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Investigating the Synergistic Potential of Combination of Natural Penetration Enhancers and a Synthetic Drug in Animal Model for Improved Transdermal Delivery

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ABSTRACT

Background: The aim of current investigation was to analyze synergistic effect of natural penetration enhancers in combination with a synthetic drug in an animal model to enhance transdermal drug delivery.

Methods: Initially, while carrying out the work in 2021-23 trial formulation batches were prepared with different penetration enhancers and then these were undergone through optimization study to optimize various parameters. A 3^2 full factorial design was utilized for study of optimization. An appropriate animal model was selected to analyze anti-inflammatory and analgesic effect of product. Statistical analysis was doneby utilizing one-way ANOVA, post hoc Tukey-Kramer test utilizing GraphPad Prism 9. Value of $p<0.05$ wasdeemed considerable.

Result: It was concluded from the study that reduction in paw licking activity was approximately found to be 69.52, 72.32 and 74.10 % with Emulgel 1, Emulgel 2 and Emulgel 3, compared with group of control ($p<0.05$ vs control). These findings showed that the analgesic effect of all three formulations was promising. Emulgel 3 *i.e.*, *Sapindus mukorossi* showed better anti-inflammatory activity in comparison to *Allium sativum* and *Zingiber officinale*.

Key words: Analgesic, Anti-inflammatory, Natural penetration enhancer, Synergistic effect, Transdermal drug delivery.

INTRODUCTION

Transdermal Drug Delivery System (TDDS) is a method of administering medication through skin, offering distinct advantages over traditional delivery routes. By utilizing patches designed with layers to gradually release medication, TDDS ensures controlled and sustained drug delivery, leading to consistent blood levels and improved efficacy (Bala *et al.*, 2014). This approach bypasses first-pass metabolism in liver, reducing risk of drug degradation and side effects. Moreover, TDDS enhances patient compliance due to its non-invasive nature and ease of application, potentially increasing adherence to treatment regimens (Jeong *et al.*, 2021).

While commonly used for medications like nicotine, contraceptives and pain relievers, careful consideration of factors such as molecular properties and skin permeability is crucial to ensure the suitability of drugs for transdermal delivery and to address potential skin irritation concerns. Thus, while offering notable advantages, the development and utilization of TDDS require thoughtful consideration and monitoring for optimal therapeutic outcomes (Bird *et al.*, 2020).

The synergistic potential of combining natural penetration enhancers with synthetic drugs in transdermal drug delivery holds promise for improving drug absorption and efficacy (Sidat *et al.* 2019). Natural penetration enhancers, derived from botanical or natural sources, possess properties that can enhance skin permeability without causing significant irritation or adverse effects (Caliskan *et al.*, 2020). When used in combination with synthetic drugs, these enhancers can facilitate drugs penetration via barrier of skin, leading to enhanced drug delivery and therapeutic outcomes (Chen *et al.*, 2016).

By leveraging the complementary properties of natural enhancers and synthetic drugs, formulations can be optimized to achieve desired drug concentrations in systemic circulation while minimizing potential side effects. Natural enhancers such as essential oils, fatty acids and plant extracts can enhance drug solubility, break the stratum corneum lipid bilayers and enhance skin hydration, thereby promoting drug permeation. This synergy allows for lower drug doses, reduced frequency of administration and improved patient compliance (*Schafer et al.*, 2023).

However, challenges such as standardization of natural enhancers, variability in their effectiveness and potential interactions with synthetic drugs must be carefully addressed during formulation development. Additionally, thorough safety assessments are necessary to ensure the

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the compatibility and tolerability of combined formulations (Suntar, 2020). Overall, the synergistic combination of natural penetration enhancers with synthetic drugs holds significant potential for enhancing transdermal drug delivery and expanding the scope of therapeutic options available to patients.

MATERIALS AND METHODS

The study was conducted between the year 2021-23 in the Animal house of Siddhartha Institute of Pharmacy and Research, Dehradun. Drug sample and carriers used in the study were obtained from various companies of repute.

Optimization study

A 3^2 full factorial design was utilized in present study for optimization study.

In-vivo study

The studies in animal were conducted after approval from Institutional Animal Ethics Committee (Protocol ID: SIP/IAEC/PCOL/01/2022).

