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Pharmaceutical Composition of a Dermal Gel Comprising Diclofenacdiethylamine and Lemongrass Oil for Anti-inflammatory and Analgesic Activity

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Abstract---*The localized reaction that results in redness caused by hyperaemia, tumor and pain caused by infection, irritation or injury may be defined as inflammation. External or internal inflammation can occur. Increasing inflammation is characterized by vasodilation, exuded fluids and infiltrated neutrophi. Analgesics are agents that function selectively in pain reduction without affecting the consciousness, working in the CNS and in the peripheral pain mediators. The analgesics can be narcotics or non-drug products, however. The study of animal suffering causes social, emotional and technical problems. In this study, we used a novel combination of diclofenacdiethylamine and lemongrass oil in the formulation of pharmaceutical dermal gel. Diclofenacdiethylamine is an established non-steroidal anti-inflammative agent commonly used for symptomatic relief of pain and inflammation in musculoskeletal disturbances, arthritis, toothache, dysmenorrhea, etc. Diclofenacdiethylammonium salt is stated for use in topical applications (Diclofenacdiethylamine). Just 10% diclofenac is clinically available from the latest topical formulations. Lemongrass oil is not only the essential skin partition, it interacts and temporarily increases skin permeability with stratum corneum constituents, but also has additional treatment characteristics, such as antiinflammatory, anti-piling and antibacterial properties that help combat nervous exhaustion, tones of the muscles and tissues. Lemongrass oils are also an essential oil. An advanced topical formulation with good penetration increases the permeation of the medicine's skin tremendously. Therefore, diclofenacdiethylamine and lemongrass oil were developed for a pharmaceutical dermal gel with film-forming capacity.*

Keywords--- *Inflammation, hyperaemia, vasodilation, fluid exudation, neutrophil infiltration, analgesics, narcotics, non-narcotics, diclofenacdiethylamine, non-steroidal, anti-inflammatory, dysmenorrhoea, lemongrass, stratum corneum, anti-fungal, anti-bacterial, nervous exhaustion*

I. INTRODUCTION

Inflammation can be characterized as a localized reaction that causes tumour, heat and pain caused by infection, irritation or injury [1]. Acute inflammation is characterized by vasodilating, fluid exudation and infiltration of neutrophil. Diclofenac is a well known anti-inflammatory non steroidal drug used for symptomatic relief from pain and inflammation. It is commonly used in musculoskeletal disorders, arthritis, toothache, dysmenorrhea etc. [2]. Diclofenac is a good NSAID candidate for topical formulations, according to several studies [3].

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In any part of the human body painful and traumatic conditions such as stresses or sprains that occur. Inhibition of enhanced prostaglandins (PGs) COX mediated in site damage is considered to be the main mechanism for anti-inflammatory action of diclofenacDiethylamine[4]. In the first cycle following oral administration, it induces local mucosal inflammation and metabolism which takes place in the liver and partly inactivates the liver. So the distribution of only 50% of the drug is reached for circulation in the body tissues. Topical injection formulations are suitable for chronic use in case of rheumatic symptoms. The efficacy of the topical diclofenac sodium depends heavily on whether the drug can penetrate the body [5]. The forms of topical doses are ideal for chronic use in rheumatic symptoms. The effectiveness of topical diclofenac sodium depends greatly on the preparedness of the medication to penetrate the skin into the body. Salts such as diclofenacdiethylammonium (DCFD) have an amphiphylic nature and are applied because diclofeanc sodium has low intact skin permeation. [7] (figure 1).

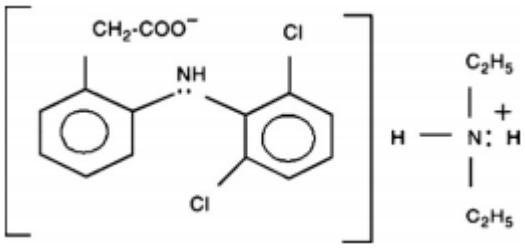

Fig.1. Chemical structure of diclofenacdiethylammonium (DCFD)

Hyperalgesics are caused by PGs, which affect the transducing properties of free nerve endings so that stimuli that usually do not produce pain can do so [8]. Diclofenacdiethylamine does not affect the sensitivity caused by direct use of PGs but blocks bradykinin, TNF-α and interleukins (ILs) sensitization mechanisms [9][10][11] and interleukins (ILs). Consequently, this is more effective against pain associated with inflammation. The use of diclofenac-diethylamine reduces neutrophil chemotaxis and the production of superoxide at the inflammatory site [12]. It has been alleged that diclofenacdiethylamine is used for topics. It is a small molecule with a short half-life of 2 hours (molecular weight of 296.14 Dalton). In theory, diclofenac is an appropriate molecule for topical formulations. Nevertheless, topical preparation in clinical practice was found to be pharmacokinetic parameter. Biologically about 10 percent of diclofenac is available from existing topical formulations. A scientist says that it can't penetrate beyond the superficial levels of the barrier even to nanoparticular, topical drug delivery systems. Several approaches are used to tackle the issue of low skin permeability. Lemongrass oil being an essential oil not only penetrates into the skin, but also interacts with the constituents of the stratum corneum to introduce a temporary, reversible increase in the skin permeability. Owing to these limitations, and considering the beneficial effects of using lemongrass oil, the treatment of inflammation and associated pain would be more advantageous as compared to other essential oils used.

