B-5459
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RESEARCH ARTICLE Indian Journal of Animal Research

Phytochemical and Pharmacological Screening of Natural Oils to Study Their Synergistic Activity with Aceclofenac

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10.18805/IJAR.B-5459

ABSTRACT

Background: Drug aceclofenac is commonly used as an analgesics both topically and orally. Though it has diverse side effects related to oral delivery. Aim of recent work is to design and optimize a transdermal gel loaded with penetration enhancer from various natural sources so as to promote the delivery *via* skin.

Methods: Carbopol was used as a gelling agent. Other ingredients used include essential oil from *Allium sativum*, *Zingiber officinale*, Triethanolamine, tween 80 *etc.* For animal studies Albino Wistar rats were employed in which analgesic as well as anti-inflammatory activity of Aceclofenac were evaluated.

Result: All the prepared Emulgel were good in appearance and viscous in nature. The pH of the Emulgel from both essential oils ranged between 5.00-7.00. The spreading coefficient ranged between 19.00 - 28.00 gm.cm/sec for FA1-FA9 and between 17-26.4 gm.cm/sec for FZ1-FZ9. All the formulations showed optimum viscosity. The drug release percent of Emulgel FA1-FA9 ranged between 75.0-89.0% and 80-90 for FZ1-FZ9. In *in-vivo* studies comparable analgesic and anti-inflammatory activity were found. As a concluding statement we can say that essential oil from both *Allium sativum* and *Zingiber officinale* can act as a potential candidate for enhancement of drug delivery through skin.

Key words: Aceclofenac, *Allium Sativum*, Emulgel, Penetration, Transdermal, *Zingiber officinale.*

Abbreviation: %: Percentage, ACF: Aceclofenac, NSAIDS: Non steroidal anti inflammatory drugs, SEM: Scanning electron microscopy.

INTRODUCTION

Aceclofenac which is a COX Inhibitors is widely used as Non-Steroidal Anti Inflammatory Drug. Its mechanism of treatment involves inhibiting the activity of cyclo-oxygenase enzyme produced in the body and responsible for prostaglandins release. This leads to commotion of pain and swelling along with inflammation in the affected part. Aceclofenac is mainly prescribed to cure and control effectively the pain and inflammation connected to rheumatoid arthritis. Combination of natural anti-inflammatory agents and aceclofenac can produce synergistic effect and give faster and effective relief in pain and discomfort when applied topically. To achieve better patient compliance, gels are preferred as they have nice soothing effects and easy water washable properties (Bhatt *et al.,* 2013).

Because of certain complexities in its structural anatomy skin displays barrier for penetration of external agents including drugs through it. Aceclofenac with molecular weight of around 354.19 is a suitable candidate for transdermal delivery. An attempt to increase the transport of drug via skin can be made by using permeation enhancement carriers commonly known known as penetration enhancers (Bashay, 2015) or sorption promoters or accelerants.

Skin penetration promoters especially naturally extracted one can alter lipid structure inter-cellularly between corneocytes and thus increase drug diffusivity. Secondly they can modify the intracellular protein domains which are present within the horny layer of skin. Also they may act by increasing the partitioning of the agent under the skin tissue. (Fox, 2011).

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How to cite this article: Kala, S. and Juyal, D. (2024). Phytochemical and Pharmacological Screening of Natural Oils to Study Their Synergistic Activity with Aceclofenac. Indian Journal of Animal Research. 1-8. doi: 10.18805/IJAR.B-5459.

Submitted: 30-07-2024 **Accepted:** 15-10-2024 **Online:** 28-11-2024

Gels form a three-dimensional matrix are mainly topical, semisolid, transparent consisting of dispersion of drug in suitable hydrophobic or hydrophilic base. (Premjeet S, 2012). Post administration of gel, liquid evaporates leaving behind the drug entrapped in a thin film.

