

Dermal Spray Formulation of Meloxicam

Sagar Singh Kang, Kiran Jyoti

Abstract--- Pain and inflammation, often seen in the form of “edema” (swelling) is one of the most common conditions which affect the animals due to injuries. Inflammation is a localised protective response stimulated by injury or destruction of tissues, which serves to dilute, destroy or wall off both the injurious agent and the injured tissue. The classic signs of inflammation are heat, redness, pain, swelling and loss of function. Numerous numbers of treatments via oral and parenteral route of administration are available for treating the conditions of pain and inflammation in animals. Though meloxicam demonstrated good oral bioavailability (76%) in cattles; however; oral and parenteral administration of meloxicam are associated with GI distress, vomiting, soft stools, diarrhea, in appetite and suboptimal concentration at the site of inflammation. NSAIDs skin delivery offers a number of benefits over the oral route associated with potential side effects. Therefore, the aim of this study is to provide a dermal spray formulation of a meglumine salt of meloxicam for the treatment of pain and inflammation in cattles. Meloxicam spray was then estimated with respect to different physicochemical parameters such as pH, drug content and Homogeneity.

Keywords--- Inflammation, edema, swelling, parenteral route, meloxicam, NSAID, dermal, meglumine

I. INTRODUCTION

Non-steroidal anti-inflammatory drugs are amongst the most commonly prescribed group of drug. These drugs are used in the treatment of various rheumatic diseases, including rheumatoid arthritis (RA), as well as in the treatment of osteoarthritis, low back pain and some joint diseases. NSAIDs’ action mechanism is reversible cyclooxygenase enzyme (COX) inhibition and reducing the prostaglandin synthesis[1]. Though, these drugs lead to the unfavorable effects particularly on stomach as an effect of inhibition of the prostaglandins (PGs), which play a main role in the protection of gastric mucosa in the systemic administration[2].

Calf survival and health are the predominant concerns of the cow-calf producers. The difficulty experienced during the birth process is an important factor that affects the health and survival of the calf. Assisted calves have a higher and less vigorous chance of injury and lack of oxygen. This can result in delayed intake of colostrum[3]. Inadequate ingestion of colostrum of good quality leads to failure of passive immunity transfer associated with mortality, preweaning morbidity, and lower ADG. For the management of cattle pain, non-steroidal anti-inflammatory drugs (NSAIDs) are increasingly being used[4]. Practical strategies that can mitigate the effects of a difficult calving and boost passive immunity transfer are important in ensuring the safety and survival of the calf and maximizing the benefit for cow-calf producers. Chemical, physical, and biological agents can trigger the inflammatory response, including mechanical trauma or infectious agents such as viruses, bacteria, and other pathogenic microorganisms[5]. Heat, swelling, redness, discomfort, and loss of function are the classic signs of

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inflammation. There are numerous oral and parenteral treatments available for the treatment of animal pain and inflammation conditions (mastitis in cattle)[6].

1.1 MELOXICAM

Meloxicam (MLX) is a powerful enolic acid class non-steroidal anti-inflammatory drug (NSAID). This prevents COX-2 preferentially and is thus used to treat osteoarthritis, rheumatoid arthritis, and other joint diseases[7]. While MLX is orally well tolerated compared with other NSAIDs, it is associated with bellyache, ulcerogenicity, and indigestion. It renders MLX administration unsafe for gastric ulcer patients[8]. Drug interactions are also associated with oral MLX administration. Skin delivery is an attractive alternative to oral administration due to its many benefits, including avoiding GI irritation, avoiding hepatic metabolism, minimizing systemic toxicity and providing steady plasma levels[9]. It has been confirmed that the local administration of non-steroidal anti-inflammatory drugs promotes analgesia.

