estimated in uterine fluid, whereas GPO was estimated in CVM.

Statistical analysis: The data of enzymatic activity in the uterine flushing of buffaloes in different groups was analyzed

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using student t test (Snedecor and Cochran 1989).

The ALP activity was significantly higher (P<0.01) in animals of group 1, group 2 and group 3 than the animals of group 14 (Table 1). ALP activity declined significantly (P<0.01) at post treatment estrus in group 1, whereas nonsignificantly in groups 2 and 3. The higher values of ALP in uterine flushing of infected buffaloes as observed in present study are similar to earlier report in buffaloes (Zain *et al.* 1997), cows (Rao and Seshagiri 1998) and mares (Katila *et al.* 1990). This ALP is present in neutrophils and the number of such neutrophils increases in the uterus due to inflammation caused by increased microbial population. On the contrary, Boos *et al.* (1988) reported that endometritis did not influence the ALP activity in uterine flushings of endometritic cows, though in purulent metritis ALP activity was significantly higher (21000 U/L).

MPO activity was significantly higher (P< 0.05) before treatment in group 1, group 2 and group 3 compared to group 4. There was significant (P<0.01) decline in MPO activity in group 1, 2 and 3 post treatment (Table 1). Higher levels of MPO were found in pretreatment uterine flushings of buffaloes of groups 1–3 which corroborated to higher bacterial load in their CVM. The increased level of MPO indicates increased number of neutrophils (Cooray 1994), which in turn also express the degree of inflammation.

LZM activity was significantly (P<0.05) higher in group 1 than group 2, group 3 and group 4 (Table 1). Significant (P<0.01) reduction was observed in LZM activity in group 1 (53.907±6.369%) and group 2 after treatment whereas nonsignificant reduction was observed in group 3. Lysozyme is found in both azurophilic and specific granules of neutrophils (Jensen *et al.* 1967). It is present in significant amount in buffalo PMN cells.

The GPO activity was significantly (P<0.05) higher in group 1 and group 2 than group 4 before treatment (Table 1). Though the post treatment enzyme activity decreased in group 1, group 2 and group 3 but the difference was nonsignificant. Srivastava *et al.* (2000) also found high GPO activity in clinical cases of metritis/endometritis. This may be comparable to present study as infection was present in oestrus cervical mucus.

The higher level of ALP, MPO and LZM in the present study coincides with the increased bacterial population present in uterine secretions. After treatment, there was a significant decline in activity of all the enzymes on subsequent estrus in uterine secretions of group 1 buffaloes, but decline was significant only in cases of MPO and LZM in group 2. This decline along with decline in bacterial population in CVM indicates that aqueous extract of *T. cordifolia* and autologous plasma have helped in reducing uterine bacterial infection. Such agents act as opsonins/ chemoattractant acted to enhance phagocytosis. In absence of any infection, the inflammatory condition in uterus may have subsided leading to reduced number of phagocytic cells (neutrophils, macrophages etc.) and hence reduced activity of enzymes.

SUMMARY

Murrah buffaloes (24), 6–8 years old, in second to fourth lactation were used to investigate immuno-modulation activity of *Tinospora cordifolia* and autologous blood plasma against genital tract infections. Amongst 24 selected buffaloes, 18 buffaloes had either clinical or genital infection as assessed by visual or by the White Side Test. These buffaloes were divided into groups 1, 2, 3, comprising 6 animals each, whereas the group 4 comprised normal animals having low bacterial load and clear genital discharge. Higher level of ALP, MPO and LZM coincided with the increased bacterial population present in uterine secretions. After treatment, there was a significant decline in activity of all the enzymes on subsequent estrus in uterine secretions of group 1 buffaloes, but decline was significant only in cases of MPO and LZM in group 2. The reduced bacterial

Table 1. Effect of different treatments on enzyme levels in uterine flushing/CVM of endometritic buffaloes (mean ±se)