The essential oils present in garlic buds are proved to be constituted mostly by sulfur rich compounds, mainly allyl polysulfides. The main components that has been reported in the essential oil obtained from the distillation of buds of *Allium sativum* include mainly sulfur-containing compounds like allyl methyl trisulfide, dimethyl trisulfide, diallyl disulfide, methyl (*E*)-1-propenyl disulfide and allyl (*E*)-1-propenyl disulfide (Al-Suwaiegh, 2023).

Essential oil present in rhizomes of ginger is rich in Terpenes *i.e.* Sesqui-terpene hydrocarbons and phenol compounds which are gingerol and shogaol which may show potential anti-inflammatory and penetration enhancement activity. Ginger contains more than 60 active

phytochemical constituents as carbohydrates, proteins, lipids, non-volatile pungent scaffolds (Gingerols, Zingerone, Shogaols and Paradols), sesquiterpene (Zingiberene, β-Sesquiphellandrene and β-Bisabolene), monoterpenoid (β-phelladrene, cineol and Citral), vitamins (B5, B6 and E) and minerals (calcium, phosphorus, magnesium, sodium, iron and manganese). (A.A. Mohammed. *et.al* 2024) Anti- inflammatory activity of ginger has also been reported earlier. Gingerol, shogaol and other similar and other related and structural substances in ginger have been proposed to inhibit biosynthesis of prostaglandin and leukotriene mainly by suppressing 5- lipoxygenase or prostaglandin synthetase (Niharika, 2017).

The current scope of this paper is to access the effect of natural penetration enhancers which is essential oil from *Allium sativum* and *Zingiber officinale* on the transdermal absorption of Aceclofenac.

Traditionally Garlic has anti-inflammatory activity. In the current study the oil extracted from Garlic *i.e. Allium sativum* and ginger *i.e. Zingiber officinale* has been used both as analgesic along with as a natural penetration enhancers. Extracted essential oil has been loaded on an Aceclofenac gel to maximise the penetration of drug.

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).

MATERIALS AND METHODS

Authentification of crude sample

Crude samples of garlic and ginger were sent for authentification to CSIR- National Institute of science communication and information resources.

The crude sample of Aceclofenac was received as a gift sample from East African Company, Dehradun.

Extraction of essential oil

Steam distillation was employed for extraction of essential oil from buds of *Allium sativum*. 100 gm of buds were crushed placed in conical flask which was connected to the Clevenger apparatus. Appropriate amount of distilled water was filled in the flask and heated up to boiling point. Steam triggered the release of the aromatic molecules present in the garlic buds. The combination of steam and essential oils was present and collected into measuring cylinder and allowed to stand for about 5 hr after which oil layer got separated and was collected. (Bagudo *et al.,* 2014).

Same procedure was adopted for crushed rhizomes of *Zingiber officinale.*

Phytochemical screening og isolated material

Various tests were performed on isolated essential oils from *Allium sativum* and *Zingiber officinale* and extract from *Sapindus mukorossi*. Various tests are explained as follows:
Specific gravity of oil (Bagudo *et al.,* 2014).

Take around 10 ml of oil in a pre-weighed weighing bottle. The specific gravity of oil can be calculated as.

$$\text{Density of oil} = W1 - \frac{W_0}{V_0}$$

Where,
W1= Weight of empty weighing bottle +oil.
W_0 = Weight of weighing bottle.
V_0= Volume of oil used.

$$\text{Specific gravity} = \frac{\text{Density of water used}}{\text{Density of oil used}}$$

Saponification value determination

Add 30 ml of ethanolic KOH to a flask containing around 2 g of the precisely weighed extracted oil. For an additional 30 minutes, it was connected to a condenser to ensure that the sample was completely dissolved. After cooling add 1 ml of phenolphthalein titrate with 0.5 M HCl until a pink endpoint.

$$\text{Saponification value} = \frac{(S-B)\times M\times 56.1}{\text{Sample weight (g)}}$$

Where,
S= Implies sample titre value.
B= Blank titre value.
M= Molarity of the HCl.
56.1 represents molecular weight of KOH.