Animals

The 200-220 g albino wistar rats were employed to assess the formulations anti-inflammatory and analgesic properties.Access to food and water was available to each of the four animals housed in each of the propylene cages.Animals werekept in standard conditions in a laboratorywith 12 h cycle of light and dark,relative humidity55±5% RH and 25°±2°C temperature.Five groups (n=6) of rats will be used to assess the anti-inflammatory impact.The study plan was presented in Table 1.

Analgesic action

With a few changes, the approach as explained by Khan *et al.*(2015) was used for investigation. In summary, five groups of animals were formed, each consisting of six rats. Group 1 was treated with vehicle base and taken as control, while group 2, 3, 4 and 5 were treated with marketed emulgel formulation and test formulations. The right hind paw's dorsal surface received 300 mg of the corresponding formulations in the treatment groups, which were then gently rubbed in. Following a half hour, 20 µl of 5% formalin were administered subcutaneously into right hind paw's plantar aponeurosis. Amount of time (measured in seconds) that the injected paw took to lick and bite was thought to be a sign of pain. After the injection of formalin, all measurements were taken for 5 minutes.

Anti-inflammatory effect
Paw edema model induced with carrageenan

Each rat in each group received 0.1 ml of carrageenan at a rate of around 1% subcutaneously applied to the right hind paw′s sub-plantar surface. The standard and test formulations were applied 30 minutes prior to the carrageenan administration. Using a digital Vernier calliper, volume of paw was measured at regular intervals of 0, 1, 2, 3 and 6 hours.

The below mentioned formula was utilized to calculate percent inhibition of volume of paw, which was then displayed as mean±SD.

$$\% \text{ Inhibition} = \frac{\text{Enhancement in paw volume (control)} - \text{Enhancement in paw volume (test)}}{} \times 100$$

Statistical analysis

The mean ± SD was used to express the values. One-way ANOVA was used to examine the data and GraphPad Prism 9 was used for post hoc Tukey-Kramer tests.The $p<0.05$value weredeemed considerable.

RESULTS AND DISCUSSION

In the present study, trial formulations utilizing different penetration enhancers and a synthetic drug were formulated and independent variables were evaluated to optimized the formulation parameters.

Optimization study

Three designs were used for the optimization study utilizing three plant extracts. For design 1 (*Allium sativum* essential oil), the spreading coefficient linear model's ANOVA revealed that the model's F-value of 13.47 was significant. There was chance of 0.60% that this high F-value could occur because of noise. Significant model terms were indicated by P-values less than 0.0500.A (concentration of gelling agent) was an important model term in this instance. When values exceed 0.1000 it means that model were not considerable. ANOVA for quadratic model related to cumulative drug release showed that F-value of Model was 126.08 implied that the model was considerable. There is just a 0.11% chance that noise may be cause of F-value this high. Model terms with P-values less than 0.0500 were deemed considerable. In this case A (Gelling agent concentration), B (Penetration enhancer concentration), A^2 were act as considerable model terms. Values more than 0.1000 indicates model terms are not considerable.

For design 2 (*Zingiber officinale* essential oil), ANOVA for linear model related to spreading coefficient showed that 129.41 Model F-value implied model was considerable. With an F-value this large, noise was just 0.01% likely to be the cause. Model terms with a P-value of less than 0.0500 were deemed considerable. In this case A (Concentration of Gelling agent), B (Concentration of Penetration Enhancers) were significant model term. The model terms are not significant if value is larger than 0.1000. Drug release 2FI model′s ANOVA revealed that model's F-value of 11.03

Table 1: Plan of study.

Group	Treatment
Group 1(Control)	Normal saline
Group 2(Standard)	Administered standard drug
Group 3 (1 Test Group)	Administered emulgel 1
Group 4 (2 Test Group)	Administered emulgel 2
Group 5 (Test Group 3)	Administered emulgel 3

specifies that it is considerable. F-value this high could be the result of noise, but only by 1.21%. Considerable model terms are specified by P-values lower than 0.0500. A (Concentration of Gelling Agent) was an important model term in this instance. Values higher than 0.1000 signify the lack of significant for the model terms.