Treatment groups	Enzymes										
	ALP			MPO			LZM			GPO	
	PRT	POT	PD	PRT	POT	PD	PRT	POT	PD	PRT	POST
G 1	51.758 ±3.488Aa	23.010 ±2.087Bab	54.338 ±5.386	21.921 ±2.564Aa	9.512 ±1.006B	54.672 ±6.217	52.126 ±6.166Aa	23.077 ±2.975B	53.907 ±6.369	0.154 ±0.017a	0.333 ±0.124ab
G 2	28.394 ±3.735b	20.735 ±2.259a	22.690 ±10.236	32.890 ±3.007Ab	14.463 ±2.189B	51.872 ±12.636	35.005 ±6.167Ab	12.007 ±2.219Bb	63.358 ±5.698	0.183 ±0.049a	0.513 ±0.189a
G 3	29.195 ±4.107b	26.349 ±3.401b	1.354 ±16.509	33.827 ±4.481Ab	17.352 ±2.951B	47.700 ±8.108	22.329 ±2.129bc	17.352 ±2.951B	20.897 ±20.839	0.222 ±0.112ab	0.053 ±0.019bc
G 4	15.781 ±2.879c	-	-	9.669 ±2.469c	-	-	11.803 ±2.325c	-	-	0.044 ±0.010b	-

PRT, Preatment; POT, post treatment; PD, percent decline; values with different superscript (A, B) within same row and with different superscript (a, b, c) within same column differ significantly at P<0.01 and P<0.05 respectively. Data are based on 6 observations.

population in CVM indicated that aqueous extract of *T. cordifolia* and autologous plasma have helped in reducing uterine bacterial infection.

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REFERENCES

Bertz U and Baggioline M. 1974. Biochemical and morphological characterization of azurophil and specific granules of human neutrophilic polymorphonuclear leucocytes. *Journal of Cell Biology* **63**: 251.

Boos A, Wittkowski G and Schwarz R.1988. Activity of alkaline phosphatase in uterine flushings of dairy cows affected with ovarian cyst or endometritis. *Theriogenology* **30**: 217–25.

Cooray R.1994. Use of bovine myeloperoxidase as an indicator of mastitis in dairy cattle. *Veterinary Microbiology* **42**: 317–26.

Cowan S T. 1974. Cowan and Steel's manual for the identification of medical bacteria, Cambridge and London, Cambridge university Press.

Jensen R S, Tew J G and Donaldson D M.1967. Extracellular b-lysis and muraminidase in body fluid and inflammatory exudates. *Proceedings of Society of Experimental Biological Medicine* **124**: 545–49.

Katila T, Lock T F, Hoffmann W E and Smith A R.1990. Lysozyme, alkaline phosphatase and neutrophils in uterine secretions of mares with differing resistance to endometritis. *Theriogenology* **33**: 723–32.

King and Armstrong. 2000. *Practical Notebook of Analytical Biochemistry.* (Ed.) Saini M Communication Centre, IVRI, Izatnagar, Bareilly, Uttap Pradesh, India.

Nair S and Kharche K G. 1988. Study of calcium, chlorine and magnesium in cervicovaginal mucous of normal and repeat breeder crossbred cows. *Indian Journal of Dairy Science* **41**: 491–92.

Rao S K and Seshagiri V N. 1998. Protein concentration and alkaline phosphatase activity in uterine flushing from cows affected with endometritis. *Indian Veterinary Journal* **75**: 369–70.

Saint-Blancard J, Chozel P, Mathieu Y, Perrot J. and Jolles P.1970. Influence of pH and ionic strength on the lysis of *micrococcus lysodecticus* by human avian lysozymes. *Biochemica Biophysica Acta* **220**: 300.

Snedecor G W and Cochran W G.1989. *Statistical Methods*. 8th edn. The Iowa State Univeristy Press, Ames, Iowa, USA.

Srivastava S K, Shanker U, Agarwal S K and Sahni K L. 2000. Effect of oestrus cervical mucous peroxidase and fern pattern on fertility in crossbred cows. *Indian Journal of Animal Sciences* **70** (7): 807–09.

Tsibris J C M, Virgin S D, Khan Dawood F S, Langenberg P N, Thomson J L and Spellacy W N. 1986. Cervico vaginal peroxidase: Markers of the fertile period. *Obstetrics and Gynaecology* **67**: 316–20.

Wittkowski G, Boos A, Ehlers J. and Roming L J P.1989. Alkaline phosphatase activities and progesterone concentrations in uterine flushings of dairy cows. Side effects and variations during oestrous. *Journal of Veterinary Medicine* **36**: 241–46.

Zain A E D, El-Ballal S S and Shehata S H. 1997. Alkaline phosphatase and transaminase activity in uterine flushing of cows affected with endometritis. *Buffalo Journal* **13**: 95–102.