TLC of ginger essential oil

Essential oil of *Zingiber officinale* was also analyzed by Thin layer chromatographic analysis method.

Mobile phase

Combination of Toluene: Ethyl Acetate: Formic Acid in the ration of 9:1:2.
TLC plates: Percolated silica gel $60F_{254}$.
visualizing agent: Anisaldehyde- sulfuric acid.
Plates were visualized both in day light and UV short and long wavelength. The *Rf* value of spots was calculated (Syed Shariq Mian, 2019).

$$Rf \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

Emulgel formulation

For the formulation take mentioned quantity of methanol and dissolve drug and essential with continuous stirring. Dissolve carbopol-934 in water into another flask. Continue stirring till carbopol is completely dissolved. To neutralize carbopol-934 add triethanolamine. Add drug solution to oily phase with little amount of Tween 80 with continuous stirring.

Finally solution was continuously stirred for upto 2 hours. Lastly add benzyl alcohol and ethylene glycol with continuous stirring.(Singh V.K 2013) Similarly nine different batches were formulated. For *Allium sativum* formulations were coded as FA1-FA9 (Table 1) and in case of *Zingiber officinale* coding were FZ1-FZ9 (Table 2).

Evaluation of emulgels

All the formulated emulgel loaded with natural oils were further evaluated for various parameters. All formulation were inspected for color and homogeneity.

For pH determination disperse 1.0 gm of formulation in 20 ml of distilled water and note pH using calibrated PH meter (Patel, 2011).

To measure spreading coefficient a circle with 1 cm diameter is marked in glass plate and spread 0.5 gm of formulated gel within circle. Place a second glass plate above it. Place around 500 gm of weight above the first glass plate for around 5 min. It cause an increase in the diameter of circle due to the spreading of the emulgel which can be calculated (Nanda, 2014). Brookfield Viscometer was used for Viscosity Measurement in cps.

For extrudability test aluminum collapsible tubes were filled with 20 g emulgel and exrtrudability was observed (Kumar, 2010).

To measure Drug content 100 mg of emulgel was dissolved in 100 ml of mixture of phosphate buffer solution pH 6.8 and methanol (60:40) solution and stirred for about 2 hrs. This solution was filtered and the absorbance was measured at 273 nm (Trivedi, 2013).

Franz diffusion cell was used to study *in vitro* drug release and artificial transdermal membrane was employed to mimic the skin membrane (Shivhare, 2009).

In the receiver compartment phosphate buffer (pH 7.4) was placed and in the donor compartment about 1 gm of Emulgel formulation was placed. The receptor phase was continuously stirred at temperature 37±0.5°C maintained during the process. Between regular time intervals 1 ml of the sample was taken and replaced with fresh buffer solution in receiver compartment. Withdrawn samples were analyzed at 273 nm.

Graphical study was done after plotting release order curves in Excel sheet to further study the type of drug release.

Based on all evaluation parameters best formulation were selected and further subjected to *in-vivo* studies.

***In vivo* studies**

For *in-vivo* studies authors took permission from IAEC with protocol ID: SIP/IAEC/PCOL/01/2022. Best formulations selected from evaluation studies were used for *in-vivo* studies employing albino Wistar rats (200–220 g) to access both analgesic as well as anti - inflammatory activity of best selected emulgel formulation. All the animals were kept under standard laboratory conditions.

All the rats were divided into different groups (n=6):

Group 1- untreated group (Control).

Group 2- received Drug (Standard).

Group 3- treatment with FAB.

Group 4- treatment with FZB.

Table 1: Formulation chart of factorial batches of for *Allium sativum* essential oil.

