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Evaluation of Wisprec Advanced Spray and Certain Phytopharmaceutical Prototypes for their Topical Anti-inflammatory Effect

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Abstract

Wisprec advanced spray, a natural essential oil based anti-inflammatory preparation and two other herbal prototypes were evaluated for topical anti-inflammatory activity. Topical anti-inflammatory activity was tested by inducing inflammation by application of 12-*O*-Tetradecanoylphorbol-13-acetate (TPA), a known inflammatory agent. Results revealed that Wisprec Advanced spray had potent topical anti-inflammatory effect.

Keywords: 12-*O*-Tetradecanoylphorbol-13-acetate (TPA), topical anti-inflammatory activity, Wisprec advanced

1. Introduction

Inflammation and pain in dairy animals is manifestation of numerous disease related conditions influencing the overall animal well-being as well as their productive and reproductive capabilities. Mastitis is defined as the inflammation of the mammary gland [1] and udder tissue and is one of the major endemic diseases of dairy cattle [2]. It typically occurs as an immune response against the bacterial invasion to the teat canal by variety of bacterial and other infectious sources. It also occurs as a consequence of chemical, mechanical or thermal injury [3, 4] to the cow's udder. There will be visible changes in the udder as a result of disease and significant alterations in the appearance, production and quality of milk.

In mastitis, inflammatory reaction is the outcome of glandular and the alveolar tissues damage, initiating the series of reaction at the cellular and molecular level. The cellular damage leads to the synthesis of prostaglandins which are potent inflammation mediators. They produce multiple responses like hyperalgesia, vasodilation and increase in permeability of blood vessels resulting in to erythema, oedema, pain and heat. White blood cells (leukocytes) are liberated into teat canal and udder tissues in response to bacterial invasion and even appear in the milk in the form of somatic cells. Milk-secreting cells and other lobular ducts all throughout the mammary gland system are destroyed by bacterial toxins with the visible reduction in milk yield and sometimes may cause the permanent damage to the udder tissues with permanent loss in milk production. Symptoms of clinical mastitis can be acute or chronic; the severely acute cases are often fatal but even in dairy animals that recover from the clinical condition there are the consequences of decreased milk production and the chances of reoccurrence of the disease in the rest of the lactation period and subsequent lactations.

Treatment and management of mastitis relies on attenuation of pain and inflammation since majority of the physiological changes and pathological lesions [5] associated with clinical mastitis are result of the inflammatory reaction to infection. Using anti-inflammatory agents

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to alleviate the inflammation and pain help inhibiting the further tissue damage. They check the spread of the infection, make management easier, hasten recovery from ailment and ensure good quality milk securing economical and welfare benefits to animals and dairy farmers.

Glucocorticoids (GC) and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are the classical anti-inflammatory agents frequently used in treatment and management of bovine mastitis disease. These agents reduce inflammation (anti-inflammatory), reduce pain (analgesic), reduce pain sensitivity (antihyperalgesic) and decrease overall body temperature (antipyretic).They inhibit eicosanoid production by checking arachidonic acid release (GC) and metabolism (NSAIDs) [6] and thus inhibit cyclooxygenase (COX) pathway and prostaglandin synthesis.

However, indiscriminate use and long term administrations of NSAID are substantially associated with high risk of lethal and toxic adverse effects causing gastric ulcers [7] and severe gastrointestinal mucosa damage [8] due to the inactivation of the protective cyclooxygenase enzyme-1 (COX-10) in gastric mucosa [3, 9]. In addition to that synthetic systemic 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 herds. It further raises the concern about the appearance of these drug residues in milk and meat making it unfit for human consumption.

In recent years, extensive research studies and pharmacological evaluation of active principles of distinct herbal species and families, used in Indian traditional system of medicine have unveiled the remarkable potency and efficacy of herbal medication in effective management of pain and inflammatory conditions. The additional advantages and benefits of indigenous ancient medicinal therapy substantiate its complementary nature to the conventional treatment. They produce minimal side effects, are well tolerated and economical remedy for inflammatory disease conditions [9, 10]. Various plant extracts and essential oils derived from plants species with formerly unknown pharmacological activities have exhibited the significant analgesic and anti-inflammatory effect in animal models used in pharmacological studies. Their effects have been anticipated owing to the presence of secondary plant metabolites (phytochemicals) monoterpenoids triterpenoids, alkaloids, glycosides, flavonoids, tannins, saponins, glucosinolates and sterols etc.