1.1.1 Anti-inflammatory effects

Standard animal models of inflammation have been used to determine the anti-inflammatory effects of meloxicam, including carrageenan or kaolin-induced rat paw oedema, granuloma formation after implantation of cotton pellets in the rat, carrageenan-induced rat pleurisy, and rat adjuvant-induced arthritis[10]. With a single dose, meloxicam was able to suppress the inflammation with a prolonged effect in all models. In a rat model of progressive and destructive joint disease, Meloxicam's anti-inflammatory activities were compared with other, established NSAIDs. The acute symptoms are associated with COX-2 expression in adjuvant-induced rat arthritis and inflammation is also immunologically mediated[11], [12]. Meloxicam is more anti-inflammatory than other compounds tested in this model. Meloxicam prevented destruction not only of oedema but also of bone and cartilage at low doses. Consequently, immunologically mediated effects could only be antagonized by meloxicam at low doses[13].

For treatment of the pain and inflammation at dose of 0.4 mg / kg (injection) and 1.5 mg / ml (suspension) for about 3 to 4 days, oral and parenteral administration of meloxicam was recommended. Although meloxicam showed good oral bioavailability (76 percent) in cattle; [14]however, meloxicam's parenteral and oral administration is associated with GI distress, soft stools, vomiting, diarrhea, appetite and suboptimal concentration at the inflammation site. Because of these drawbacks, meloxicam administration by topical route for pain and inflammation treatment would be more effective than the parenteral or oral route of administration[15]. Therefore, a prescription dermal spray was developed in this study to treat pain and inflammation effectively. Meglumine, a glucose-derived amino sugar, is a drug excipient used to enhance meloxicam's solubility in ethanol. As a permeation enhancer, this dimethylsulfoxide (DMSO) was used. Meloxicam's optimized pharmaceutical dermal spray was characterized by a set of stringent parameters in vitro and in vivo to determine its therapeutic effectiveness against inflammation and pain due to injuries in the animals. Powder X-ray diffraction (PXRD) pattern and Differential scanning calorimetry (DSC) revealed that meloxicam was in fact crystalline and showed the melting point at 262.64°C.

II. MATERIALS AND METHODOLOGY

2.1 Drugs and reagents

Meloxicam dermal spray comprises of the active pharmaceutical ingredient meloxicam along with a pharmaceutical excipient, meglumine and a pharmaceutically acceptable permeation enhancer, di-methyl sulfoxide (DMSO). Along with these following ingredients ethanol has been used as a vehicle. The ethanol used as vehicle also acts as penetration enhancer and also contributes to the proper absorption of the spray solution topically. The di-methyl sulfoxide (DMSO) used here in the formulation is now a well established penetration enhancer used in topical formulations.

2.2 Formulation of meloxicam dermal spray

The composition of meloxicam dermal spray for veterinary use is specified in **Table 1**. A clear solution was prepared by dissolving meglumine in ethanol and this solution was heated to about 90°C. Meloxicam was then added to the hot solution that ultimately forms meglumine salt of meloxicam. Finally the permeation enhancer dimethyl sulfoxide was added to this solution to obtain the optimized pharmaceutical dermal spray formulation.

Table 1: Composition of dermal spray of meloxicam

Sr. no.	Ingredients	Quantity (g)	Quantity (%)
1.	Meloxicam	0.2	2.15
2.	Meglumine	0.25	2.69
3.	Dimethylsulfoxide	3.30	35.56
4.	Ethanol	5.53	59.59

2.3 Characterization of dermal spray

2.3.1 Measurement of color, state, texture and homogeneity, pH and specific gravity:

The optimized pharmaceutical dermal spray of meloxicam was characterized under a set of stringent parameters to establish its stability and therapeutic efficacy against the condition of pain and inflammation due to injuries. All the measurements regarding the characterization parameters were taken in triplicate (n=3). The colour state, texture and homogeneity, pH and specific gravity of developed spray were noticed.

2.3.2 Drug content

To ensure the quantity of active pharmaceutical ingredients, formulated spray was assayed for the drug content by HPLC method. For this purpose, meloxicam aliquots were prepared by using the diluents (Acetonitrile: Phosphate buffer, pH 3.4, 50:50). A highly sophisticated analytical instrument was used for the HPLC estimation (Agilent 1220 LC). The diluted samples of the formulation were run through a C₁₈ column in the presence of mobile phase, Acetonitrile: Phosphate buffer (pH 3.4) at a ratio of 60:40, the wavelength was set to 270 nm and the run time was fixed to 15 minutes.