The pharmaceutical topical gel of diclofenacdiethylamine and lemongrass oil has been formulated that contains the active ingredient and pharmaceutically acceptable lemongrass oil that not only enhance the penetration of drug through the layers of the skin but also has additional anti-inflammatory, anti-fungal, anti-bacterial and astringent properties beneficial for the skin. Lemongrass oil helps to combat nervous exhaustion, tones muscles and tissue, help’s in correcting poor circulation, relieves muscle pain by making it more subtle, gel gives localised action

thereby reducing toxicity and side effects. Desirable actions are produced with least side effects and toxicity.

II. METHODS AND RESULTS:

For topical delivery, it is highly desirable that the required dose must be bioavailable, reaches to the inflammatory cells and relieves inflammation and associated pain. Thus, in present investigation, a topical formulation of diclofenacdiethylamine was developed with the essential oil of *Cymbopogonflexuosus* or lemongrass oil (LO) as it has anti-inflammatory properties proved by several scientific studies and being an essential oil, it improves and enhances permeability in the treatment of inflammation. Citral has reported anti-inflammatory effects as an active compound of the Lemongrass oil. Lemongrass oil, as penetration enhancer works to enhance the penetration of diclofenacdiethylamine from the topical gel into the lower skin layers using different mechanisms of action based on (1) As an active ingredient in Lemongrass oil, Citral has reported anti-inflammatory effects, (2) interaction with the intercellular protein domain causing their adaptation, (3) increases the penetration of a drug.

Diclofenacdiethyamine, Lemongrass oil and the physical mixture of both diclofenacdiethylamine and lemongrass oil were characterized under a set of parameters. We characterized the active ingredients using FT-IR spectroscopy. Spectra was recorded to analyze the alteration of stretching frequencies. The assignments of FT-IR stretching frequencies of diclofenacdiethylamine, lemongrass oil, and physical mixture are presented in Table 1**.** Next,DSC was used to measure the endothermic peak of diclofenacdiethylamine. Our data demonstrated that the endothermic peak of diclofenacdiethylamine was detected at 154.63°C close to the melting range of diclofenacdiethylamine which is 137-140°C (Figure 1). Our next step was to define the crystalline state of diclofenacdiethylamine by the PXRD technique. The XRD pattern of diclofenacdiethylamine showed intense and sharp peaks indicating its crystalline structure (Figure 2).In the first step of the development of formulation, diclofenacdiethylamine was dissolved in distilled water with continuous stirring till the whole drug gets solubilized. Then the solution was homogenized in agitator/mixer homogenizer during which CarbopolUltrez was added by sprinkling slowly on surface of above solution, till carbopol gets mixed completely. After complete mixing, HPMC ES and PEG 1500 were added to the above mixture. Let this mixture be named as ‘A’. Simultaneously, the remaining diclofenacdiethylamine was dissolved in the mixture of Glycerol, Benzyl alcohol and Propylene glycol (separately mixed before adding the drug) with the help of magnetic stirrer to form a single phase clear solution. The resultant solution was added through the side walls in the homogenising solution ‘A’ and the solution was stirred for 30 minutes and then left to stay for another 30 minutes without agitation. After the completion of 30 minutes, Lemongrass oil was added and pH was adjusted by the addition of dropwisetriethanolamine till the pH reaches 7 (Table 2).

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Table 1. FT-IR assignment of diclofenacdiethylamine and physical mixture

Functional group	Molecular Motion	Wavenumber (cm^{-1})
Amine	N-H bend	~800
Alkanes	C-H stretch CH_2 bend CH_3 bend	2950-2800 ~1465 ~1375
Aromatics	C-H stretch C=C stretch C-H bend (mono) C-H bend (ortho) C-H bend (meta)	3020-3000 ~1600 & ~1475 770-730 & 715-685 770-735 ~880
Carboxylic acids	O-H bend	1440-1400
Acid chlorides	C-Cl stretch	730-550

Table 2. FT-IR assignment of lemmon grass oil and physical mixture

Functional Group	Molecular motion	Wavenumber (cm^{-1})
Alkene	C=C stretch	1680-1600
Aromatic	C-H stretch C=C stretch C-H bend (mono) C-H bend (ortho) C-H bend (meta)	3020-3000 ~1600 & ~1475 770-730 & 715-685 770-735 ~880
Carboxylic Acid	O-H stretch C-O stretch	3400-2400 1320-1210
	C=O stretch	1786-1650

