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ORIGINAL ARTICLE

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Evaluation of a polyherbal topical aerosol spray as a supportive therapy for clinical mastitis in dairy cows

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A B S T R A C T

The present study was designed to evaluate the polyherbal topical aerosol spray Wisprec® Advanced (M/S. Natural Remedies Private Limited, India) as a supportive therapy for clinical mastitis in dairy cows. A total of 41 dairy cows suffering from clinical mastitis were selected, and Wisprec® Advanced was sprayed on mastitis affected quarters of udder two times a day along with a parenteral antibiotic till complete recovery. The rectal temperature, pain on palpation of udder, swelling of udder, consistency of milk, recovery period and product satisfaction score were assessed to evaluate the efficacy of Wisprec® Spray. Topical application of Wisprec® Advanced Spray have shown a significant improvement (P<0.001) in alleviation of rectal temperature, pain on palpation of udder and swelling of udder, and the consistency of milk was restored to normal after 3 to 4 days of treatment. The results demonstrate that the Wisprec® Advanced spray could be considered as an alternative to non-steroidal anti-inflammatory drugs (NSAIDs) as a supportive therapy for clinical mastitis of dairy cows.

Keywords

Anti-inflammatory, Clinical mastitis, Dairy cows, Polyherbal, Supportive therapy, Wisprec® Advanced

ARTICLE HISTORY

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INTRODUCTION

The term mastitis refers to the inflammation of the mammary gland caused by microorganisms, usually bacteria which enter the udder, multiply and produce toxins which are detrimental to the mammary gland. Mastitis is characterized by physical, chemical and bacteriological modifications in the milk as well as pathological changes in the glandular tissues of the udder (Radostitis et al., 2006). Globally mastitis is one of the important diseases in dairy cattle because of the high incidence and its associated production losses. Until recent past, mastitis was primarily a concern of dairy farmers and dairy processors; because of worries about antimicrobial resistance, antimicrobial residues, milk quality and animal welfare, it has also become a concern to consumers and society (Hogeveen et al., 2011).

The pain suffered by cows due to mastitis is an obvious welfare issue and moreover cows suffering from pain often have a reduced appetite, are less willing to move to sites of available food and have reduced milk yields, with concomitant deleterious effects on farm economics. In mastitis, inflammatory reaction and pain is the outcome of glandular and the alveolar tissues damage, initiating a series of reaction at the cellular and molecular level. The cellular damage leads to the synthesis of prostaglandins which are potent mediators of inflammation and in turn produce multiple responses like hyperalgesia, vasodilation and increase in permeability of blood vessels resulting in erythema, edema, pain and heat. White blood cells (leukocytes) are emigrated into the teat canal and udder tissues in response to bacterial invasion and appear in the milk in the form of somatic cells (Souza et al., 2012).

Treatment and management of mastitis relies on attenuation of pain and inflammation since majority of the physiological changes and pathological lesions associated with clinical mastitis is result of the inflammatory reaction to infection. Hence administration of anti-inflammatory agents to alleviate the inflammation and pain along with antibiotics, checks further tissue damage and spread of infection, facilitates management of mastitis easier, accelerate recovery from ailment and ensures good quality milk securing welfare and economic benefits to animals and dairy farmers respectively. Glucocorticoids and Non-steroidal Anti-inflammatory Drugs (NSAIDs) are the classical anti-inflammatory agents frequently used in treatment and management of bovine mastitis. Glucocorticoids and NSAIDs inhibit the release and metabolism of arachidonic acid respectively (Morin, 2004) thus hindering the synthesis and release of prostaglandins, leukotrienes and thromboxanes thereby exerting their anti-inflammatory, analgesic, antihyperalgesic and antipyretic effects.

However, indiscriminate and long term use of NSAIDs are associated with a broad spectrum of adverse effects such as gastrointestinal and cardiovascular events, renal toxicity, hypertension and deterioration of congestive heart failure among others (Sostres et al., 2013) due to inactivation of the protective physiological responses exerted by prostaglandins on gastric mucosal membranes, vascular endothelium and renal tissues. Adverse effects of these drugs can be life threatening and due diligence by the prescribing veterinarian in drug and dose selection and monitoring is always indicated. Moreover synthetic parenteral anti-inflammatory formulations are quite expensive and repetition of the doses makes the treatment probably cost-prohibitive. Also many reports have suggested that there are certain categories of prescribed anti-inflammatory drugs which are not labeled for their application in lactating dairy cows (Morin, 2004). It further raises the concern about extra-label drug use and appearance of these drug residues in milk and meat making it unfit for human consumption and increases the milk and meat withdrawal period.