III. RESULTS

3.1 Characterization of gel

The color, state, texture and homogeneity were recognized by visual appearance and noticed to be yellow, liquid, smooth, soothing and uniform, respectively. A particle size analysis was performed for the dermal spray. The particle size of the formulation was determined to be 56.72 ± 33.85 nm. The drug content analysis was performed with reference to a well established HPLC method. Therefore, through this estimation the drug content of the pharmaceutical dermal spray was estimated to be 106.8 ± 15.95 %. Further the formulation was subjected to centrifugation tests at 10,000 rpm (20 C) for 15 minutes and no phase separation was observed. The pH and specific gravity of meloxicam dermal spray were noticed to be 6.5 ± 0.3 and 0.91 ± 0.05 g/ml, respectively (**Table 2**).

Table 2: Characterization of meloxicam dermal spray

Sr. No.	Characterization of spray	Formulated Spray
1	Color	Yellow
2.	State	Liquid
3.	Texture	Smooth
4.	Homogeneity	soothing and uniform
5.	Particle size (nm)	56.72 ± 33.85
6.	pH	6.5 ± 0.3
7.	Drug content	106.8 ± 15.95 %
8.	Specific gravity	0.91 ± 0.05 g/ml

3.2 Solubility Analysis

Solubility analysis indicated that 7.34 ± 0.97 mg of meloxicam/10 ml of ethanol that was not sufficient for achieving an effective therapeutic concentration. Therefore, it was necessary to make use of a solubility enhancing agent which could increase the solubility of meloxicam in ethanol to achieve the effective concentration. Meglumine, an amino sugar derived from glucose is a pharmaceutical excipient which was used for enhancing the solubility of meloxicam in ethanol. Powder -X-ray diffraction pattern (PXRD) indicated that meloxicam was crystalline in nature (**Figure 1**). The endothermic peak of meloxicam was noticed at 262.48°C equivalent to the melting point (**Figure 2**).

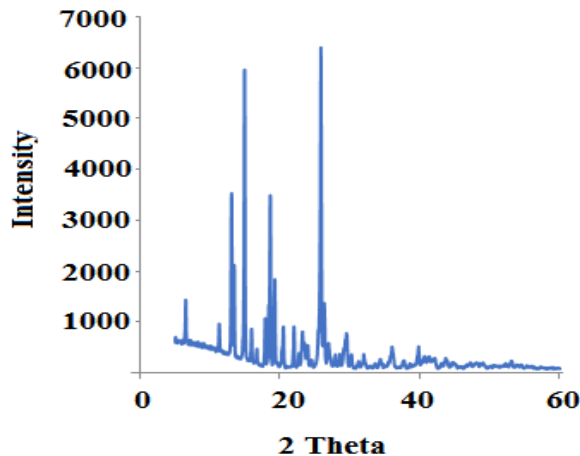


Figure1: Powder-X-ray diffraction pattern of meloxicam

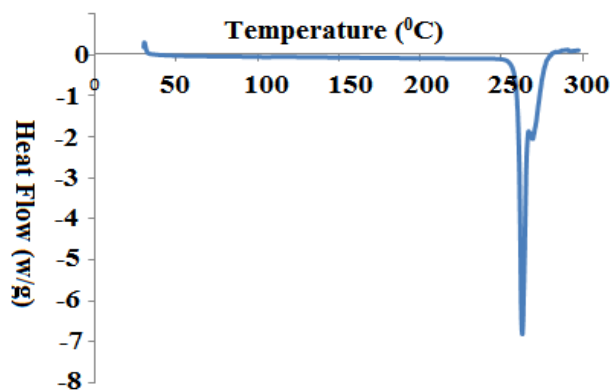


Figure 2: Differential scanning calorimetry (DSC) of meloxicam

3.3 Efficacy of the meloxicam dermal spray

The therapeutic efficacy of the meloxicam dermal spray was tested *in vivo* against carrageenan induced oedema in Wistar rats. For this purpose, 30 male/female rats were divided into 5 groups and each group contained 6 rats.