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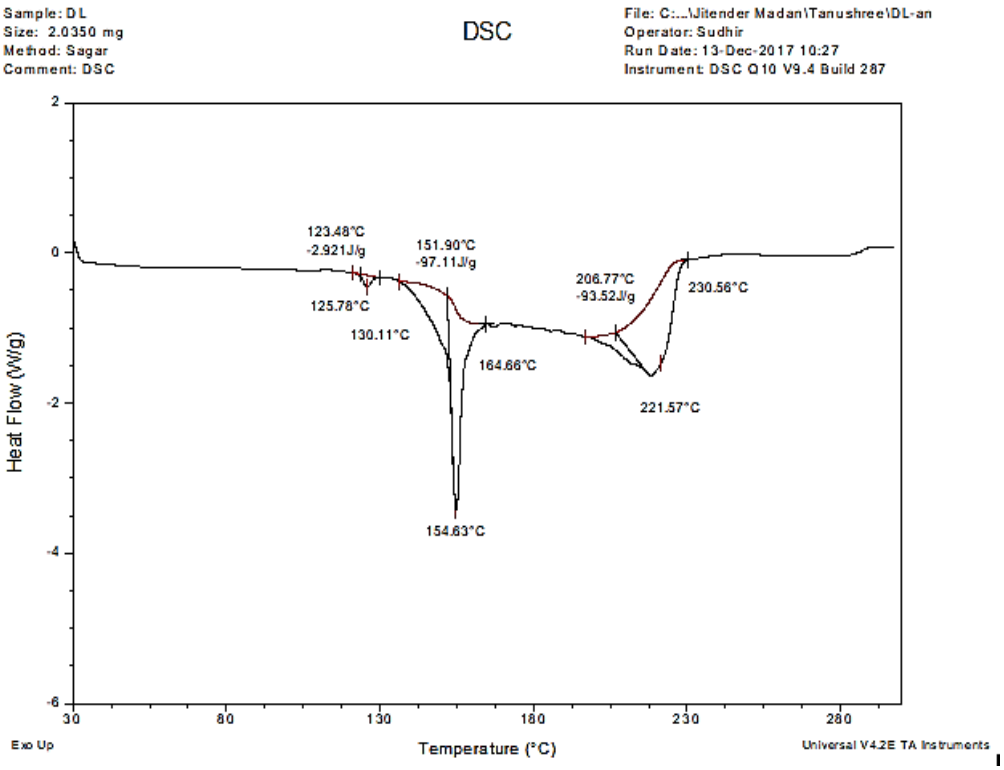

Fig.1. Differential scanning calorimetry of diclofenacdiethylamine

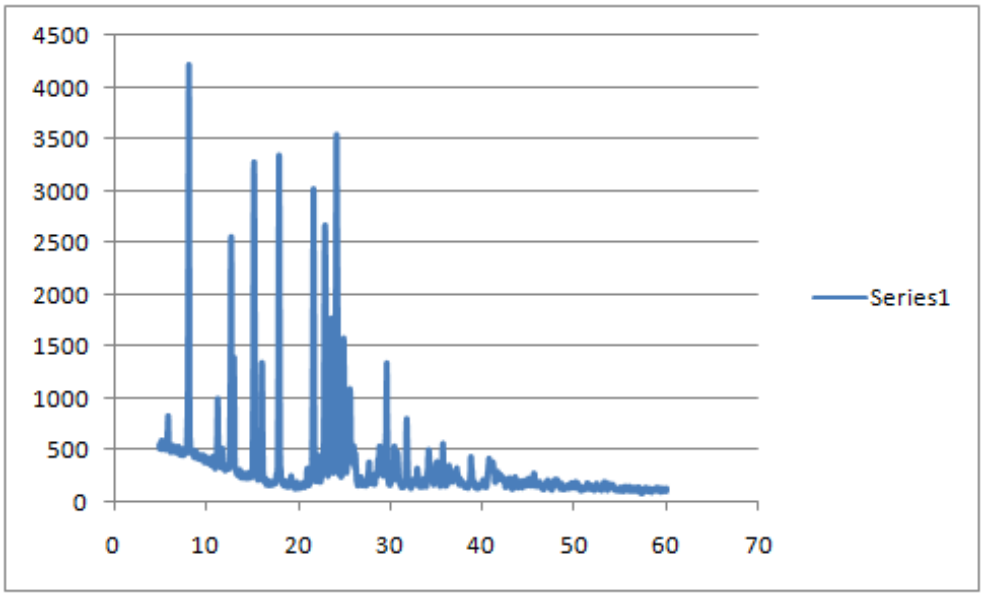

Fig.2. PXRD curve of diclofenacdiethylamine

The optimized topical gel was then characterized under a set of stringent parameters to establish stability and therapeutic efficacy against inflammation. Having formulated the pharmaceutical topical gel of diclofenacdiethylamine and lemongrass oil, our first step was to determine the color, state, texture and homogeneity by visual appearance and noticed to be pale yellowish, semi-solid, smooth and uniform, respectively. Most of the