In recent years, extensive research and pharmacological evaluation of active principles of distinct herbal species and families have unveiled the remarkable potency and efficacy of herbal medications in effective management of pain and inflammatory conditions with additional advantages and benefits such as free from adverse effects, tolerance and economical remedy. Various extracts and essential oils derived from plants species have exhibited the significant analgesic and anti-inflammatory effect in animal models (Benni et al., 2011; Ashraf et al., 2013; de Cassia da Silveira e Sa et al., 2014) and used in the treatment of mastitis in dairy animals (Hase et al., 2013; Waghmare et al., 2013). Their activities have been anticipated because of the presence of secondary plant metabolites (phytochemicals) such as monoterpenoids, triterpenoids, alkaloids, glycosides, flavonoids, sterols, tannins, saponins and glucosinolates *etc*. With this viewpoint and growing acceptance of alternative herbal therapy, the present study was carried out to evaluate the topical polyherbal aerosol formulation Wisprec® Advanced Spray as a supportive therapy for clinical mastitis in lactating dairy cows.

MATERIALS AND METHODS

Ethical approval: The study conducted complies with the guidelines laid down by the Institutional Ethics Committee. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. In addition, an informed consent was taken from dairy farmers regarding the inclusion of their dairy cows in this clinical trial.

Study design: Wisprec® Advanced Spray (M/S. Natural Remedies Private Limited, Bangalore, Karnataka, India) is a combination of essential oils of oil of castor (*Ricinus communis*), tulasi (*Ocimum sanctum*), eucalyptus (*Eucalyptus globulus*) and menthol isolated from peppermint (*Mentha piperita*) as excipients (**Table 1**). An open-label veterinary clinical trial comprising a total of 41 lactating cross-bred dairy cows (Holstein-Friesian and Jersey) suffering from clinical mastitis presented at Government Veterinary Dispensaries of Tamilnadu and Kerala states, India were conducted between December 2013 to June 2014. The mastitis was diagnosed based on the clinical signs exhibited by dairy cows. Wisprec® Advanced was sprayed in sufficient quantity to cover the affected quarters of udder two times a day along with a parenteral antibiotic. The dairy animals were observed for the parameters like rectal temperature, pain on palpation of udder, swelling of udder and consistency of milk; mean product satisfaction score and recovery period were calculated in order to evaluate the efficacy of Wisprec® Advanced Spray following topical application. The investigational parameters were quantified and graded using a scoring system as presented in **Table 2**.

Table 1. Composition of Wisprec® Advanced spray

Each 10 mL contains	
Ricinus communis seed oil	0.890 g
Ocimum sanctum leaf oil	0.089 g
Eucalyptus globulus leaf oil	0.445 g
Menthol as excipients	*q.s.*

q.s. quantum sufficit

Table 2. Scoring System for Study Parameters

Parameters	Description
Rectal Temperature Score	
1	Normal (101 - 103° F)
2	Fever (>103° F)
Pain Score	
1	No pain on palpation of udder
2	Presence of pain on palpation of udder
Swelling Score	
1	No swelling (completely diminished)
2	Mild reduction
3	Swelling observed
Milk Consistency Score	
1	Presence of blood
2	Presence of clots and flakes
3	Watery milk
4	Normal consistency
Product Satisfaction Score	
1	Not satisfied
2	Satisfied
3	Highly satisfied

Data collection and statistical analysis: All study parameters were assessed and recorded by Veterinarians using raw data sheets controlled by the Principal Investigator (PI). All raw data sheets were verified by the PI for clarity and completeness and duly signed by PI. Upon completion of the trial, these raw data were compiled, processed and expressed as Mean ± S.E.M. The data were subjected to statistical analysis using paired t-test (IBM SPSS Statistics Version.21.0; SPSS Inc., Chicago, IL, USA) to draw a comparison between pre-treatment and post-treatment values for each investigational parameter and $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Rectal temperature, pain and swelling score: Of 41 dairy animals enrolled in the study, 66% (n=27) were presented with fever (rectal temperature above 103° F) and 34% (n=14) exhibited a normal rectal temperature (101 to 103° F) prior to initiation of treatment with Wisprec® Advanced Spray. Following Wisprec® Advanced Spray treatment, a significant improvement (reduction, $P<0.001$) was observed in temperature score as compared to pre-treatment and rectal temperature was restored to normal in about 98% animals (**Table 3**). There was a statistically significant reduction in pain score ($P<0.001$) and swelling score ($P<0.001$) upon treatment with Wisprec® Advanced Spray (**Table 3**). About 35 and 31 animals were found to be completely relieved from pain and swelling of udder respectively. However, nine cows have shown a mild to moderate reduction and one cow have shown no improvement in swelling of udder upon treatment with Wisprec® Advanced Spray.

Table 3. Effect of Wisprec® Advanced Spray in Dairy Cows suffering from Clinical Mastitis.

