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POLYHERBAL FORMULATION EXAPAR LIQUID IMPROVES LOCAL IMMUNITY IN POST PARTUM COWS

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Summary

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A study on eighteen cows in advanced pregnancy was conducted in a dairy farm in Bidar Karnataka. The animals were randomly divided in two groups, treated (n=12) and control (n=6). Treated animals were administered Exapar liquid for 5 days while no treatment has been given to control animals. Non-specific and specific immune response in blood samples and lochial discharge were studied on 0, 6th, 15th and 21st day post-calving. It was observed that the administration of Exapar Liquid significantly improved the local immune response in animals as evident by a significant increase in total immunoglobulins, phagocytic response and polymorphonuclear cells in treated animals.

Key words: cattle, immunity, immunoglobulin, immunodiffusion, postpartum

INTRODUCTION

Post partum fertility has a profound impact on the economic viability of dairy industry. Post-partum period is the most crucial transitory phase in bovine life when various physiological, gynaecological, biochemical and immunological changes are occurring. During this phase, cattle are exposed to increased risk of infection of uterus, as during parturition the anatomical barriers are broached and remain open for several days (Goff & Horst, 1997).

Infection of the uterus is largely influenced by the balance between bacterial contamination, the local and systemic immune status during pregnancy and parturition. Infectious diseases are more prevalent during this period because of an impaired immune status before and immediately after parturition (LeBlanc *et al*., 2002). The major classes of immunoglobulins (IgM, IgA and IgG), either by passive diffusion or local production, play an important protective role in the uterus by acting as opsonins to enhance phagocytosis, stimulating the complement pathways or blocking pathogens from adhering to mucous surfaces. Other cellular components activated in the uterus during this period include neutrophils, lymphocytes, eosinophils, mast cells and macrophages. A scientific approach towards reproductive health management of animals during this crucial phase had al-

ways been emphasized by the scientific community and a lot of research has been done to potentiate overall immune response of cows during postpartum phase (Wheeler & Wait, 1993). Immune response at local sites with the help of cellular invasion such as B- and T-lymphocytes, neutrophils, macrophages and other polymorhonuclear cells is an important defense mechanism for cows against various post partum pathogenic infections.

Indiscriminate usage of antibiotics for treatment of uterine infections has led to emergence of resistant strains. As a result of overuse and misuse, attention is now focused towards alternative treatments viz. herbal medicine (Hemiaiswarya *et al*., 2008). Many herbs are known for their strong immunopotentiating properties. Hu *et al.* (1992) have studied the influence of medicinal herbs on phagocytosis by bovine neutrophils. Herbal preparations as Uterotone, Exapar, Replanta, Involon etc. are claimed to have therapeutic efficacy (Nehra, 1987; Srinivas *et al*., 1998). The drugs capable of inducing myometrial contractions, potentiating immune response, may be useful for hastening the expulsion or to prevent the retention of foetal membranes (Singal, 1996; Tandle *et al.,* 2002).

Exapar Liquid is a unique combination of potent medicinal herbal extracts that tones up uterus for better post partum reproductive efficiency. Cleansing and restorative effects therefore, appears to be a safe and effective option both therapeutically and prophylactically. It is a combination of herbs with documented action profile e.g. *Plumbago zeylanica* (Oyedapo, 1996), *Aloe barbadensis* (Hutter *et al*, 1996), *Aristolochia indica* (Chopra *et. al*., 1982), *Gloriosa superba* (Tiwari *et. al.*, 1967), *Lepidium sativum* (Nadkarni, 1954), *Leptadenia reticulata* (Satyavati *et. al.*, 1976), *Rubia cordifolia* (Nadkarni, 1954), and others.

The current study was undertaken to explore the efficacy of the polyherbal formulation Exapar Liquid in improving the local immune response in cattle. Some earlier reports have also validated efficacy of Exapar in expulsion of membranes, regulation of lochial discharge, treatment of various uterine and involution of uterus in bovines (Singal, 1996) and in improvement of reproductive efficiency in buffaloes (Khanna *et al*., 1997).

MATERIALS AND METHODS

The research programme was conducted in the department of Gynaecology & Obstetrics, College of Veterinary and Animal Sciences, Bidar, Karnataka, India. The methodology of research trial and protocol was legally approved by the Committee for the purpose of control and supervision on experiments in animals (CPCSEA) No. 164/2005 (India) and Institutional Ethics Committee, C.V.S, Bidar, Karnataka, India. Eighteen crossbred cows in late gestation of second to fourth lactation were selected from a university dairy farm, for the experiment and were randomly allocated to two groups, control (n=6) and treatment (n=12). The cows from the treatment group were administered Exapar Liquid herbal uterine tonic and cleanser (supplied by M/S Ayurvet Ltd, Baddi, India) at a dose of 100 mL/cow twice daily orally on the day of calving followed by 50 mL Exapar Liquid daily from 2^{nd} to 5^{th} post partum days.