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characteristic parameters were compared against standard topical gel. The pH of the gel was measured by a pH measured pen. (Hanna Instruments, Germany). In brief, 1 gm of optimized topical gel sample was diluted up to 50 ml with distilled water and pH was measured. All measurements were carried out in triplicate (n=3). The pH of optimized gel sample was determined to be 7.73±0.0360 and that of standard gel was found to be 7.55±0.0115. The spreadability of topical pharmaceutical gel was measured to be 55.801±011.28 g.cm/sec and 83.0388 ± 6.148593 g.cm/sec of Standard diclofenacdiethylamine gel by glass plate method. Good spreadability implies a shorter period of time. Extrudability is a common empiric test for measuring the strength needed to extrude the tube material. The method was used to determine applied cording to a cording rate above the yield and consequent plug flow for one such apparatus in the rheogram region. In this study, on application of weight to grams, at least 0.5 cm of gel in 10 seconds, the method for assessing gel for extrudability was based on the percentage of gel and gel extruded from lacquered plastic collapsable tube. The extrudability calculation of each formulation was three times that measured the average values. Extrusion was measured using the equation below. It was estimated that the extrudability is 242.61g.cm2.

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm^2).

Viscosity of gel was evaluated by Brookfield viscometer using CP-75 spindle. The topical pharmaceutical gel was poured into the adaptor of the viscometer and the angular velocity increased gradually from 100 rpm. The rheological analysis of the gel followed the non-Newtonian fluid behavior (Figure 3a-d). This indicated that the gel is easily spreadable by small amounts of shear. All measurements were carried out in triplicate (n=3), along with the marketed standard topical gel. Further, no phase separation upon centrifugation at 10,000 rpm (20°C) for 15 min was noticed in any batch of the topical pharmaceutical gel.

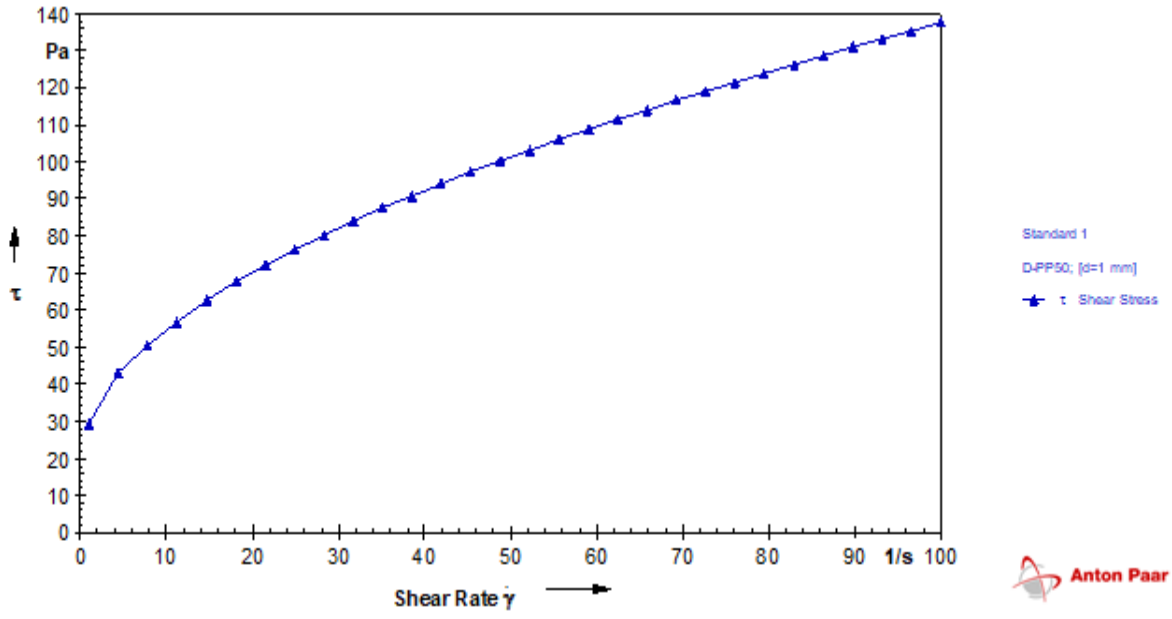

Fig.3a. Rheology curve of standard diclofenacdiethylamine gel.