1. Normal Group (n=6).
2. 0.1ml Carrageenan (1.5% w/v) induced paw edema (n=6).
3. 0.1ml Carrageenan (1.5% w/v) induced paw edema treated with blank dermal spray once a day for three days/72h (n=6).
4. 0.1ml Carrageenan (1.5% w/v) induced paw edema treated with meloxicam dermal spray (n=6).
5. 0.1ml Carrageenan (1.5% w/v) induced paw edema treated with oral meloxicam (n=6).

Carrageenan at the dose of 1.5% w/v (in normal saline) as 0.1 ml/paw, in the left hind paw was injected for inducing the inflammation condition called oedema and pain. The weight of all the animals was taken in normal condition. The paw volume of all the animals was measured using a mercury plethysmometer before injecting

carrageenan in the paws. Treatment with meloxicam dermal spray significantly (One way ANOVA test, $P < 0.05$) reduced the paw volume from 1.225 ± 0.24 mm to 0.92 ± 0.09 mm at 48 h post treatment and 0.84 ± 0.12 mm at 72 h post treatment. Correspondingly, oral administration of meloxicam significantly (One way ANOVA test, $P < 0.05$) reduced the paw volume from 1.28 ± 0.33 mm to 1.09 ± 0.27 mm at 48 h treatment and 0.97 ± 0.22 mm at 72 h post treatment. Treatment with blank dermal spray did not exhibit remarkable difference in reduction of paw volume (**Figure 3**). Apart from these, meloxicam dermal spray also exhibited improved analgesic activity. Treatment with meloxicam dermal spray increased significantly (One way ANOVA test, $P < 0.05$) time taken to withdraw paw from normal 6 ± 1.26 seconds to 11.67 ± 1.36 seconds post 72 h treatment. Correspondingly, oral administration of meloxicam also significantly (One Way ANOVA test, $P < 0.05$) increased the time taken to withdraw paw from normal 5.5 ± 1.04 seconds to 8.5 seconds post 72 h treatment. Treatment with blank dermal spray did not exhibit remarkable difference in increment in time taken to withdraw paw (**Figure 4**).

