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Immunotherapeutic potential of *Ocimum sanctum* (L) in bovine subclinical mastitis

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Abstract

Immunotherapeutic potential of aqueous extract of *Ocimum sanctum* (*O. sanctum*) leaf in bovine sub-clinical mastitis (SCM) was investigated. Somatic cell count (SCC), total bacterial count (TBC), milk differential leukocyte count (DLC), phagocytic activity and Phagocytic index and leukocyte lysosomal enzymes like myeloperoxidase and acid phosphatase content were evaluated after intramammary infusion of aqueous leaf extract of *O. sanctum*. The results revealed that the aqueous extract of *O. sanctum* treatment reduced the TBC and increased neutrophil and lymphocyte counts with enhanced phagocytic activity and phagocytic index. Similarly, the lysosomal enzymes contents of the milk polymorphonuclear cells (PMNs) were also enhanced significantly in animals treated with the extract. The results suggest that the crude aqueous extract of *O. sanctum* (leaf) possesses some biologically active principles that are antibacterial and immunomodulatory in nature. As such, the present wok substantiates the therapeutic use of medicinal herb and also emphasizes on the potential of the commonly available non-toxic substances to enhance the mammary immunity.

Keywords: Acid phosphatase; Myeloperoxidase; *Ocimum sanctum*

1. Introduction

Plant based products constitute a major source of alternative therapy, as these medicinal plants have been exploited since centuries by various communities, for their activities against wide spectrum of diseases in man and animals. The herb *Ocimum sanctum (Lamiaceae)* belongs to a group of medicinal plant that grows in tropical region of India, where it has been called by various names depending on the geographic location, although very commonly it is known as 'Tulsi' (holy basil). The extracts of different parts of the plant have found wide use in herbal medicine. The fixed oil of the herb was found to possess significant analgesic (Singh and Majumdar, 1994), anti-inflammatory (Singh et al., 1996), immunomodulatory (Sadekar et al., 1998) and antimicrobial activity (De et al., 1999).

Mastitis is the most costly disease in dairy cattle and is usually caused by bacterial infections, eventually damages the udder tissues (Yagi et al., 2002). Antibiotics are the only proven method for treatment of mastitis, however antibiotic therapy of established mammary infection are only moderately efficacious and require prolonged milk withdrawal due to residue in milk (Daley and Hayes, 1992). Polymorphonuclear cells (PMNs) that are the primary cellular defenses of the mammary gland are depressed during periparturient period (Cai et al., 1994), whereas, most of the antibiotics

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used for the treatment of mastitis further depress the activity of the PMNs defense (Hoeben et al., 1997). One possible approach to control mastitis involves manipulation of host defense mechanism. Hence, recent strategies aimed at improving the immune cells of the diseased udder during immunosuppressive stages would greatly impact the ability of the animal to resist pathogenic infection.

World Health Organization has recommended all member countries to actively promote native medicines of their respective country (Kamboj, 2003). The use of conventional plant products described in ancient literature in modern medicine suffers from the fact that scientific evidence and explanation are lacking in most cases. Michell (2000) reviews the roots of comparative medicine and argues that during the 20th century it failed to realize its full potential. The present study, therefore, is an effort to investigate the immunotherapeutic potential of *O. sanctum* L (leaf) aqueous extract against bovine subclinical mastitis. Accordingly, the antibacterial efficacy of the *O. sanctum* leaf extract as well as its immunomodulatory potential in bovine sub clinical mastitis (SCM) was studied.

2. Materials and methods

2.1. Collection of plant material

Fresh leaves of *O. sanctum* were collected during October and November from the campus of Indian Veterinary Research Institute (IVRI), Izatnagar. The plant material was identified at National Botanical Research Institute, Lucknow (India). A voucher specimen has been kept in the laboratory for future references. The leaves were washed, shade dried and pulverized by a grinder, passed through mesh sieve and stored in sealed container.

2.2. Preparation of extract

The powdered leaves of *O. sanctum* were extracted with distilled water in soxlet apparatus upto 4 cycles (Paech et al., 1956). The extracted material was filtered through sterile muslin cloth and the filtrate obtained was evaporated to dryness in vacuo below 40 °C.The yield was 9.25% w/w with respect to dry powdered material for *O. sanctum.* The dried powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4, 0.01 M) at the rate of 20 mg of extract per ml of phosphate buffer saline. The dose of *O. sanctum* extract was standardized in the pilot study, by taking 3 cows in 2 batches having sub-clinical mastitis. Finally, the filtrate was filtered through membrane filter (pore size 0.45 μm) and stored at −20° till used for intramammary infusion.

2.3. Selection of animals and experimental protocol

Sixty crossbred lactating cows were selected from an organized dairy farm (Cattle & Buffalo), IVRI, Izatnagar. These cows were maintained in the animal shed of the institute under identical environmental conditions and were divided in 4 equal groups. Group I and Group II consisting of 30 clinically healthy cows served as healthy control. Fifteen cows in Group III and 15 cows in Group IV (30 cows) positive for SCM, screened on the basis of CMT positive reaction (Schalm et al., 1971) were taken for the drug trial. 100 mg of sterile *O. sanctum* extract was infused per teat by intramammary route in Group II and Group III cows, after diluting the drug in 5 ml sterile phosphate buffer saline, once daily for 7 days, similarly 5 ml sterile phosphate buffer saline was infused by intramammary route in Group IV cows for 7 days.

2.4. Milk sampling

Fifty millilitre of milk from each cow was collected in sterile vials after cleaning the teat orifice with 70% ethyl alcohol and after discarding few streams of milk. The milk was collected on day 0, 3, 7, 15 and 30 post treatment. The somatic cell count (SCC) of the milk samples was done as per method of Schalm et al. (1971). Total bacterial count (TBC) was carried out by the method of Griffin et al. (1977), on 5% bovine blood agar plates. The organisms were identified on the basis of colony morphology, characteristic hemolytic pattern and Gram's staining.

2.5. Milk differential leukocyte count

Milk differential leukocyte count (DLC) in milk was done as previously described by Dulin et al. (1982). Numbers of neutrophil and lymphocyte were counted in 100 cells and expressed in percentage.

2.6. Isolation of the milk polymorphonuclear cells

The isolation of isolation of the milk polymorphonuclear cells (PMNs) from the milk samples was carried out as per the method of Daley et al. (1991). In brief, 50 ml of milk was passed through cheese cloth and then the milk was poured into 50 ml conical tubes and centrifuged at 200*g* for 30 min at 4 °C (Sorvall RT 6000, DUPONT). The fat was removed, and the skim milk was poured off and discarded. PMN cell pellet was washed twice resuspending in sterile PBS. After final wash, cells were resuspended in 1 ml of PBS. Differential leukocyte count was performed to ascertain the PMN cells. Viability of the cells was checked by trypan blue (SRL, India) exclusion technique (Colligan et al., 1994), the cell suspension was adjusted to 1×10^7 and 1×10^6 cells/ml, in