With this viewpoint and growing acceptance of alternative herbal therapy, the present investigation was undertaken to comparatively evaluate the poly herbal formulations for their possible potency and efficacy in the alleviation and management of pain and inflammation.

The polyherbal formulation NR-WR-01 *contains* extracts of herbs viz. *Cymbopogon citratus, Ocimum sanctum and Cucurma longa*, NR-W1-01 (WISPREC ADVANCED SPRAY) contains *Eucalyptus globulus Ocimum sanctum, Ricinus communis, Menthol* and NR-MP-01 contains extracts of *Eucalyptus globulus, Cucurma longa, Paedaria foetida, Glycyrrhiza glabra, Cedrus deodara*.

2. Materials and Methods

TPA-induced mouse ear edema method [11] had been used to evaluate the anti-inflammatory activity of the phytopharmaceutical formulations. TPA was obtained from Sigma., USA. Animals were procured from CPCSEA certified supplier and were housed at institute's animal house for quarantine before experiment. Animals were grouped in to three groups comprising seven animals in each. Edema was induced in ears of each mouse by the topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer ear surfaces. Thirty minutes after the application of TPA, the inner and outer surface of right ear was treated with test candidate (10mg). The left ear was left untreated and considered as reference control. The test candidate's ability to reduce the inflammation was taken as measure for calculating the anti-inflammatory activity and was detected by measuring change in ear edema thickness. The thickness of ear was measured using a micrometer (Mitutoyu PK-1025) before applying TPA and at 4hrs and 24hrs after TPA application. The measurement was taken near the top of the ear distal to the cartilaginous ridges. Rubor (redness) at the site of inflammation was measured visually and scored among the groups.

3. Statistical Analysis

The TPA induced edema in right treated ear was compared with left untreated ear and analyzed by using student 't' test with the significance set at 0.05. The results had been

expressed as mean ± SEM. Statistics were calculated with the help of online GraphPad statistical software.

4. Results and Discussion

TPA induced edema in applied ears of mice in all groups. The edema observed after 4 hours of TPA application was found to be low in treated ears of two groups; NR-WI-01 and NR-WR-01 whereas edema increased in one group treated with NR-MP-01. Out of all the formulations tested NR-WI-01 was found to be most effective in reducing topical inflammation induced by TPA by up to 55% at 4 hrs. (Table 1 and Fig.1). After 24 hrs, all the formulations seemed to be protective and showed anti-inflammatory activity. It was interesting to note that NR-MP-01 an ointment which did not show any protection at 4 hrs. interval had shown protection up to 38% closer to 44% offered by NR-WR-01 which is a liquid formulation whereas NR-WI-01 had shown the highest protection of up to 68% a day after TPA application (Table 2 and Fig. 2).

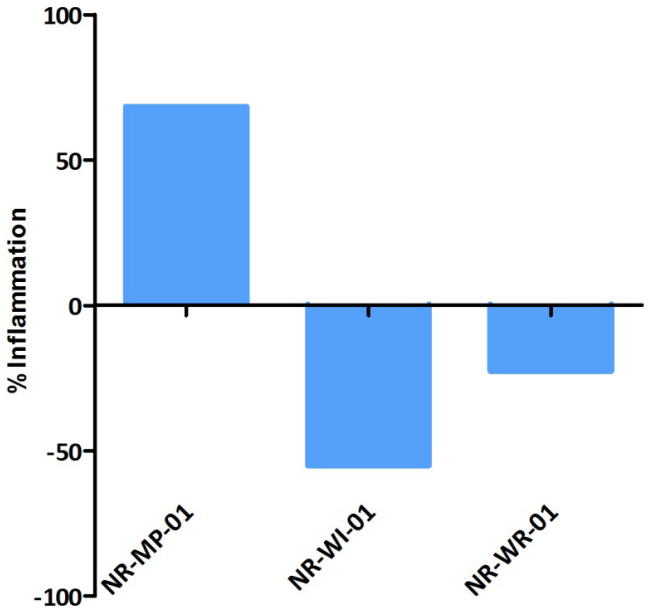


Fig. 1. Percentage inhibition of topical inflammation at 4hrs interval after TPA application by three herbal formulations.