For design 3 (*Sapindus mukorrisi* extract), ANOVA for quadratic model associated with spreading coefficient, 11.28 Model F-value indicated that the model was significant. The probability that an F-value this significant might beoutcome of noise was only 3.68%. Model terms are considerable if P-value is smaller than 0.0500. In this instance A (Concentration of Gelling agent), A^2 were significant model terms Values higher than 0.1000 signify the lack of significance for the model terms. ANOVA for 2FI model related to drug release showed 83.57 Model F-value which implies that the model is significant. Only 0.01% of cases might have an F-value this high due to noise. Model terms with P-values less than 0.0500 are deemed significant. In this case, A, B and AB were significant model terms. If the value is greater than 0.1000, the model terms become irrelevant.

In-vivo study

All the prepared formulations were investigated for anti-inflammatory and analgesic action.

Analgesic action

Outcomes of formalin test (paw licking activity) wasmentioned in Table 2 and Fig 1. Each formulation decreased the amount of paw licking($p<0.05$ vs control). However, standard formulation was found to produce significant analgesic activity. From the results it was shown that the test formulations (Emulgel 1, Emulgel 2 and Emulgel 3) showed significant analgesic activity comparable to the standard formulation. The decrease in paw licking action was found to be 69.52, 72.32 and 74.10% with Emulgel 1, Emulgel 2 and Emulgel 3, as compared to control group ($p<0.05$ vs control) in paw licking in animals. These findings indicated that there was an analgesic effect in all three formulations.

ANOVA summary was shown in Table 3 and Fig 2. It shows the paw lickings number observed in different treatment groups in formalin test, $p<0.05$ v/s control (ns).

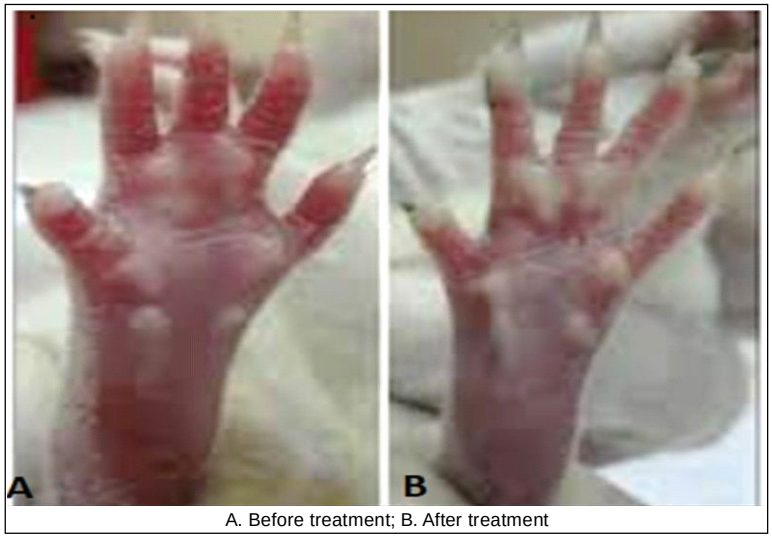
Anti-inflammatory action

Histamine and carrageenan-induced paw edema model

Paw edema model created by carrageenan is frequently used to determine anti-inflammatory properties of different synthetic and natural materials. It is the unique acute

Table 2: Paw lickings by animals after formalin injection.

Control (Normal saline)	Standard-aceclofenac gel	Emulgel 1	Emulgel 2	Emulgel 3
0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
35.75±0.12	17.68±0.47	28.25±0.19	34.81±0.26	15.73±0.13
48.65±0.22	30.66±0.56	32.38±0.27	45.13±0.51	28.15±0.32
98.45±0.15	35.63±0.44	40.76±0.15	57.41±0.35	35.39±0.28
141.0±0.27	49.71±0.29	53.23±0.38	63.79±0.18	46.21±0.11
215.0±0.18	58.82±0.25	65.52±0.47	59.51±0.31	55.68±0.45



A. Before treatment; B. After treatment

Fig 1: Carrageenan induced paw oedema.