Parameters	Score		Success (%)
	Pre–treatment	Post–treatment	
Rectal Temperature	1.66 ± 0.07	$^{\$}$1.05 ± 0.03	95.12
Pain	1.98 ± 0.02	$^{\$}$1.20 ± 0.06	80.49
Swelling	3.00 ± 0.00	$^{\$}$1.29 ± 0.09	95.12
Milk Consistency	2.59 ± 0.10	$^{\$}$3.95 ± 0.05	97.56
Product Satisfaction	-	2.51 ± 0.08	100.00
Recovery Period (d)	-	3.68 ± 0.15	NA

Values are expressed as Mean ± S.E.M; n=41; d – days
$^{\$}P<0.001$ as compared to pre-treatment by paired t-test

Inflammation is an intrinsically beneficial event leading to removal of offending factors and restoration of tissue structure and physiological function and is characterized by the rapid influx of blood granulocytes (Nathan, 2002) and these cells in turn secretes the inflammatory mediators such as prostaglandins, leukotrienes and thromboxanes. Prostaglandins play a vital role in the generation of the inflammatory response and their biosynthesis is contributing to the development of the cardinal signs of inflammation such as erythema, edema and pain (Funk, 2001). The essential oils of tulasi (*O. sanctum*), eucalyptus (*E. globulus*), menthol isolated from peppermint (*Mentha piperita*) and oil of castor (*R. communis*) present in Wisprec® Advanced Spray have been scientifically well known to possess anti-inflammatory activities in *in vivo* and *in vitro* systems. In the present study, mastitis cases were presented with two cardinal signs of inflammation, swelling of udder and presence of pain on palpation of udder; the topical application of Wisprec® Advanced Spray produced a significant relief of dairy animals from pain and swelling of udder demonstrating the peripheral analgesic and anti-inflammatory effect of constituents present in the Wisprec® Advanced Spray. The peripheral analgesic effect is generally mediated through inhibition of cyclooxygenase and/or lipoxygenase and other inflammatory mediators or inhibition of pain responses mediated by peripheral nociceptors (Duarte et al., 1988). Wisprec® Advanced Spray was also found to be very effective at alleviation of inflammation in TPA

Table 4. Effect of Wisprec® Advanced Spray in Dairy Cows suffering from Clinical Mastitis at Different Stages of Lactation.

SL	Stage of Lactation	Parameter	Score		Success (%)
			Pre-treatment	Post-treatment	
1	Early (n=22)	Rectal Temperature	1.64 ± 0.10	$1.09 ± 0.06	90.91
		Pain	1.95 ± 0.05	$1.23 ± 0.09	77.27
		Swelling	3.00 ± 0.00	$1.50 ± 0.14	90.91
		Milk Consistency	2.59 ± 0.14	$3.91 ± 0.09	95.45
		Product Satisfaction	-	2.59 ± 0.11	100
		Recovery Period	-	3.68 ± 0.20	NA
2	Mid (n=14)	Rectal Temperature	1.71 ± 0.13	$1.00 ± 0.00	100
		Pain	2.00 ± 0.00	$1.07 ± 0.07	92.86
		Swelling	3.00 ± 0.00	$1.07 ± 0.07	100
		Milk Consistency	2.64 ± 0.17	$4.00 ± 0.00	100
		Product Satisfaction	-	2.57 ± 0.14	100
		Recovery Period	-	3.50 ± 0.25	NA
3	Late (n=05)	Rectal Temperature	1.60 ± 0.24	1.00 ± 0.00	100
		Pain	2.00 ± 0.00	*1.20 ± 0.20	80
		Swelling	3.00 ± 0.00	$1.20 ± 0.20	100
		Milk Consistency	2.40 ± 0.24	^4.00 ± 0.00	100
		Product Satisfaction	-	2.00 ± 0.00	100
		Recovery Period	-	4.20 ±0.49	NA

Values are expressed as Mean ± S.E.M; d - days; NA - Not Applicable
*P<0.05, ^P<0.01 & $$P$<0.001 as compared to pre-treatment by paired t-test

induced ear edema model in *Swiss albino* mice (Babu et al., 2014) wherein it produced a significant reduction in rubor score and ear edema following topical application and it further supports the analgesic and anti-inflammatory effect of Wisprec® Advanced Spray observed in the present study.

Linolenic acids (Gopinath et al., 2011) and eugenol (Daniel et al., 2009) present in *O. sanctum* oil has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism, thereby synthesis of prostaglandins and leukotrienes and could be responsible for anti-inflammatory activity. The phytochemical active principle, 1, 8-cineole present in eucalyptus oil is a novel natural antagonist of TRPA-1 receptors (Takaishi et al., 2012) which are known to be involved in processing of noxious cold temperature signals and inflammatory processes. Menthol present in Wisprec® Advanced Spray, after topical application causes a sense of coolness due to the stimulation of cold receptors by inhibiting Ca^{++} currents of neuronal membranes, has anti-inflammatory properties by inhibition of release of leukotrienes, prostaglandins and interleukins from monocytes and of serotonin and neuropeptides (Peier et al., 2002).

