Development, characterization and evaluation of ginger extract loaded microemulsion: *In vitro* and *Ex vivo* release studies

Ayesha Akram[1], Naveed Akhtar[2], Muhammad Khurram Waqas[3], [*]Akhtar Rasul[1], Kashif ur Rehman[3], Jahangir Khan[4], Muhammad Iqbal[5] and Barkat Ali Khan[5]

[1]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Government College University Faisalabad, Pakistan
[2]Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur, Pakistan
[3]Institute of Pharmaceutical Sciences, University of Veterinary & Animal Sciences, Lahore Pakistan
[4]Department of Pharmacy, University of Malakand, Chakdara, KPK, Pakistan
[5]Faculty of Pharmacy, Gomal University Dera Ismail Khan, KPK, Pakistan

Abstract: *Zingeber officinale* (ginger) has been used for a long time in conventional medicine for the management of many diseases most important of which is inflammatory diseases. The aim of this study was formulation of topical microemulsion system to enhance the solubility, stability and release profile of ginger extract, as it is unstable in the presence of light, air, heat and long term storage. The solubility of ginger extract in different oils, surfactants, and co-surfactants was determined in order to find the optimal components for microemulsion. Isopropyl myristate (IPM) was selected as oil phase, tween 80 and PEG 400 were selected as surfactant and co-surfactant respectively based on highest solubility values. Pseudo-ternary phase diagram was constructed in order to find out the microemulsion region. The prepared microemulsions were evaluated for pH, viscosity, conductivity, refractive index, globular size, zeta potential, polydispersity index, ginger extract content, *in-vitro* and *ex-vivo* release profiles. The formulation GE1 showed best physicochemical properties with smallest globular size (19.75nm), highest release rate and flux value. It also showed significant ($p<0.05$) anti-inflammatory effect as compared to reference piroxicam drug solution. It is concluded that ginger extract can be used to develop stable microemulsion system with better skin permeation and promising anti-inflammatory activity.

Keywords: Ginger extract, O/W microemulsion, pseudo-ternary phase diagram, drug release, permeability.

INTRODUCTION

Microemulsions (ME) are stable systems thermodynamically and this property allows self-emulsification of that system whose properties are independent on process used (Lucia *et al*., 2016). This system acts as super-solvent for the drug molecule (Puri *et al*., 2016). ME can solubilize both lipophilic and hydrophilic drugs thus improving the bioavailability of poorly soluble drugs. This property is due to presence of nanodomains of varying polarity within same single phase system (Kawakami *et al*., 2002; Okur *et al*., 2011).

Some important challenges for drug delivery are poor water solubility of drug, short half-life (in vitro and in vivo), low bioavailability, low stability, large number of side effects and other regulatory issues. This solubility issue is the major barrier in the drug delivery of new drug and many existing drugs. But microemulsions appear to be the best vehicles for the delivery of these poorly soluble entities (Lv *et al*., 2018). The small droplet size of microemulsions has the advantage of adhering to biological membranes and to transport these entities in more controlled manner. Using ME as drug delivery vehicles, lipophilic components from different plant extracts can be co-solubilized to obtain synergistic effect for various therapeutic purposes (Kogan & Garti, 2006). *Zingeber officinale* (Ginger) is a herbal plant which belongs to family Zingeberaceae and is widely used as herb, condiment and spice (Chan & Wong, 2015). It has been used as traditional medicine from ancient times for the management of several diseases including inflammatory diseases. The active compounds present in ginger are gingerols, shagaols, and paradol that have anti-inflammatory, anti-oxidant, anti-cancer and anti-atherosclerotic properties (Habib *et al*., 2008).

Repetitive daily applications of conventional topical dosage forms may result in poor patient compliance especially in the cure of chronic skin disorders. Therefore, development of novel topical drug delivery systems is of great interest for dermatological therapy like microemulsions, nanoemulsions, hydrogels, emulgels etc. These delivery systems are of great interest because they may facilitate less frequent dosing by increasing contact time, maintaining drug delivery across the skin and by other mechanisms (Frederiksen, 2015).

MATERIALS AND METHOD

Ginger rhizome was purchased from the local market, Faisalabad, PakistaGinger rhizome was purchased from

*Corresponding author: e-mail: akhtar.rasul@gcuf.edu.pk, barki.gold@gmail.com

the local market, Faisalabad, Pakistan and authenticated by Dr. Adnan Amin, Department of Pharmacognosy, GU. The herbarium reference is 45/Phg-GU/2018. Tween-60, Tween-80, Span-20, Span-80, Polyethylene glycol 400, Propylene glycol, Ethyl acetate, Mineral oil and Isopropyl myristate were obtained from Daejung chemicals (Korea). Cremophore EL and Miglyol 812N were purchased from Pakistan scientific store, Faisalabad (Pakistan). Coconut oil, Soybean oil, Almond oil, Eucalyptus oil, Castor oil and Olive oil were purchased from Al-Barkat Pharmaceuticals LTD (Lahore, Pakistan). Potassium dihydrogen phosphate (KH_2PO_4) was acquired from E. Merck (Germany). Sodium hydroxide (NaOH) was obtained from Riedel-de Haen (Germany).