inflammatory model with higher repeatability. Carrageenan is a non-antigenic phlogistic substance with no appreciable systemic effects.Carrageenan′s sulphated sugars are what cause complement system and inflammatory mediators to become active.Outcomes of anti-inflammatory action are summarized in Table 4 and 6. Paw volume was found to significantly decreased ($p<0.0001$) in animal treated with Aceclofenac emulgel (Fig 3). With increase in time on 1st, 3rd and 5th hour, test samples considerably reduced the volume while more reduction was found in Emulgel 3 ($p<0.0001$). The specified results were summarized in Table 4. Early phase response of % reduction in paw edema was determined after 1hof paw edema induction. Outcomes indicated that Emulgel 3 formulation reduced early phase paw edema by 38.68%. The decrease of paw oedema was observed 21.47% and 24.86% with Emulgel 1 and Emulgel 2. Late phase response was even larger where 75.92%, 58.33% and 56.48% paw edema inhibition was observed with formulations; Emulgel 3, Emulgel 1 and Emulgel 2. The paw edema inhibition in groups administered with standard was found to be 71.29% in comparison to the test formulations. Fig 4 showed the anti-inflammatory effect of Aceclofenac emulgel in comparison to control ($p<0.001$) in carrageenan-induced paw oedema models. ANOVA summary is given in Table 5 and 7.

Table 3: ANOVA summary.

ANOVA SUMMARY					
F	2.258				
P Value	0.0914				
Significant diff among means (P<0.05)?	No				
R square	0.2654				
Brown-forsythe test					
F (DFn, DFd)	4.557(4, 25)				
P Value	0.0067				
Are SDs significantly different (P<0.05)?	Yes				
Bartlett's Test					
Bartlett's statistic (corrected)	16.36				
P value	0.0026				
Are SDs significantly different (P<0.05)?	Yes				
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	14730	4	3682	F (4, 25)=2.258	P=0.0914
Residual (within columns)	40774	25	1631		
Total	55504	29			
Data summary					
Number of treatments (columns)	5				
Number of values (total)	30				

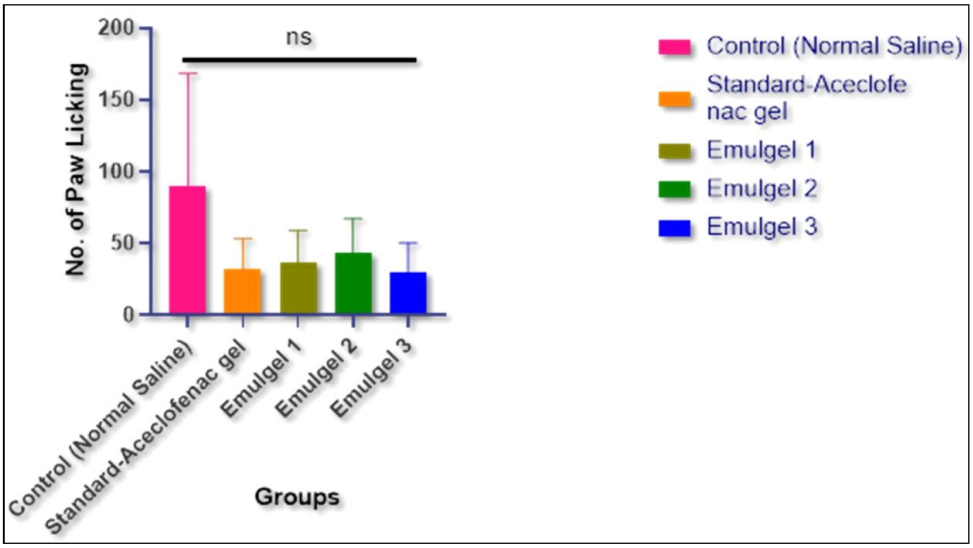


Fig 2: Paw licking numbers observed in different groups of treatment for formalin test, $p<0.05$ v/s control (ns).