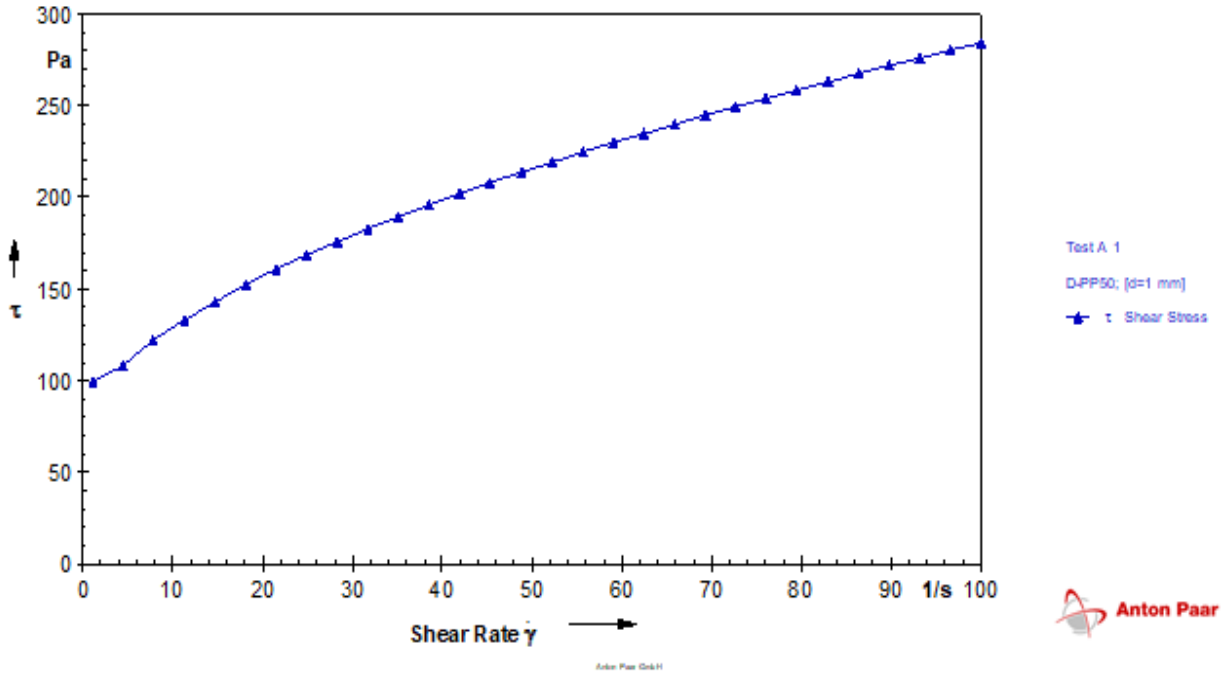

Fig.3b. Rheology curve of Batch A of Test formulation.