Table 1: Showing TPA induced edema in treated ears and control ears after 4hrs of TPA application

	NR-MP-01		NR-WI-01		NR-WR-01	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Mean	**0.2886***	0.1714	**0.1217***	0.2714	**0.2443***	0.3157
SD	**0.0782**	0.0273	**0.0739**	0.0418	**0.0752**	0.0556
SEM	**0.0296**	0.0103	**0.0302**	0.0158	**0.0284**	0.021
% Inhibition	**68.37806301**		**–55.15843773**		**–22.61640798**	

*Statisitcally significant with 'p' value <0.02

Table 2: Showing TPA induced edema in treated ears and control ears after 24hrs of TPA application

	NR-MP-01		NR-WI-01		NR-WR-01	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Mean	**0.31***	0.50	0.23*	0.72	0.39*	0.69
SD	**0.06**	0.09	0.07	0.12	0.10	0.15
SEM	**0.02**	0.03	0.03	0.05	0.04	0.06
% inhibition	**-37.92**		**-68.06**		**-44.45**	

*Statistically significant at 'p' value <0.01

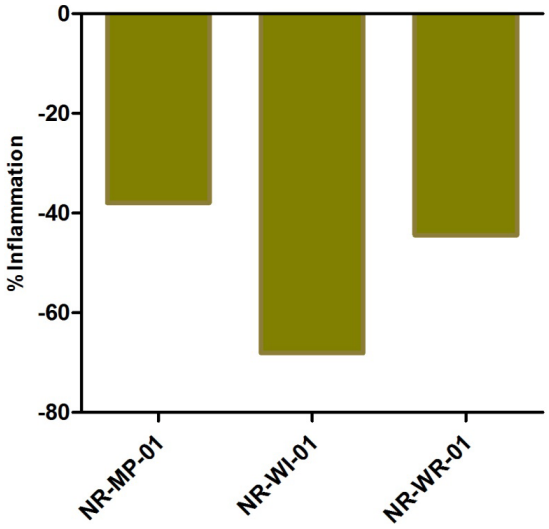


Fig. 2. Percentage inhibition of topical inflammation at 24hrs interval after TPA application by three herbal formulations.

Rubor (Redness): One of the cardinal signs of inflammation is rubor due to increased blood supply to the site of damage. During the study period the ears of all groups were visually observed, scored and were found that mice treated with NR-WI-01 had less reddening of the ears compared to other groups indicating low inflammation. (Fig. 3)

Table 3: Rubor scoring measured visually by investigator (Higher the + means higher the rubor)

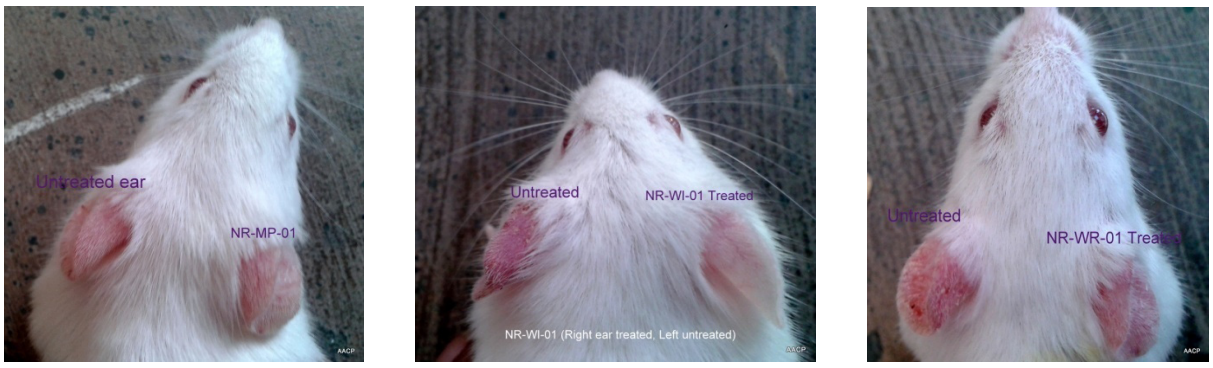
Treatment	Untreated	NR=MP-01	NR-WI-01	NR-WR-01
Rubor score @ 4 Hr	++++	++++	++	+++
Rubor Score @ 24 Hr	+++++	++	+	++

+: more number of plus sign indicates redness

NR-WI-01 (Wisprec Advanced) is an unique combination of essential oils of *Eucalyptus globulus, Ocimum sanctum* and *menthol* formulated in castor oil base. It is a clinically proven and excellent aerosol spray endowed with potent anti-inflammatory activity and helps in the effective management of pain and inflammation in clinical mastitis [18]. The oils derived from *Eucalyptus globulus* have magnificent anti-inflammatory action which is well demonstrated by Silva J *et al.*, in the variety of animal models [12]. They proposed that the oils of *Eucalyptus globulus* have analgesic property through peripheral and central actions.