Ingredients	FA1	FA2	FA3	FA4	FA5	FA6	FA7	FA8	FA9
Allium sativum oil (ml)	1	3	5	1	3	5	1	3	5
Aceclofenac (mg)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methanol (ml)	5	5	5	5	5	5	5	5	5
Carbopol 934 (gm)	0.15	0.15	0.15	0.325	0.325	0.325	0.5	0.5	0.5
Tween 80 (ml)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Benzyl alcohol (ml)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Ethylene glycol (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine (ml)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Purified water (qs)	20	20	20	20	20	20	20	20	20

Table 2: Formulation chart of factorial batches of for *Zingiber officinale* essential oil.

Ingredients (%)	FZ1	FZ2	FZ3	FZ4	FZ5	FZ6	FZ7	FZ8	FZ9
Zingiber officinale oil	1	3	5	1	3	5	1	3	5
Aceclofenac (mg)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methanol (ml)	5	5	5	5	5	5	5	5	5
Carbopol 934	0.15	0.15	0.15	0.325	0.325	0.325	0.50	0.50	0.50
Tween 80 (ml)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Benzyl alcohol	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Ethylene glycol	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Purified water (qs)	20	20	20	20	20	20	20	20	20

For analgesic activity method described by Khan *et al* was used and around 300 mg of formulation was gently rubbed on dorsal surface of the rat's right hind paw. After about 30 min subcutaneously 5% formalin (20 µl) was injected over plantar aponeurosis of the right hind paw of rat. The time taken by rats for responses like licking and biting was an indicator of pain response was recorded in seconds for 5 min after injection (Khan *et al.,* 2015).

Carrageenan-Induced Paw Edema Model was used to evaluate the anti-inflammatory effect of emulgel. 0.1 ml of 1% of carrageenan was administered subcutaneously to right hind paw of rat in the sub-plantar surface. All the formulations were rubbed around half an hour before the carrageenan administration. The paw volume was measured at regular time intervals with the help of a digital Vernier caliper (Panthong *et al.,* 2007).

SEM analysis

After the *in-vivo* studies the best formulation was applied to excised rat skin and sent for SEM analysis.

RESULTS AND DISCUSSION

Phyto-chemical analysis of extracted bio- material

All the natural materials extracted were further subjected for phyto chemical analysis study. Color, odour, specific gravity and saponification value was studied for essential oils. Values for each oil are given in Table 3.

TLC analysis for *Zingiber officinale*

Presence of terpenes was confirmed by identification of spots of identical Rf value and about equal magnitude obtained with an unknown and a reference sample chromatographed on the same plate as shown in Fig 1.

All the optimization batches of emulgels from essential oil from *Allium sativum (*FA1-FA9) and *Zingiber officinale* (FZ1-FZ9) were formulated as per the formulation table and further evaluated for different evaluation parameters. Results of all the parameters are shown in Table 4 and 5.

Appearance

The prepared gel formulations showed good viscous, off white appearances and were viscous in nature.

pH

All the formulations showed optimum pH between 5.5 to 7.10 (FA1-FA9) and around 5.7-7.2 for emulgel batches FZ1-FZ9 which is around the PH of skin which showed that the product will show less irritability to the skin.

Viscosity

Viscosity of all formulation FA1-FA9 lied between 20350 to 39741 cp and in case of FZ1-FZ9 20259-38069 cp depending on the concentration of gelling agent concentration for various batches. High concentration batches showed high viscosity. FA9 and FZ9 with highest concentration of gelling agent showed viscosity of 39741 cps and 36450 cps respectively.

Extrudability

All the batches showed good extrudability as shown in Table 4 and 5. FA4 showed highest extrudability of 341 $gm.cm^2$ while FZ8 showed extrudability of 385.59 $gm.cm^2$.

Spreading coefficient

All gel formulation showed good spreadibility which showed that gel will be easily spread in the skin without any inconvenience.

Drug content

All formulations showed good drug content with FA5 having best drug content of 90.1%. Also FZ8 showed best drug content of 90.3%.