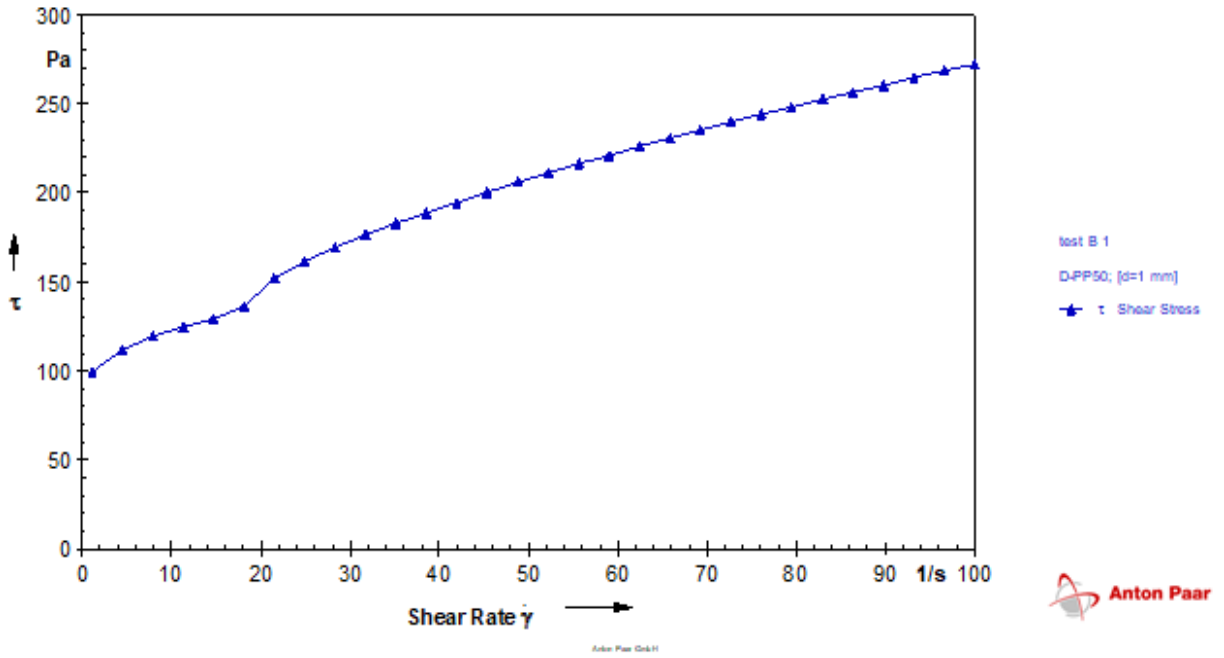

Fig.3c. Rheology curve of Batch B of Test formulation

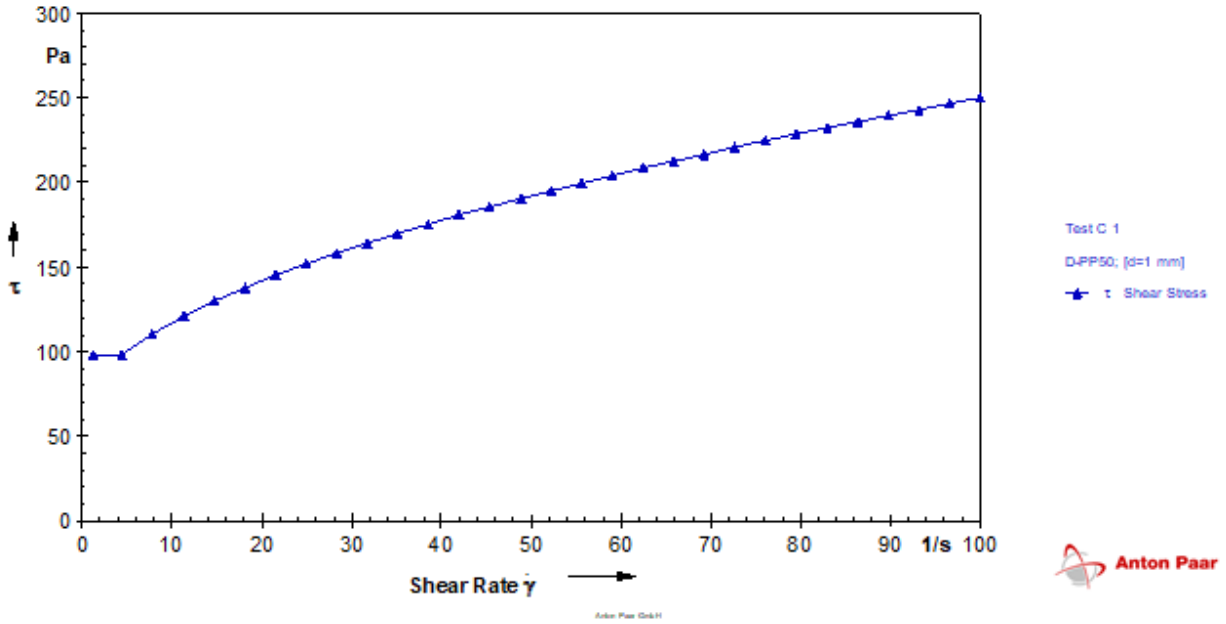

Fig.3d. Rheology curve of Batch C of Test formulation

The therapeutic efficacy of diclofenacdiethylamine and lemongrass oil topical gel was established by *in vivo* estimations using carrageenan induced paw oedema method. Adult males Wistar Rats were housed at controlled temperature (4-6 weeks, body weight 150–200 g) (22 ± 2 ° C) with access to food or water free. The animal experiments were carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Culture, Government of India. The "Institutional Animal Ethical Committee of Chandigarh College of Pharmacy", formed under "Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA)" approved the pharmacological protocols. The animals were used only once and then rehabilitated. Paw edema was induced in a left hind paw of each rat, except normal control group, by intraplantar injection of 0.01 ml of 1.5 w/v% (suspension in saline) carrageenan. Rats were divided into 5 groups of six animals each. Group A was normal control male Wistar rat and was not treated. Group B was induced with inflammation using carrageenan and was not treated. Group C was induced inflammation using carrageenan and was treated with novel diclofenacdiethylamine and lemongrass oil gel. Group D was Standard group treated with marketed diclofenacdiethylamine formulation (VOLONI Gel) and Group E was inflammation induced with carrageenan control group treated with blank gel Parameters used for the evaluation were:

1. Paw volume
2. Body weight
3. Inflammatory score
4. Histopathology

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 diclofenacdiethylamine and lemongrass oil dermal gel significantly (One way ANOVA test, P<0.05) reduced the paw volume from 0.184±0.04 mm to 0.111 ±0.09 mm at 48 h post treatment and 0.098±0.12 mm at 72 h post treatment. Correspondingly, topical administration of standard diclofenacdiethylamine gel significantly (One way ANOVA test, P<0.05) reduced the paw volume from 1.1±0.33 mm to 0.98±0.27 mm at 48 h treatment and 0.88 ±0.19 mm at 72 h post treatment. Treatment with blank dermal gel did not exhibit remarkable difference in reduction of paw volume. Apart from these, diclofenacdiethylamine and lemongrass oil dermal gel also exhibited improved analgesic activity. Treatment with diclofenacdiethylamine and lemongrass oil dermal gel increased significantly (One way ANOVA test, P<0.05) time taken to withdraw paw from normal 4.6±1.03 seconds to 9.5±1.04 seconds post 72 h treatment. Correspondingly, topical administration of diclofenacdiethylamine dermal gel also significantly (One Way ANOVA test, P<0.05) increased the time taken to withdraw paw from normal 4.6±1.03 seconds to 9.5±1.04 seconds post 72 h treatment. Treatment with blank dermal dermal gel did not exhibit remarkable difference in increment in time taken to withdraw paw (Figure 4).