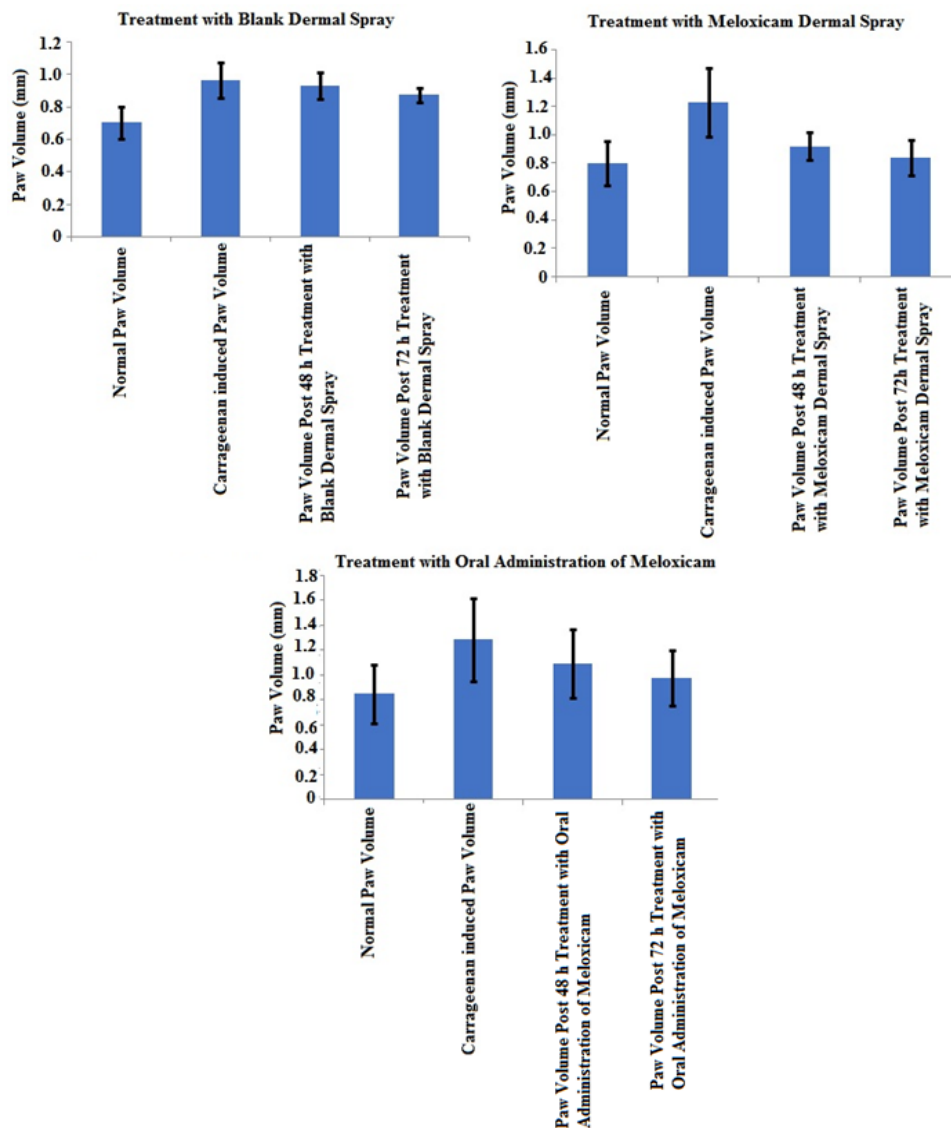


Figure 3: Measurement of Paw Oedema Volume in all treatment Groups

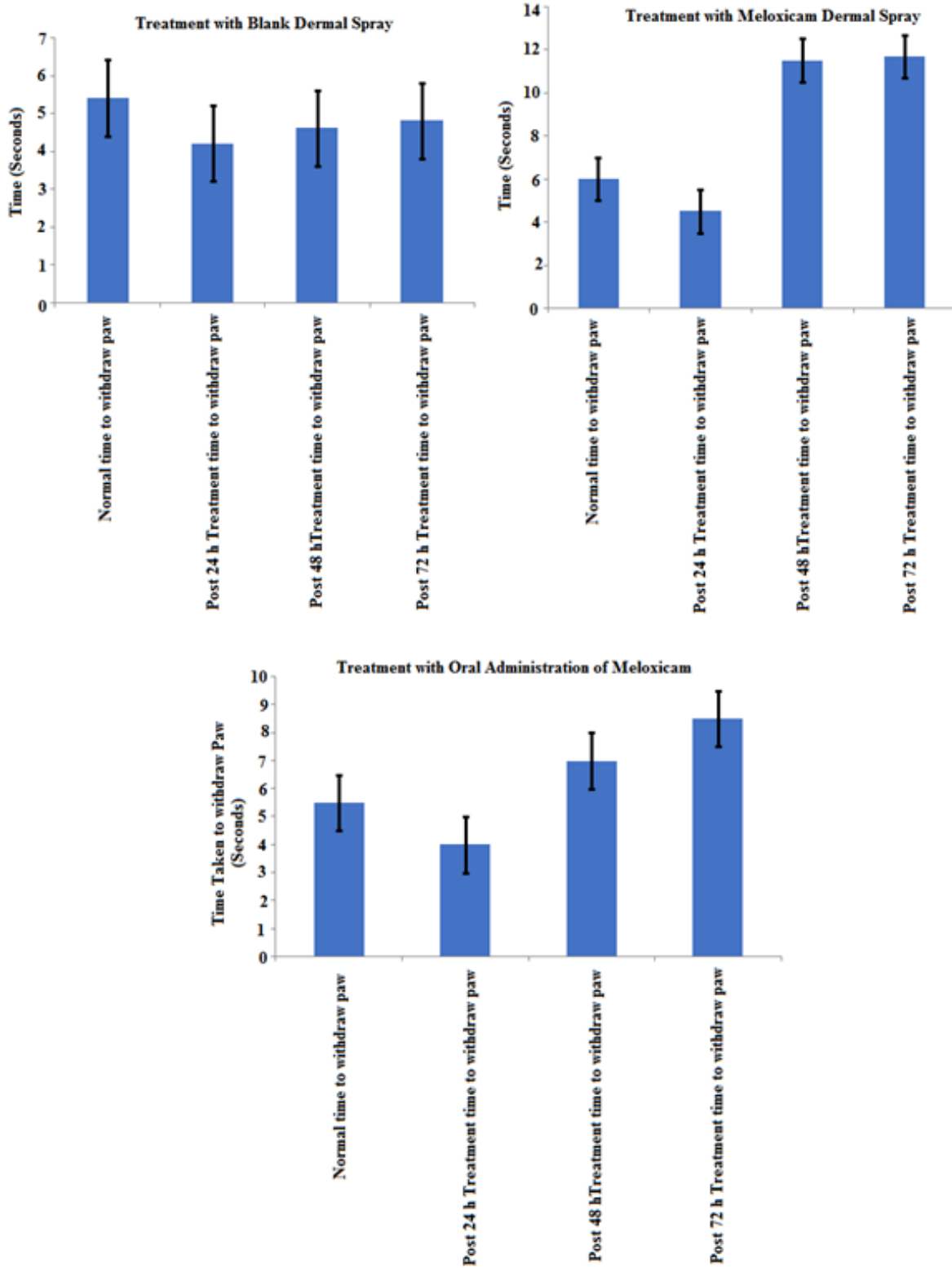


Figure 4: Measurement of Latency Period in all treatment Groups

3.4 Histopathological Evaluation

Histopathological studies of rat paw skin stained with haematoxylin and eosin dye indicated that administration of carrageenan produced neutrophil infiltration accompanied by oedema. On the other hand, stained micrographs of paw skin treated with meloxicam dermal spray remarkably reduced neutrophil population at the site of application as compared to carrageenan treated group. Additionally, although oral administration of meloxicam also considerably reduced the neutrophil population and oedema as compared to carrageenan treated group, however; this reduction was significantly lower than meloxicam dermal spray treated group (**Figure 5**).