Drug release

In *in vitro* drug release studies all the emulgel formulations FA1-FA9 depicted good drug release profile with almost 82% of drug release upto 8 hr as shown in Fig 2. Similarly FZ1-FZ9 showed release of about 84% upto 8 hr. This showed the penetration enhancers can increase the release of drug effectively.

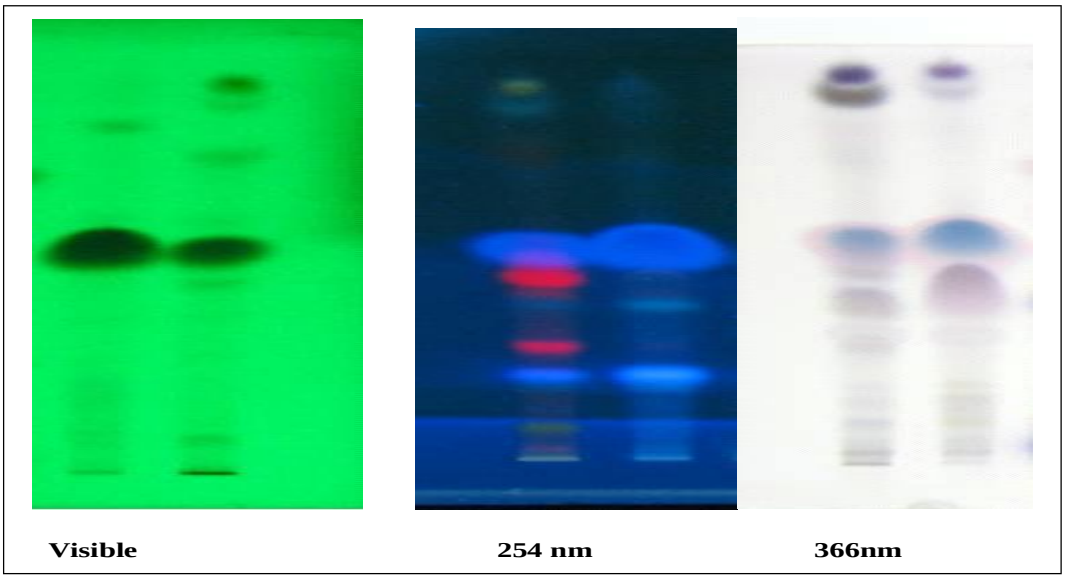
After the statistical analysis the Model F-value of 126.08 implied that formulation model was significant and there are only 0.11% chances that this large value of F could occur due to noise. P-values were always less than 0.0500 indicating that model terms are significant. Based on statistical analysis software gave the best possible concentration of penetration enhancer to be used for maximum effect. So again emulgel with the concentration suggested by software were formulated (FAB and FZB) and evaluated.

Analgesic activity

All the formulations reduced paw licking activity ($p<0.05$ vs. control). Results of the paw licking activity are shown in Table 6. However, the standard formulation was found to produce significant analgesic activity. Comparison of standard with test formulations showed that the effect of formulations; FAB and FZB were comparable to the standard

Table 3: Phyto-chemical analysis of essential oils.

Parameters	Characteristics (Essential oil from *Zingiber officinale*)	Characteristics (Essential oil from *Allium sativum*)
Color of oil	Light yellow	Light yellow
Specific gravity	0.85	0.89
Odour	Characteristic	Characteristic
Saponification value (mgKOH/g)	93	98



Compound	Rf	Colour (after post derivitazation)
Essential oil from *Zingiber officinale*	0.67	Pink

Fig 1: TLC analysis for ginger oil.

Table 4: Evaluation of emulgel batches from *Allium sativum* FA1-FA9.

