

EFFECT OF THERAPY OF *WITHANIA SOMNIFERA* ON IMMUNE STATUS OF CROSSBRED COWS AFFECTED WITH SUBCLINICAL MASTITIS

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ABSTRACT

The present study was conducted to determine the impact of *Withania somnifera* (WS) on immune status in crossbred cows suffering from sub clinical mastitis. For this milk samples from apparently healthy Holstein Friesian crossbred cows were collected and examined for subclinical mastitis (SCM) by the diagnostic tests viz., California mastitis test (CMT), somatic cell count (SCC) and cultural examination. The effect of WS on immunomodulation related biochemical parameters including immunoglobulins (IgA, IgG and IgM) and phagocytic activity of polymorphonuclear (PMN) cells were evaluated. The therapeutic study was conducted on eight cows and administered oral alcoholic extract of WS@ 125 mg/kg body weight and intramammary 500 mg WS extract dissolved in 5 ml NSS twice daily for 7 days. The results of this study showed that WS exhibited marked immunomodulatory property by showing significant improvement in mean values of IgG, IgA, IgM, phagocytic activity and phagocytic index in post treatment mean values in comparison to their respective pre-treatment mean values. Therefore, on the basis of results it was concluded that the herbal extract of WS possesses immunomodulation property for SCM.

Keywords: Cow, Immunomodulation, Subclinical mastitis, *Withania somnifera*

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Mastitis causes huge loss to the dairy industry in terms of quality and quantity of milk. It involves an inflammatory response in the mammary tissue to multifactorial etio-pathological factors. The use of antibiotics are the only choice of treatment for mastitis, nevertheless antibiotic therapy in intra mammary infection often fails due to immune-suppressions and concurrent infections. Multidrug resistance is a worldwide problem attributed to the extensive and indiscriminate use of antimicrobials. For this reason, the concept of using non-antibiotic strategies for controlling mastitis is gaining attention. Therefore, alternatively to fight this distressing disease researchers are investigating and weightage is being given to the use herbal products to enhance the immunity of mammary gland and to improve the oxidative stress of mammary parenchyma from reactive free radicals. One such strategy is based on enhancement of the animal's natural defence mechanism by use of non-specific immuno-modulators such as plant materials. The herbal therapy generally does not contaminate the milk and hence there is no loss of milk withdrawal. *Withania somnifera* (Ashwagandha) is an important herb in the Ayurvedic and indigenous medicinal system for over 3000 years, and its root, leave and whole plant are used for medicinal purposes. Ghosal *et al.* (1989) reported that roots of *W. somnifera* possess immuno-modulatory properties like activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal

enzymes secreted by the activated macrophages. Therefore, the present study was carried out to investigate immuno-modulatory effects of *W. somnifera* in bovine subclinical mastitis.

MATERIALS AND METHODS

The study was conducted in eight HF crossbred cows (Group I) found positive for subclinical mastitis by testing of milk samples by California mastitis test (CMT), somatic cell count (SCC) and bacterial cultural examination. Roots of *W. somnifera* after identification and confirmation from Botanical Survey of India, Jodhpur, Rajasthan (Registration No. - BSI/AZRC/1.12012/Tech./2019-20(PI.Id.)/712) were used for preparation of ethanolic extract by hot continuous Soxhlet extraction procedure. For therapeutic trials the prepared extract was administered @ 125 mg/kg body weight by oral route and @ 500 mg dissolved in 5 ml NSS by intramammary infusion in each affected quarter twice daily for 7 days. In addition, eight apparently healthy HF crossbred cows (Group II) found negative for subclinical mastitis by CMT, SCC and bacterial cultural examination were kept as control for comparative study. Immunomodulation study was carried out by estimation of pre-treatment (day 0) and post treatment (day 8) milk immunoglobulins (IgG, IgA and IgM) by using sandwich ELISA kits. IgG was estimated by using test kit supplied by Genxbio Health Sciences (P) Ltd., H-26, Site C, Surajpur Industrial Area, Gautam Budh Nagar, Greater Noida-201306, U.P., India and IgA and

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IgM were estimated by using test kit supplied by Bethyl Laboratories, Inc., P.O. Box 850, Montgomery, TX 77356, USA. Phagocytic activity and phagocytic index of isolated milk polymorphonuclear cells (PMNs) (Fox *et al.*, 1987) and differential leukocyte count (Dulin *et al.*, 1982) of milk samples were estimated. The isolation of PMNs from milk samples was carried out as per the method of Daley *et al.* (1991) and viability of cells was tested by trypan blue (SD'S Lab Chem Industry, Bombay, India) exclusion technique (Colligan *et al.*, 1994). The cell concentration in PMN suspension was adjusted at 1.0×10^7 cells/ml following the method of Gentle and Thompson (1990). Phagocytic activity of milk PMN cells was expressed by the per cent phagocytosed neutrophil in 100 cells and phagocytic index was determined by the unit of *S. aureus* ingested by single neutrophil, counted in 100 cells. The data obtained in the research work undertaken were statistically analyzed and compared using standard formulas given for mean, standard error by student's t-test. The IBM SPSS 20.0 software statistical package program was used for these tests. The level of $p < 0.05$ was used to determine the statistical significance.

RESULTS AND DISCUSSION

In the present study, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae* and *E. coli* were the pathogens isolated during bacterial cultural examination of pre-treatment milk samples of cows found positive for subclinical mastitis. Pre- and post-treatment mean values of various parameters of milk estimated during the present investigation for immunomodulatory effects of *W. somnifera* in subclinical mastitis are presented in Table 1.