Immune systems reaction to harmful stimuli, like injured cells, pathogens, poisonous substances, or irradiation, is called as inflammation, which works by eliminating damaging stimuli and starting process of healing. Inflammation is therefore a crucial defense mechanism for overall health. Most of the time, the risk of injury or infection is successfully decreased by molecular and cellular interactions and events while acute inflammatory reactions. This mitigating procedure aids in the resolution of the acute inflammation and the return of tissue homeostasis. Many chronic inflammatory disorders can arise from uncontrolled acute inflammation, which can also turn into a chronic condition (Medzhitov, 2010). Carrageenan-induced inflammation is well-researched, nonimmune,acute and highly reproducible. Edema, erythema and hyperalgesiaare primary indicators of inflammation that appear right after subcutaneous injection. These indications are caused by proinflammatory substances such as bradykinin, tachykinins, histamine, nitrogen speciesand reactive oxygen species (Sarkhel, 2016). In this investigation, an Aceclofenac emulgel was successfully formulated by utilizing penetration enhancers that lead to enhanced release of drug and solubility. Furthermore, when examined in animal models of inflammation, the developed emulgel showed an anti-inflammatory activity, decreasing inflammation in paw edema animal model by more than 50% compared tocontrol, demonstrating how the enhancement in drug release and solubilitytranslated into an effective biological action.It has also been shown that a variety of herbal remedies alter the expression of pro-inflammatory genes (Asif *et al.*, 2020). Various plant extracts have demonstrated analgesic and anti-inflammatory properties in numerous animal models (Panthong *et al.*, 2007). The three formulation ingredients have been carefully chosen from formerly reported and conventionally established herbal penetration enhancers which have increased the penetration of anti-inflammatory drug. Various researches show the immunotherapeutic and immunomodulatory effects of garlic, comprising free radical-mediated anticancer, anti angiogenic and anti-inflammatory activity, enhancing dyslipidemia, hyperglycemia, cardiovascular,

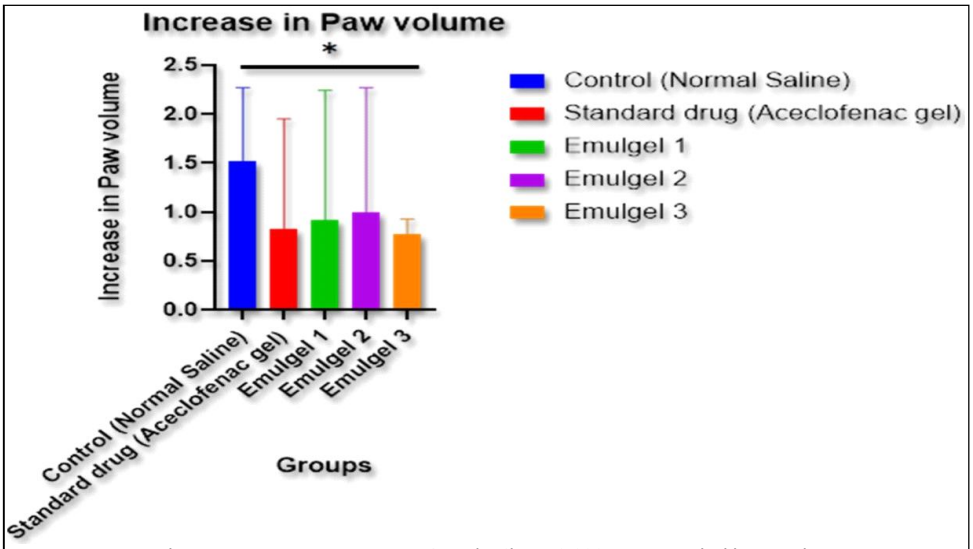
Table 4: Effect of Aceclofenac emulgel on paw volume in carrageenan induced paw oedema.

Control (Normal saline)	Standard drug (Aceclofenac gel)	Emulgel 1	Emulgel 2	Emulgel 3
1.52±0.75	0.83±1.12	0.92±1.32	0.99±1.28	0.78±0.15
1.45±0.16	0.75±1.15	0.84±1.13	0.88±1.78	0.71±0.23
1.36±0.53	0.68±1.07	0.71±1.08	0.76±0.11	0.61±0.18
1.28±0.41	0.57±1.23	0.63±0.24	0.69±0.24	0.52±0.22
1.19±0.32	0.49±1.11	0.54±0.37	0.58±0.45	0.46±0.63
1.08±0.12	0.31±0.88	0.45±0.18	0.47±0.32	0.26±0.57

Table 5: ANOVA summary.