Formulation	Appearance	pH	Viscosity (cps)	Extrudability ($gm.cm^2$)	Spreading coefficient (gm.cm/sec)	Drug content (%)
FA1	Good, Viscous	6.60	21570	283.13	25.4	89.2
FA2	Good, Viscous	6.53	20350	375.41	21.5	88.7
FA3	Good, Viscous	6.67	21045	258.36	23.6	86.8
FA4	Good, Viscous	5.87	36612	341.46	28.5	87.9
FA5	Good, Viscous	5.80	35320	317.29	20.1	90.1
FA6	Good, Viscous	7.10	36213	319.65	22.4	86.4
FA7	Good, Viscous	5.70	38901	278.97	19.56	88.9
FA8	Good, Viscous	6.63	37916	338.71	21.2	90.8
FA9	Good, Viscous	6.73	39741	279.98	19.6	88.1

Table 5: Evaluation of emulgel batches from *Zingiber officinale* FZ1-FZ9.

Formulation	Appearance	pH	Extrudability ($gm.cm^2$)	Viscosity (cps)	Spreading coefficient (gm.cm/sec)	Drug content (%)
FZ1	Good , Viscous	6.51	383.29	21450	26.4	80.1
FZ2	Good , Viscous	5.57	295.79	20259	25.5	84.7
FZ3	Good , Viscous	6.67	289.09	21075	24.6	83.5
FZ4	Good , Viscous	5.97	349.24	34665	18.5	83.5
FZ5	Good , Viscous	5.91	347.19	32517	21.3	89.4
FZ6	Good , Viscous	7.20	343.95	31775	22.7	85.6
FZ7	Good , Viscous	5.79	279.97	37987	17.16	88.1
FZ8	Good , Viscous	6.03	385.59	38069	20.3	90.3
FZ9	Good , Viscous	6.43	287.18	36450	18.5	85.3