The pre-treatment mean values of milk immunoglobulins *viz.*, IgG, IgA and IgM of milk of cows affected with subclinical mastitis (Group-I) were significantly ($P < 0.05$) higher than control animals (Group-II). These findings were in agreement with previous similar studies carried out by Kocina *et al.* (2012) and Saat *et al.* (2016). The possible reason of increase in immunoglobulins in animals affected with subclinical mastitis might be due to functional IgG1, IgG2 and IgM acts as opsonins and facilitate phagocytosis by PMNs and macrophages, while IgA plays roles in toxin neutralisation and bacterial agglutination, thereby hindering bacterial spread and colonization (Paape *et al.*, 2000 and Korhonen *et al.*, 2000). There was significant ($P < 0.05$) decrease in the post-treatment mean values of IgG, IgA and IgM of Group-I animals as compared to their respective pre-treatment mean values.

During differential leukocyte count estimation, it was observed that pre-treatment mean values of neutrophils

and lymphocytes of Group-I were significantly ($P < 0.05$) higher and lower than in milk of Group-II cows, respectively. These findings were in agreement with previous similar investigations conducted by Gupta (2010) and Marcel *et al.* (2017). Elevated neutrophils in milk of animals affected with SCM might be attributed to natural defence mechanism of the body against infectious agents. More immature and band-shaped neutrophils were observed in the milk of infected cows. This might be found because bone marrow releases more immature neutrophils and sent to fight against infections to the mammary glands. There was significant ($P < 0.01$) decrease and increase in the post-treatment mean values of neutrophils and lymphocyte in milk of SCM affected cows as compared to their corresponding pre-treatment mean values, respectively. These post-treatment mean values of neutrophils and lymphocyte of Group-I were approaching towards their corresponding levels in animals of Group-II.

The pre-treatment mean values of phagocytic activity and phagocytic index of isolated milk polymorphonuclear cells (PMNs) of Group-I were significantly ($P < 0.05$) lower in comparison with Group-II. These observations of present study were in agreement to the findings of Vishnoi *et al.* (2007) and Alhussien *et al.* (2015). Post-treatment mean values of phagocytic activity and phagocytic index of Group I animals were significantly ($P < 0.01$) increased as compared to corresponding pre-treatment values. Inflammatory chemo-attractants guide PMN toward foci of infection during mastitis. Interleukin-8 plays a role not only in chemotaxis but inducing release of alkaline phosphatase from secondary granules and production of reactive oxygen species by PMN during chemotaxis (Galligan and Coomber, 2000). The first event to occur in the process of phagocytosis is contact and recognition of bacteria. Bacteria resist recognition but recognition or opsonization of bacteria essentially needed before phagocytosis (Ohman *et al.*, 1988). Immunologic recognition is principally accomplished by specific antibodies that recognize bacterium through Fab regions and bind to PMN via Fc-receptors (FcR) on the plasma membrane (Burvenich *et al.*, 1994).

Withania somnifera widely used as a general tonic to increase energy and prevent diseases may be pertaining to its effect on the immune system. Glycowithanolides and a mixture of sitoindosides IX and X isolated from *W. somnifera* were found to effectively mobilization and activation of peritoneal macrophages, phagocytosis, and increased activity of the lysosomal enzymes (Ghosal *et al.*, 1989). *W. somnifera* root extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages (Davis and Kuttan, 2000).

Table 1. Pre and post-treatment mean±SE values of Ig G(mg/ml), Ig A (mg/ml), Ig M (mg/ml), milk differential leukocyte count, phagocytic activity and phagocytic index in milk of SCM affected group-I and healthy group-II cows

Parameter	Group-I		Group-II (Control)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
IgG (mg/ml)	0.50±0.008 ^{ca}	0.39±0.015 ^{bb}	0.28±0.005 ^{aa}	0.28±0.005 ^{aa}
IgA (mg/ml)	0.37±0.035 ^{bcA}	0.19±0.021 ^{bb}	0.11±0.007 ^{aa}	0.12±0.009 ^{aa}
IgM (mg/ml)	0.52±0.072 ^{ba}	0.23±0.034 ^{abB}	0.14±0.009 ^{aa}	0.15±0.011 ^{aa}
Lymphocyte (%)	16.89±0.498 ^{abA}	19.28±0.387 ^{abB}	18.57±0.22 ^{ca}	19.29±0.212 ^{abA}
Neutrophils (%)	44.28±0.646 ^{bcA}	34.39±1.271 ^{bcB}	17.54±0.225 ^{aa}	17.69±0.208 ^{aa}
Phagocytic activity	16.89±0.37 ^{aa}	19.45±0.459 ^{abB}	21.88±0.625 ^{ba}	21.82±0.566 ^{ca}
Phagocytic index	1.56±0.146 ^{aa}	2.17±0.203 ^{ab}	2.22±0.141 ^{ba}	2.25±0.135 ^{aa}

Means bearing different superscript in a row (capital letter) and in columns (small letter) differ significantly (P<0.05) for each parameter

CONCLUSION

The results of this study vividly indicated that subclinical mastitis causes exertion on the immunity of the lactating animals resulting lowering the immune status in terms of variations of immunoglobulins and phagocytic activity of the PMNs cells. The herbal extract of *W. somnifera* possesses immunomodulation property and exhibited marked immunomodulatory property by showing significant improvement in post treatment mean values in comparison to their respective pre treatment mean values of IgG, IgA, IgM, phagocytic activity and phagocytic index.

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