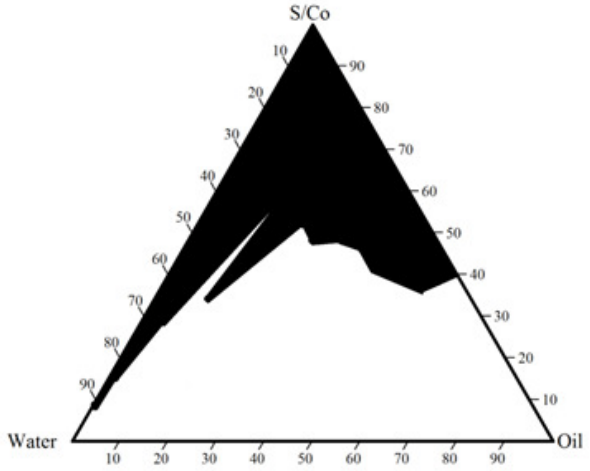

Fig. 1: Pseudo-ternary phase diagram composed of IPM as oil phase, Tween 80 / PEG 400 as Surfactant / Co-surfactant mixture (1:1)

Ginger extract was prepared in ethanol by maceration process. The solubility of ginger extract in various oils (Isopropyl Myristate, Miglyol 812N, Soybean oil, Eucalyptus oil, Coconut oil, Almond oil, Castor oil, Olive oil, and Mineral oil), surfactants (Tween 80, Tween 60, Span 80 and Cremophore EL), and co-surfactants (PG, PEG 400, Span 20 and Ethyl acetate) was determined. Then, pseudo ternary phase diagram was constructed to find the ME region. From pseudo ternary phase diagram, five formulations were selected on the basis of region obtained from phase diagram. Ginger extract was dissolved in Tween 80 and PEG 400 (1:1) mixture. Isopropyl myristate (IPM) as oil phase was added to the mixture. Then, water was added and the final mixture was vortexed until a clear solution was obtained.

Characterization of microemulsions

The average droplet size, polydispersity index and zeta potential of microemulsions were measured in triplicate using photon correlation spectrometer (Malvern Zetasizer, UK). The ME sample was placed in a cuvette in a thermostatic chamber. Before taking the readings, the ME formulations were diluted with distilled water. The viscosities of formulations were determined at 25 ± 0.5ºC using rotational viscometer at 50 rpm with help of spindle 64 for 1 min (Brookfield DV-II+ Pro UK). Conductivities, pH and refractive indexes were also determined at similar conditions using Conductivity meter (EcoScan con5, Eutech Instruments), pH meter (HI 2210 Hanna, USA) and Abbe Refractometer (PCE instruments UK) respectively. Experiments were performed in triplicate for each sample. *In-vitro* and *Ex-vivo* release studies were performed to optimize the formulations for anti-inflammatory study.

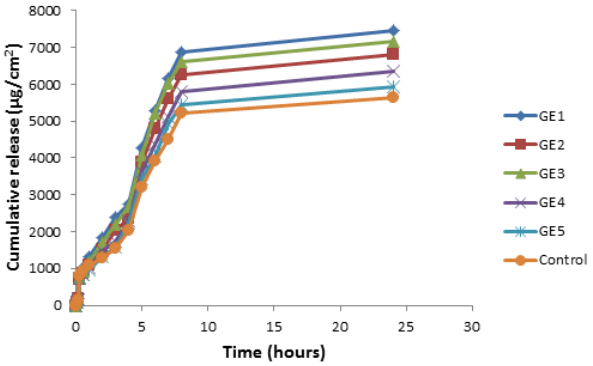

Fig. 2: Permeation profiles of five ME formulations in comparison with control

In-vitro release studies

The *in-vitro* release of ME formulations containing ginger extract were examined using Franz diffusion cells. The cell consists of donor compartment and a receptor compartment. The cell's diffusion area was 1.539 cm^2. The volume of receptor compartment was 9mL. The membrane was hydrated for 24 hours in phosphate buffer before start of study. The medium used in the receptor compartment was phosphate buffer having 7.4 pH. The temperature of medium was maintained by circulating water bath at controlled temperature of 32.0 ± 0.1ºC. Cells were placed on magnetic stirrer hot plate for continuous stirring of cell medium. When the temperature was maintained, 1 gram of ME formulation was applied on cellulose acetate membrane (Micropore, USA) that was placed over donor compartment of cell having pore size 0.45 µm and diameter of 13mm. 1mL of sample was taken from receptor nozzle after predetermined time intervals of 5, 10, 15, and 30 minutes, and then at 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours. 1mL of phosphate buffer was added into receptor compartment after each sampling interval to maintain the sink conditions. The obtained samples were then analyzed for ginger extract content using UV spectrophotometer at respective *λmax*. Three replicates were performed for each formulation. Mixture of ginger extract in tween 80 was served as control for drug permeation studies.

Cumulative amount of drug released per unit area (Qt)

The cumulative drug permeated through the membrane is denoted by Q_t having units of µg/cm^2 and was calculated by using Eq. (1) (Salerno *et al*., 2010).