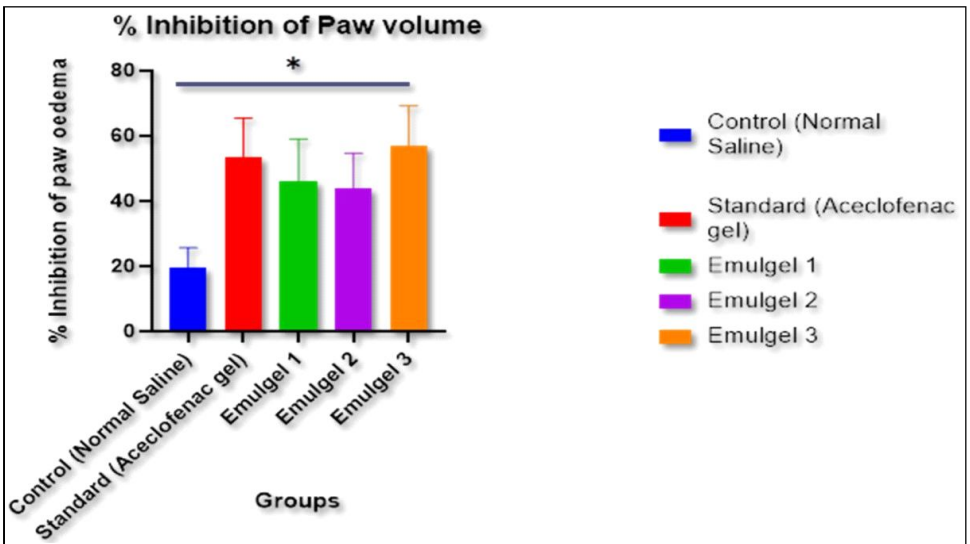
ANOVA SUMMARY					
F	17.05				
P Value	<0.0001				
Significant diff among means (P<0.05)?	Yes				
R square	0.7317				
Brown-Forsythe test					
F (DFn, DFd)	0.03765 (4, 25)				
P Value	0.9971				
Are SDs significantly different (P<0.05)?	No				
Bartlett's Test					
Bartlett's statistic (corrected)	0.1423				
P value	0.9976				
Are SDs significantly different (P<0.05)?	No				
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2.263	4	0.5659	F (4, 25) = 17.05	P<0.0001
Residual (within columns)	0.8298	25	0.03319		
Total	3.093	29			
Data Summary					
Number of treatments (columns)	5				
Number of values (total)	30				

autoimmune, infectious diseases and allergy, that have been observed in both cell lines and animal models (Chandrashekar*et al.*, 2009), (Yin *et al.*, 2014). Mechanism of action of aqueous garlic extract as an antioxidant is well-established; it involves scavenging reactive oxygen species (ROS) and augmenting cellular antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and catalase. Additionally, because it contains phytochemicals like SAMC andDAS, garlic is a significant source of antioxidants (Arreola *et al.*, 2015). The anti-inflammatory activities were indorsed in a decrease in production and expression of proinflammatory cytokines IL-1and TNF (Hodge *et al.*, 2002).Garlic oil treatment may regulate anti-inflammatory and proinflammatory cytokine levels in colon because it contains bioactive ingredients like allyl methyl sulfide, that has been shown to stimulate the IL-10 production and proinflammatory cytokine which inhibits IL-1,TNF and diallyl sulfidesecretion, a proinflammatory cytokine which inhibits IL-1 and TNFsecretion(Balaha *et al.*, 2016). All these researches have suggested that it induced anti-inflammatory and analgesic effect. Another formulation (Emulgel 2) was



The outcomes represent mean±SEM (n=6), *p<0.0001 compared with control.

Fig 3: Effects of aceclofenac emulgels on paw oedema induced with carrageenan in rats.



Dotted capped line indicated comparison between Aceclofenac emulgel and standard (Aceclofenac) treatment group. *p<0.0001 compared to control. Emulgel 3 indicatedbetter anti-inflammatory action as compared to Emulgel 1 and 2, pertaining to the presence of Sapindus mukorossi.

Fig 4: Anti-inflammatory activity of Aceclofenac emulgel in comparison to control (p<0.001) in paw edema models induced with carrageenan.

Table 6: Anti-inflammatory action (Percent Inhibition) of Aceclofenac emulgel on paw edema in rats inducedwith carrageenan.