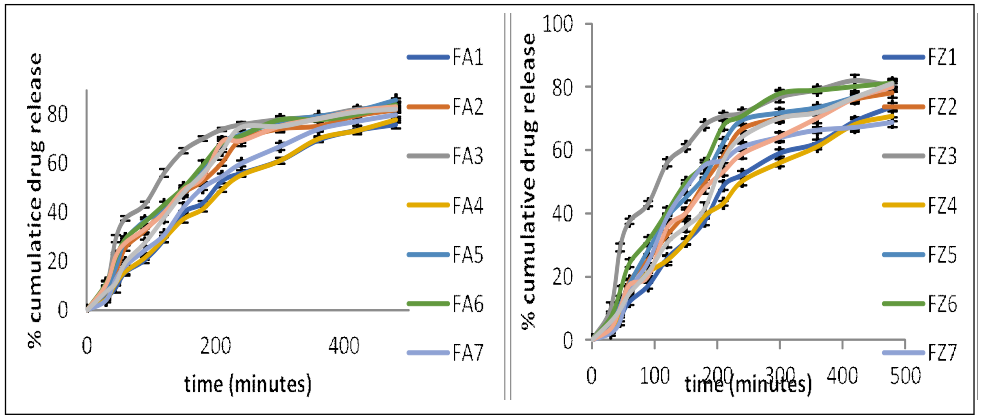
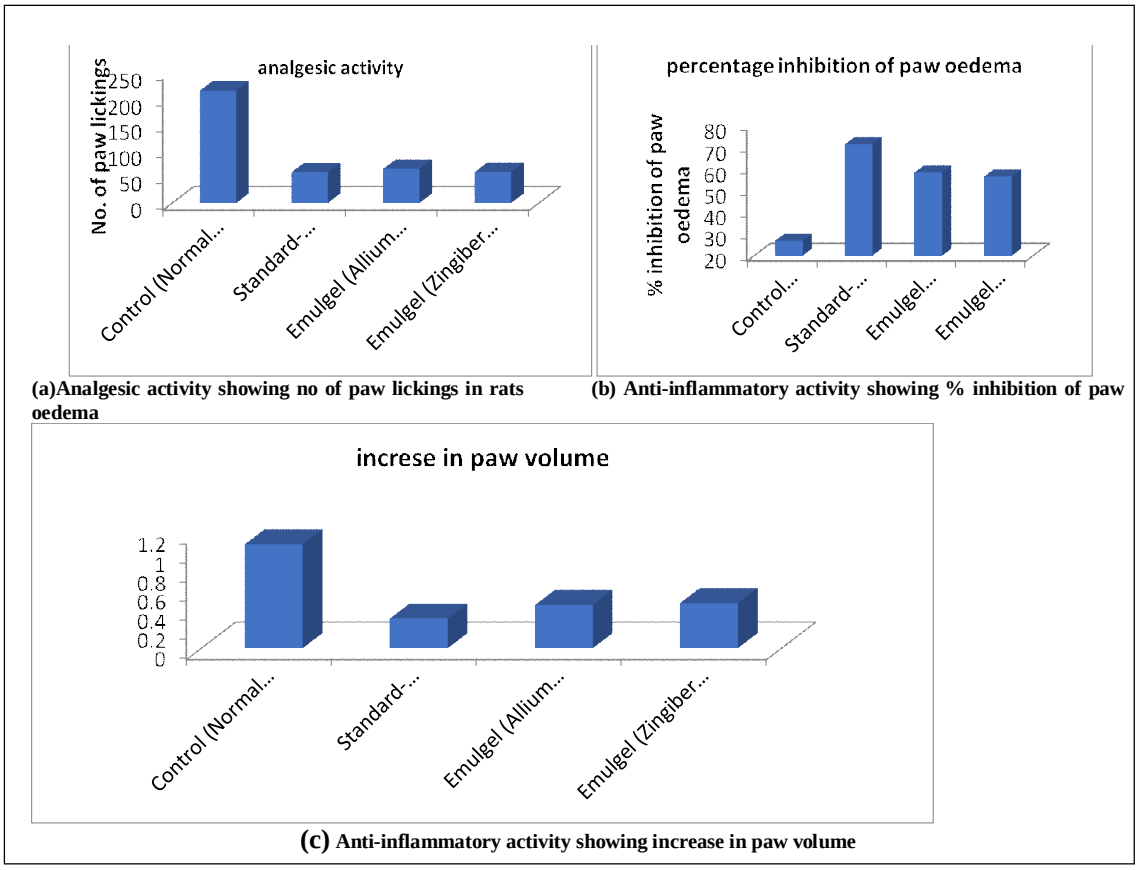


Fig 2: Cumulative drug release of various formulations.



(a)Analgesic activity showing no of paw lickings in rats oedema

(b) Anti-inflammatory activity showing % inhibition of paw oedema

(c) Anti-inflammatory activity showing increase in paw volume

Fig 3: *In-vivo* activity of the best selected formulations.

Table 6: Number of Paw lickings by animals after formalin injection.

Control (Normal Saline)	Standard-Aceclofenac gel	Emulgel 1(FAB)	Emulgel 2(FZB)
0	0	0	0
35.75±0.12	17.68±0.47	28.25±0.19	34.81±0.26
48.65±0.22	30.66±0.56	32.38±0.27	45.13±0.51
98.45±0.15	35.63±0.44	40.76±0.15	57.41±0.35
141.0±0.27	49.71±0.29	53.23±0.38	63.79±0.18
215.0±0.18	58.82±0.25	65.52±0.47	59.51±0.31