To study the efficacy of the polyherbal formulation Exapar in improving local immune response, samples of lochial discharge and whole blood were collected before administration of test drug (day 0) and on 6^{th}, 15^{th} and 21^{st} day

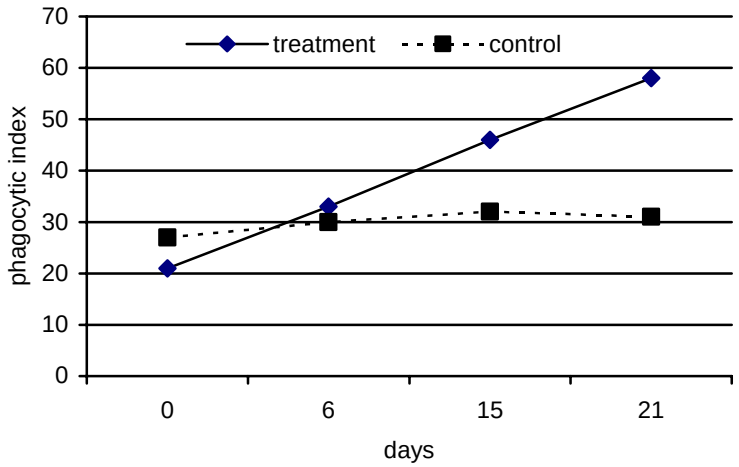

Fig. 1. Average phagocytic index (NBT-test) in post parturient cows, treated with Exapar (n=12) and untreated controls (n=6).

post calving. Various non-specific immunity parameters such as phagocytic index, total and differential leukocyte counts (TLC, DLC) were assessed in whole blood, while more specific parameters for cell mediated immune response, IgA and IgG were assessed in blood serum and lochial discharge. Phagocytic index was assessed by the nitroblue tetrazolium assay (NBT) as per Park *et al.* (1968). TLC and DLC was estimated by method of Nambiar (1960). IgA and IgG were estimated by Ouchterlony technique of agar gel immunodiffusion using anti-bovine immunoglobulins raised as per the procedure described by Hari Babu & Panda (1993).

The data was statistically analyzed using one-way ANOVA as described by Snedecor & Cochran (1980).

RESULTS

The results revealed a remarkable increase in phagocytic index from day 0 (22.25± 1.36) to day 21 (58.42±2.92) in treated cows ($P \leq 0.05$) as compared to controls, in which a non-significant variation in phagocytic index value was observed from day 0 (26.33±2.59) to day 21st (31.67±2.17) (Fig. 1).

Total and differential leukocyte counts in the treated group exhibited a significant increase from day 0 to 21, vs. the insignificant increase in untreated controls (Table 1).

Lochial discharge, collected on 21st day after calving, showed 91.67% of positive reactors for IgA content vs 16.67% in control cows (Table 2). There was not however much significant difference in serum IgA content in both treated and control groups. In the lochial discharges of the treated group, the IgA positive reactors increased from day 0 (66.67%) to day 21st (91.67%) while controls showed 66.67% positive reactors on day 0 followed by a decrease up to 16.67% on day 21. The serum samples showed 83.33% positive reactors in treated group and 66.67% in controls.

Table 1. Total and differential leukocyte counts in post parturient cows, treated with Exapar (n=12) and untreated controls (n=6). Data are presented as mean ± SEM

	Days of sampling after calving			
	0	6	15	21
Total leukocyte counts				
Control group	9.69±1.51	9.81±1.29	9.48±1.30	8.73±1.27
Treated group	9.63±0.94 [a]	10.61 ±0.91	11.53±0.88 [ab]	13.16±0.94 [bc]
Differential leukocyte counts				
Neutrophils, %				
Control group	25.00±2.16	25.83±1.17	25.00±1.24	25.00±1.37
Treated group	26.50±2.16 [a]	29.92±0.76 [b]	34.50±1.08 [c]	40.00 ±0.54 [d]
Lymphocytes, %				
Control group	62.83±1.62	58.5±1.48	63.33±1.36	63.5 ±1.31
Treated group	61.17±1.21 [a]	58.17±1.30 [b]	54.42±1.01 [c]	52.25±0.72 [d]

The values in the same column and within the same group (treatment and control) with different superscripts differ significantly (P≤0.05).