Time (hr)	Control (Normal saline)	Standard drug (Aceclofenac gel)	Emulgel 1	Emulgel 2	Emulgel 3
1	11.18±0.86	35.39±1.84	21.47±1.17	24.86±1.28	38.68±1.48
2	15.13±1.11	48.27±2.51	42.06±1.74	39.31±1.47	51.01±2.79
3	17.76±1.05	51.47±1.68	47.79±0.87	44.11±1.36	55.14±3.01
4	21.71±1.61	55.46±2.16	50.78±2.68	46.09±2.28	59.37±4.37
5	25.01±0.32	58.82±2.78	54.62±3.71	51.26±3.07	61.34±5.56
6	26.97±2.73	71.29±3.65	58.33±4.09	56.48±4.13	75.92±3.89

Table 7: ANOVA summary.

ANOVA SUMMARY					
F	10.27				
P Value	<0.0001				
Significant diff among means (P<0.05)?	YES				
R square	0.6216				
Brown-forsythe test					
F (DFn, DFd)	0.2738(4, 25)				
P Value	0.8921				
Are SDs significantly different (P<0.05)?	No				
Bartlett's Test					
Bartlett's statistic (corrected)	2.840				
P value	0.5850				
Are SDs significantly different (P<0.05)?	No				
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	5121	4	1280	F (4, 25) =10.27	P<0.0001
Residual (within columns)	3118	25	124.7		
Total	8239	29			
Data summary					
Number of treatments (columns)	5				
Number of values (total)	30				

comprised of *Zingiber officinale*. Ginger is said to provide numerous health benefits, including the reduction of pain, swelling and inflammation.Dried ginger extract, dried gingerol-enriched extract and [6]-gingerol showed a strong anti-inflammatory and analgesic properties.Previous investigations on animals have suggested that rats' hind limbs administered with [6]-gingerol produced more heat, which was linked to higher oxygen demand and efflux of lactate.Ginger larger dose reduces the consumption of oxygen,that was linked to a disturbance in mitochondrial activity(Young *et al.*, 2005). A further investigation suggested that the rats received a [6]-gingerolsingle intraperitoneal injection (2.5 or 25 mg/kg) showed a fast, noticeable decrease in temperature of body as well as a considerable decrease in rate of metabolism, which supported these findings(Ueki *et al.*, 2008). Findings suggests that ginger may show anti-inflammatory activity through calcium levels modulation mediated with transient receptor potential vanilloid subtype 1 (TRPV1), a heat and pain sensitive receptor which interact with [6]-gingerol (Dedov *et al.*, 2002). The Emulgel 3 comprised of *Sapindus mukorossi*, also called as soap nut is used as a surfactant in many pharmaceutical preparations. Soapnuts' surfactant effectiveness is similar to that of sodium octanesulfonate, dioctyl sodium sulfosuccinate and sodium dodecylben zenesulfonate. Researchers found that *Sapindus mukorossi* affected the ethyl cellulose-free filmspermeability, which helped them create rate-controlling membranes that were appropriate for transdermal application. With an increment in concentration of soapnut powder, the both medications permeability increased, suggesting that concentration of soapnut powder affects free film permeability more so than films created without soapnut.Overall, these data suggest that the intricate interactions between these components are responsible for test formulationsanalgesic and anti-inflammatory effects(Katakam *et al.*, 2010). From the obtained results, it was observed that emulgel 3 showed better anti-inflammatory activity pertaining to the presence of surfactant *i.e.*, *Sapindus mukorossi* which caused more permeability in comparison to *Allium sativum* and *Zingiber officinale*.

CONCLUSION

The present study concluded thatthe decrease in paw licking action was found to be 69.52, 72.32 and 74.10% with Emulgel 1, Emulgel 2 and Emulgel 3, compared to control group ($p<0.05$ vs control) in paw licking in animals. These outcomes suggested that all 3 formulations have promising analgesic effect. Emulgel3 showed better anti-inflammatory activity pertaining to the presence of surfactant *i.e.*, *Sapindus mukorossi* as penetration enhancer which caused more permeability in comparison to *Allium sativum* and *Zingiber officinale*.

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Conflict of interest

The authors affirm that there is no conflict of interest.

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