The phytochemical active principle possessing the anti-inflammatory effect in eucalyptus oil is identified as 1, 8 Cineole. A variety of biological models studies have indicated the potent anti-inflammatory effect of 1, 8 cineole. Takaishi M *et al.,* have shown that 1, 8 cineole is a novel natural antagonist of TRPA-1 receptors [13]. TRPA-1 receptors are known to be involved in processing noxious cold temperature signals. These receptors are also known to be involved in inflammatory processes. Molecular mechanism studies have shown that 1, 8 cineole inhibits the activity of NF-kappa B, a key regulator of inflammation in cells. More specifically 1,8 cineole has been shown using reporter gene assays to prevent the translocation of NF-kappa B P65, which plays a vital role in initiating gene expression of proteins involved in inflammation [14]. Production of tumor necrosis factor-alpha, interleukin-1beta, leukotriene B4 and thromboxane B2 are also inhibited by 1,8 cineole in monocytes stimulated by LPS [15].

Eugenol a phenylpropanoid found in essential oil of *Ocimum sanctum* has been shown to inhibit prostaglandin E2 in lipopolysaccharide stimulated RAW



NR-MP-01 NR-WI-01 NR-WR-01

Fig. 3. Photographs showing rubor in each treated group mouse.

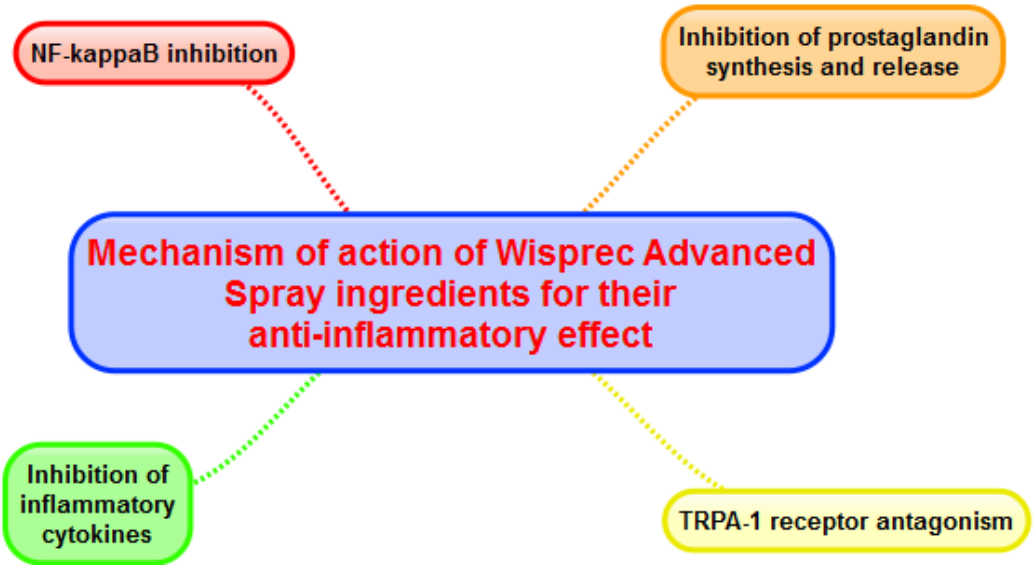


Fig. 4. Illustration of mechanism of action of active principles present in Wisprec Advanced Spray.

264.7 cells. Eugenol also inhibits the cyclooxygenase-2 gene expression in LPS stimulated macrophages. Thus, inhibition of cycloxygenase pathway may be the mechanism of action of *Ocimum sanctum* essential oil [16]. The essential oil extract, menthol present in NR –WI-01 (Wisprec Advanced spray) is well known for its cooling and local anesthetic effect. Menthol is also reported to have local anesthetic effect in vivo in the rabbit conjunctivial reflex test and in vitro in a rat phrenic nerve hemidiapharagm preparation [17]. The probable mechanism of action of Wisprec advanced spray as deduced from its ingredients and phytochemical actives is summarized in Fig. 4.

5. Conclusion

Udder inflammation and pain are the classical manifestations in bovine clinical mastitis. Among all the three topical anti-inflammatory formulations compared, NR-WI-01 (Wisprec Advanced Spray) is found to be most potent. It is clinically proven to reduce udder inflammation, it is further supported by the current invivo study. 1, 8-Cineole, Eugenol and Menthol are the key phytochemical active principles present in Wisprec advanced spray.

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