Table 2. Number (percentage) of immunoglobulin A positive reactors in lochial and serum samples from- post parturient cows, treated with Exapar (n=12) and untreated controls (n=6) in the agar gel immunodiffusion test

	Days of sampling after calving			
	0	6	15	21
Lochial discharge				
Control group	4 (66.67%)	3 (50.00%)	4 (66.67%)	1 (16.67%)
Treated group	8 (66.67%)	9 (75.00%)	8 (66.67%)	11 (91.67%)
Blood serum				
Control group	3 (50.00%)	3 (50.00%)	2 (33.33%)	4 (66.67%)
Treated group	8 (66.67%)	8 (66.67%)	6 (50.00%)	10 (83.33%)

IgG levels in both lochial discharge and serum samples exhibited a trend, similar to that of IgA (Table 3).

DISCUSSION

The salient findings of the current experimental study are relevant and have established a unique property of polyherbal formulation Exapar Liquid in improving local immune response in dairy cattle, in addition to its uterine cleansing activity.

Significantly increased phagocytic index is desirable for the sequestration of pathogenic organisms and is an important parameter of local immunity (Lee *et al*., 1992). The observed increase in the phagocytic index in treated cows is suggestive of the probable role of Exapar Liquid in immunoprotection. The results are quite encouraging and there is no literature available to compare present findings on the PI values after administration of Exapar. The constituent herbs of Exa-

Table 3. Number (percentage) of Immunoglobulin G positive reactors in lochial and serum samples from- post parturient cows, treated with Exapar (n=12) and untreated controls (n=6) in the agar gel immunodiffusion test

	Days of sampling after calving			
	0	6	15	21
Lochial discharge				
Control group	4 (66.67%)	2 (33.33%)	1 (16.67%)	2 (33.33%)
Treated group	6 (50.00%)	5 (41.66%)	7 (58.33%)	9 (75.00%)
Blood serum				
Control group	4 (66.67%)	5 (83.33%)	4 (66.67%)	2 (33.33%)
Treated group	11 (91.67%)	10 (83.33%)	9 (75.00%)	12 (100.00%)

par Liquid: *Plumbago zeylanica, Citrullus colocynthis, Aloe barbadensis, Tribulus terristris etc.* are well known for their antibacterial and immunostimulant activity (Satyawati *et al*., 1987; Hutter *et al*., 1996). The total and differential leukocyte counts were determined to study the effect of Exapar Liquid on non-specific immunity.

It can be suggested that administration of Exapar Liquid increases polymorphonuclear cells, particularly leukocytes and neutrophils, thus potentiating non-specific immune response. 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 β2-integrin and l-selectin (Tizard, 2000). The results are in accordance with that of Gilbert *et al*. (1993) revealing prophylactic use of herbal preparations in increasing the polymorhonuclear counts. Hu *et al.* (1992) also recorded accelerated phagocytosis by an increase of blood neutrophils after administration of a herbal drug in bovines. The herbal ingredients of Exapar liquid namely *Piper longum, Tribulus terristris, Cyperus rotundus* and many more are well known for their potent anti-inflammatory activity (Hussain *et al*., 1992).

The ingredients of Exapar Liquid viz. *Uraria picta, Tribulus terristris, Plumbago zeylanica, Leptidium sativum* etc. are scientifically proven to have adaptogenic and immunomodulatory properties (Satyavati *et al*., 1987). The results in present study are concomitant with those of Kumar *et al.,* (1999) who studied the immunomodulatory effect of Rasayanas – a herbal drug. Administration of Rasayanas significantly enhanced the proliferation of spleen cells and increased the number of antibody forming cells thereby improving humoral immune response through high titre of circulating antibodies.

The increase in immunoglobulin levels are compared to the normal levels of IgG and IgA in cattle as reported by Ogunrinde *et al.* (1984). Results revealed that treated post-partum cows exhibited significantly higher IgG levels in serum than in lochial discharge indicating an improvement in specific immune response by opsonization.

Evaluation of specific immune parameters in lochial discharge and blood serum has shown augmentation of both local and systemic immune response in post partum cow uterus after treatment

with Exapar Liquid. There was a remarkable increase in polymorphonuclear cells, phagocytic index and total serum immunoglobulins in the group treated with Exapar Liquid. The immunoprotective and immunomodulatory activity of Exapar Liquid can be attributed to the various herbal constituents present in the formulation which make it a unique choice for veterinarians and farmers for enhancing local immunity and prevention of various reproductive disorders in postpartum dairy cattle.

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