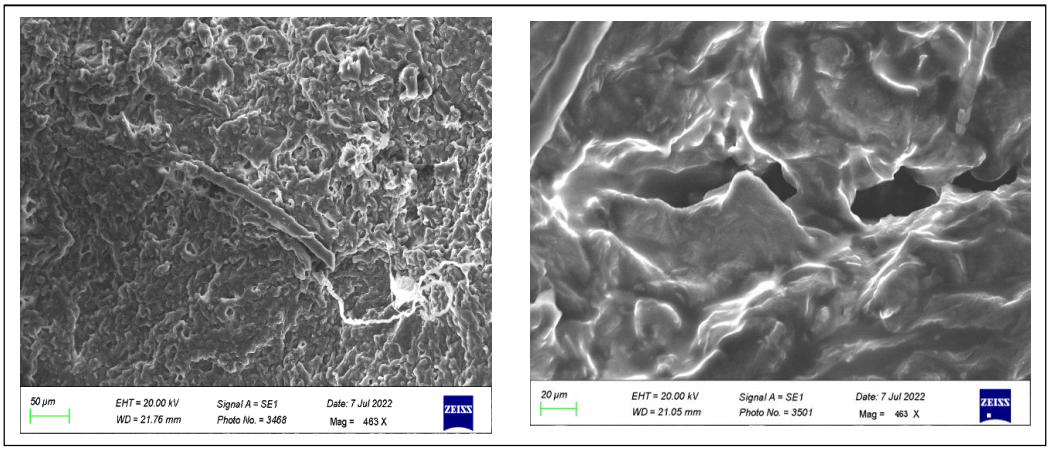


Fig 4: (a) SEM picture of rat skin (b) SEM picture of rat skin after application of emulgel FZB.

formulation. These results implied that all three formulations showed promising analgesic activity. The paw licking activity was reduced to approximately 69.52% and 72.32% with FAB and FZB, respectively compared to control group ($p<0.05$ vs. control).

Anti-inflammatory activity

To assess the anti-inflammatory activity Carrageenan-induced paw edema model was used. Paw volume was found to significantly decreased ($p<0.0001$) in animal treated with Aceclofenac emulgel (Fig 3). With progress in time on 1st, 3rd and 5th hr, test samples significantly decreased the volume while more reduction was found in Emulgel from *Zingiber officinale* ($p<0.0001$). The detailed results are shown in Fig 3. Early phase response of % reduction in paw edema was measured 1 hr after induction of paw edema. The reduction of paw edema was 21.47% and 24.86% with FAB and FZB, respectively. However, late phase response even higher where 58.33% and 56.48% inhibition of paw edema was observed with formulations; FAB and FZB respectively. The inhibition of paw edema in groups treated with standard was found to be 71.29% in comparison to the test formulations.

SEM analysis

Fig 4 demonstrates the SEM pictures of excised rat skin both before and after application of emulgel from *Zingiber officinale*.

In animal testing the inflammation markers produced by carrageenan is fast, non immune and also highly reproducible. Main inflammation symptoms includes edema, hyperalgesia along with erythema. Inflammation and pain develops immediately after SC injection due to action of pro inflammatory agents like bradykinin, histamine, *etc.* Embodiments of the present invention generally relate to a delivery method for a chemical substance for the controlled dispensing of the chemical substance to and through a surface, respectively skin. More specifically the invention relates to a method, *i.e.* for transdermal drug delivery of Aceclofenac. Evaluation results suggest that FA5 and FZ8 showed the best evaluation parameters and same were used for *in-vivo* testing on Wistar rats. FZ8 showed the best analgesic and anti-inflammatory activity. Thus the extractes oils can act a synergist to enhance the activity of Aceclfenac.

CONCLUSION

After all results and discussions it can be concluded that along with its anti inflammatory activity, essential oil from both *Zingiber officinale* and *Allium sativum* can act as potential penetration enhancer for various classes of drug. However essential oil from *Zingiber officinale* can give better penetration enhancement.

Summary

The main aim of this work is to find out the possibility of *Allium sativum* and *Zingiber officinale* essential oil to act as penetration enhancer for Aceclofenac loaded emulgel formulation. The optimized formulation from both the natural sources showed comparable *in-vivo* activity to that in standard dosage form of Aceclofenac.

ACKNOWLEDGEMENT

The author acknowledges the Siddhartha Institute of Pharmacy, Dehradun to provide all the facilities and chemical required for the animal research. Other finances required were managed by authors.

Informed content

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there is no conflict of interest.

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