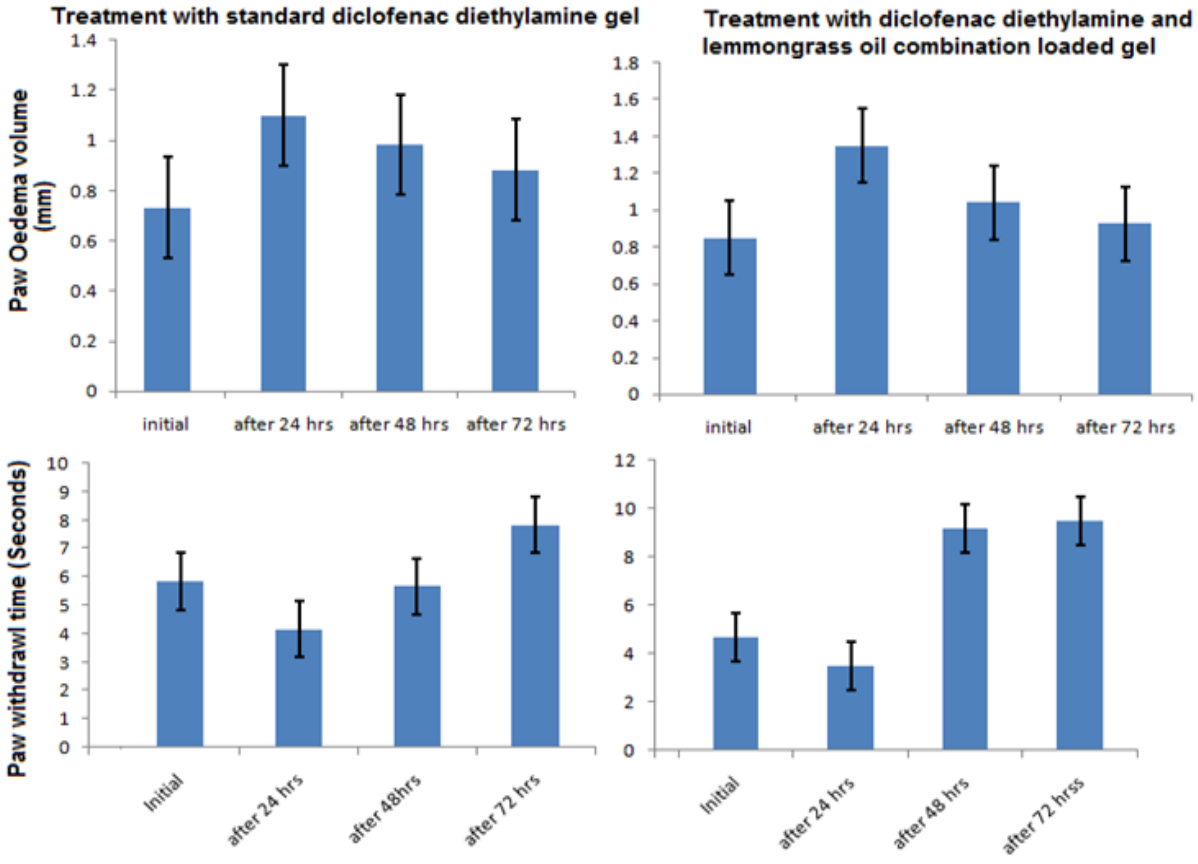

Fig.4. Measurement of paw oedema volume and analgesic activity post standard diclofenacdiethylamine and diclofenacdiethylamine and lemongrass oil combination loaded gel

Histopathological studies of rat paw skin stained with haemotoxylin 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 diclofenacdiethylamine and lemongrass oil dermal gel remarkably reduced neutrophil population at the site of application as compared to carrageenan treated group. Additionally, although topical administration of diclofenacdiethylamine gel also considerably reduced the neutrophil population and oedema as compared to carrageenan treated group, however; this reduction was significantly lower than diclofenacdiethylamine and lemongrass oil dermal gel treated group (Figure 5).