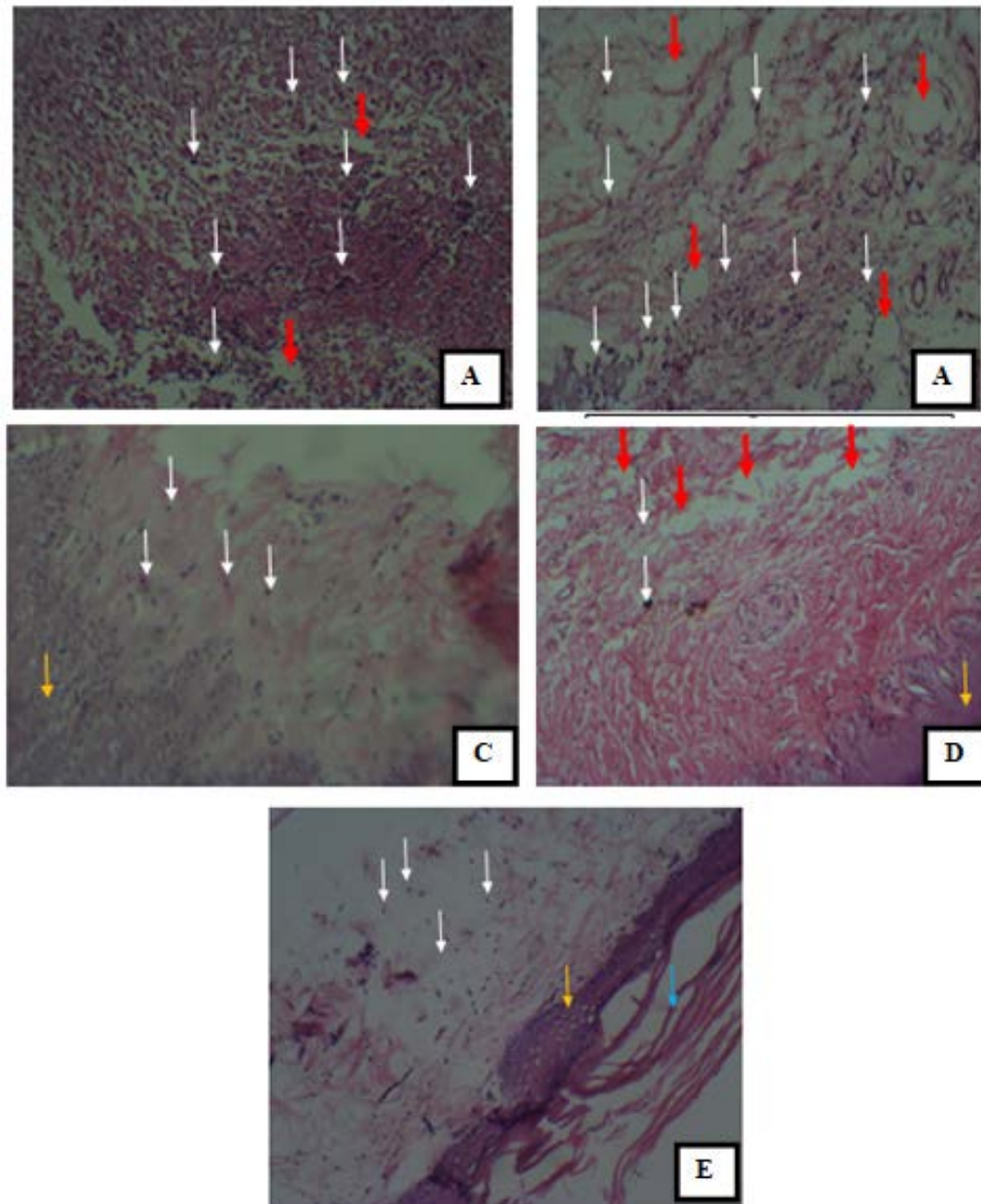


Figure5: Histopathological analysis of inflamed tissue

As shown in **Figure 5**, A represents Blank Dermal Spray, B represents Carrageenan Induced Inflammation, C represents Meloxicam Dermal Spray, D represents Oral Administration of Meloxicam and E represents Normal Group. The white, yellow, blue, and red arrows denoted the neutrophils responsible for the inflammation, epidermis, dermis, and the developed edema or swelling.

IV. CONCLUSION

The results present a Meloxicam dermal spray that is physicochemically stable and non-irritant and could spread a significant amount of meloxicam across the skin. The developed meloxicam topical spray is very efficiently and effectively treats the condition of pain and inflammation (edema) or swelling due to physical injuries suffered by the animals. This can be easily and confidently depicted from compiled and the calculated data which was obtained from the characterization parameters laid down for the evaluation of the pharmaceutical meloxicam topical spray. The spray has the potency to visibly as well as physiologically reduce the pain and inflammation at a very rapid rate. The pharmaceutical meloxicam topical spray can be adequately used in cases of pain and inflammation symptoms ranging from mild to severe and also chronic cases of edema and pain. Histopathological further supported that topical spray of meloxicam at the site of oedema reduced the accumulation of neutrophils equally as compared to oral administration of meloxicam. Thus pharmaceutical meloxicam dermal spray had very significant and promising results with respect to the marketed oral meloxicam formulation available for treating the condition of pain and inflammation caused due to injuries..

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