Milk consistency score, recovery period and product satisfaction: Prior to treatment, of 41 dairy cows enrolled the milk consistency was found to be normal in 3 cows, watery in 18 cows; clots and flakes were observed in milk of 20 dairy cows. Following treatment with Wisprec® Advanced Spray, there was a significant improvement (P<0.001) in the milk consistency score. Milk consistency was restored to normal in all dairy cows treated topically with Wisprec® Advanced Spray between two to five days. The recovery period (Mean ± S.E.M, time taken in days for complete recovery of milk consistency to normal) in dairy animals treated with Wisprec® Advanced Spray was 3.68 ± 0.15 (**Table 3**). Milk consistency was restored to normal as early as 2 days in 2 cows, 3 days in 18 cows, 4 days in 9 cows and 5 days in 9 cows. As a whole the veterinarians involved in the investigation were satisfied with the performance of the product as a supportive therapy for the treatment of their respective clinical mastitis cases (93%). Of 38 cases of successful recovery, three cows with udder pain and seven with mild reduction in swelling of udder have also been graded as satisfied by veterinarians as there was restoration of milk consistency to normal.

In mastitis, the invading bacterial pathogens in the mammary gland triggers the masses of granulocytes pass between the milk producing cells into the lumen of the alveoli, consequently increasing the somatic cell counts and also damaging the secretory cells. The increased number of leukocytes in milk will cause an increase in the number of somatic cells. The clots or flakes are formed by aggregation of leukocytes and blood clotting factors which may block the ducts and prevent complete removal of milk (Jones, 2006). In the present study, topical application of Wisprec® Advanced Spray was found to improve the consistency

of milk to normal from watery and clots / flakes form indicating the improved health status of mammary glands, resolution of infection and inflammation in the udder

Effects of Wisprec® Advanced spray in mastitis of dairy cows at different stages of lactation: Of 41 dairy cows enrolled in the study, 22, 14 and 5 dairy cows were at early, mid and late stage of lactation respectively. The response of mastitis affected animals at different stages of lactation to topical application of Wisprec® Advanced Spray is presented in **Table 4**. The topical application of Wisprec® Advanced Spray two times a day produced a statistically significant improvement in all study parameters in dairy cows suffering from clinical mastitis at all stages of lactation.

Antibiotics are routinely used for treatment of mastitis, but efficacy of supportive treatment such as non-steroidal anti-inflammatory agents has also been studied in combination with antibiotic treatment and known to be beneficial at reducing the inflammation, pain and fever in clinical mastitis cases (Hogeveen et al., 2011). It is also proven that administration of combination of antibiotic and anti-inflammatory drugs resulted in a lower somatic cell count and reduced risk of culling of lactating dairy cows from the herd as compared with the antibiotic monotherapy (McDougall et al., 2009). Our results demonstrate that topical application of Wisprec® Advanced Spray twice a day could alleviate the symptoms of pain, inflammation and fever and complete recovery of cows from mastitis between three to four days of treatment; the veterinarians involved in the study found it performing satisfactorily against treatment of their respective mastitis cases in lactating dairy cows. The anti-inflammatory and analgesic effects of Wisprec® Advanced Spray observed in the present study could be attributed to phytoactive compounds, 1, 8-cineole, eugenol, linolenic acids and menthol.

Topical applications offer advantages of blocking the pain at the site of inflammation, with maximum active drug concentration and minimal systemic effects (de Paula et al., 2010) and are known to have better safety profile and avoid issues associated with oral, intramuscular or intravenous routes such as gastric disturbances, first-pass hepatic metabolism and inconstant serum concentrations (Jorge et al., 2011). Topical applications also have advantages over hypodermic injections, which are known to be painful, generate dangerous medical wastes and pose the risk of disease transmission by needle re-use (Miller and Pisani, 1999). In addition, topical application is generally inexpensive, non-invasive and can be applied to the animals by farmers themselves obviating the help of veterinarian.

CONCLUSION

The topical polyherbal aerosol spray Wisprec® Advanced was effective at alleviation of fever, pain and swelling of udder and restoration of milk consistency to normal after three to four consecutive days of application and our results also indicates Wisprec® Advanced possesses the anti-inflammatory drug-like properties and could be an alternative to parenteral NSAIDs as a supportive therapy for clinical mastitis in dairy cows. Further studies need to be carried out in a large population to evaluate the relative and comparative efficacy and advantages of Wisprec® Advanced spray over NSAIDs.

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