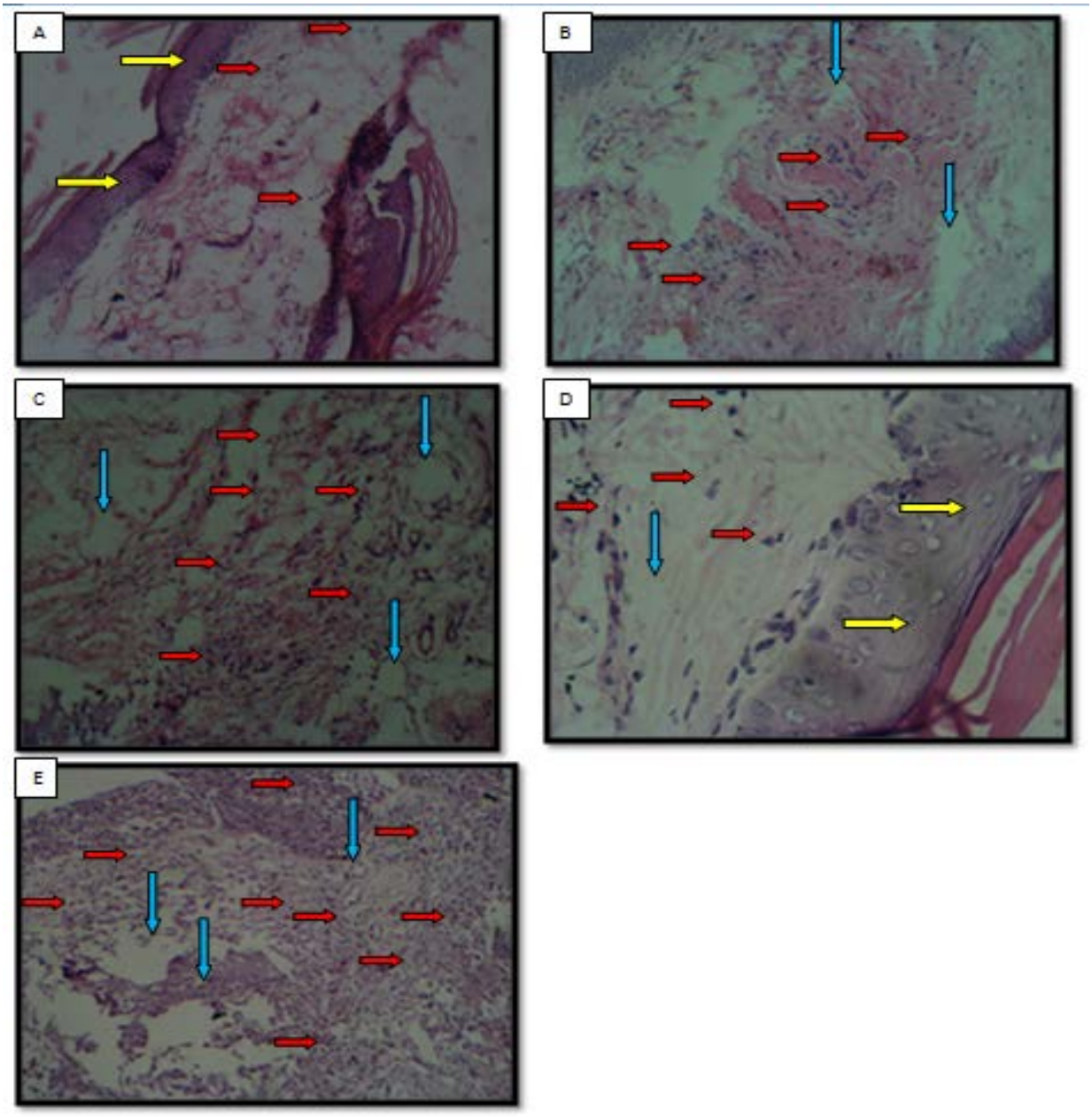

Fig.5. Photomicrographs of the histopathological analysis of paw tissue from rats, A – Normal group, B – Marketed (standard) Treatment group, C – Inflammation control group, D – Test (formulation) treatment group, E – Blank gel treatment group. Red arrow indicates Neutrophil, Blue arrow indicates Oedema, Yellow arrow indicates epithelium.

III. CONCLUSION:

An advanced topical approach with efficient penetrators can make a huge difference in drug molecule skin permeation. Hence, a pharmaceutical dermal gel with film forming ability was formulated by using diclofenacdiethylamine and lemongrass oil. The pH of tailored gel was determined to be 7.73±0.0360 while the pH of standard gel was found to be 7.55±0.0115. The spreadability of topical pharmaceutical gel was measured to be 55.801±12.053 g.cm/sec as compared to 83.0388±6.14 g.cm/sec of standard gel. The drug content was estimated to be 99.32%. The rheological analysis of the gel followed the non-Newtonian fluid behavior. The *in vitro* release of dermal gel through rat skin followed the first order kinetic. Moreover, tailored dermal gel exhibited promising *in vivo* results. Diclofenacdiethylamine dermal gel reduced the paw edema volume remarkably as compared to standard diclofenacdiethylamine dermal gel with high therapeutic efficacy. Moreover, diclofenacdiethylamine dermal gel remarkably reduced the inflammation in histological results. In conclusion, tailored diclofenacdiethylamine dermal gel showed higher therapeutic potential as compared to standard diclofenacdiethylamine dermal gel in preclinical analysis.

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