Table 1: The composition of selected ME formulations from pseudo-ternary phase diagram

Formulation code	IPM (%w/w)	Tween 80/PEG 400 (%w/w)	Water (%w/w)
F1	4.0	40.0	56.0
F2	7.0	45.0	48.0
F3	10.0	50.0	40.0
F4	15.0	55.0	30.0
F5	20.0	55.0	25.0

Table 2: *In vitro* permeation parameters of ME formulations

Microemulsion codes	Flux (µg/cm^2/hr)	Permeability coefficient (cm/hr)	Lag time (min)
GE1	871.78 ± 6.43	0.109 ± 0.0008	1.25
GE2	796.74 ± 10.84	0.099 ± 0.001	2.87
GE3	846.92 ± 14.05	0.106 ± 0.002	1.77
GE4	733.88 ± 12.84	0.092 ± 0.002	3.23
GE5	694.87 ± 8.46	0.087 ± 0.001	4.18
Control	655.11 ± 8.34	0.082 ± 0.001	4.32

Table 3: *Ex-vivo* permeation parameters of selected ME formulations

Microemulsion codes	Flux (µg/cm^2/hr)	Permeability coefficient (cm/hr)	Lag time (min)
GE1	842.07 ± 16.4	0.105 ± 0.002	2.05
GE2	758.55 ± 13.99	0.095 ± 0.002	3.05
GE3	791.1 ± 16.16	0.099 ± 0.002	1.96
Control	622.54 ± 14.95	0.078 ± 0.002	4.49

$$Qt = [Cn\,V + \sum_{i=1}^{n-1} Ci\,S] \quad (1)$$

Where, C_n = ginger extract concentration (µg/mL) present at receiver compartent at nth sampling time
V = Volume of receiver compartment solution (9mL)
$\sum_{i=1}^{n-1} Ci$ = Sum of concentrations of ginger extract at *i*th sampling points through n-1
S = Volume of sample
The obtained results of cumulative drug release per unit area Q_t/A (A= 1.539 cm^2) were plotted against time.

Steady State Flux (Jss)
For calculating steady state flux values of each ME formulation, the linear regression interplotation of experimental data was obtained at steady state and flux was calculated from the slope as mentioned in Eq. (2). Steady state flux is denoted by units of µg/cm^2/hr (Sunitha *et al*., 2013).

$$J_{ss} = (\Delta Q_t/\Delta t)/ A \quad (2)$$

J_{ss} = Steady state flux
$\Delta Q_t/\Delta t$ = cumulative drug permeated per unit time
A = diffusion area of membrane (1.539 cm^2)

Permeability Coefficient (Kp)
Apparent values of permeability coefficient were calculated by dividing the flux with drug concentration as given in Eq. (3) (Sintov and Botner, 2006).

$$Kp = J_{ss}/Co \quad (3)$$

Where, Co represents the drug concentration that was applied on the membrane in the donor compartment (8.0×10^3 ug/mL). The unit of Kp is cm/hr.

Lag time (Tlag)
The lag time is the time of first drug detection in the medium is calculated from x-intercept of linear portion of plot of cumulative drug release per unit time in steady state conditions (Vlaia *et al*., 2016).

Ex-vivo release studies
Ex-vivo studies for drug release were performed using rat abdominal skin as the membrane. The study was carried out with the approval of institutional review board, Government College University Faisalabad. *Ex-vivo* skin permeation studies were also carried out using Franz diffusion.

Preparation of skin
Mice of weight 250-300 g were taken and abdominal skin samples were excised. The hairs were removed carefully by applying hair removing cream at the abdomen. After 15 minutes, hair was removed gently and carefully. Hairs were removed on the day when release studies were to be carried out in order to get optimal results. The rats were anesthetized before sacrificing them. Skin was removed and epidermis layer was separated from dermis layer carefully using scalpel. Subcutaneous fats were also removed. Then the skin was washed with distilled water.

To start the release studies, the excised skin was clamped between donor and receptor compartment of diffusion cell in such a way that epidermis or stratum corneum part should face the donor compartment. Same procedure was used as mentioned in *in-vitro* permeation studies. The cumulative drug release per unit area (µg/cm^2), steady state flux (J_{ss}), permeability coefficient and lag time were calculated as described above.

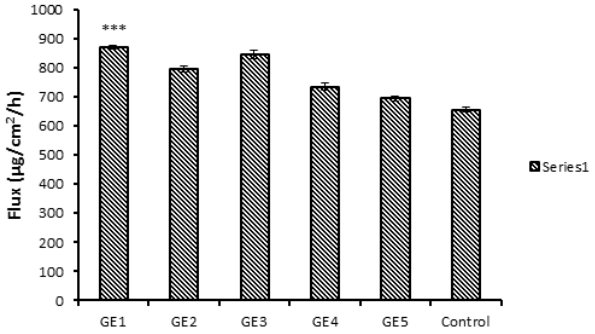

Fig. 3: Permeation rate (flux) of five ME formulations in comparison with control. (*) represents significant difference of ME formulations flux from control

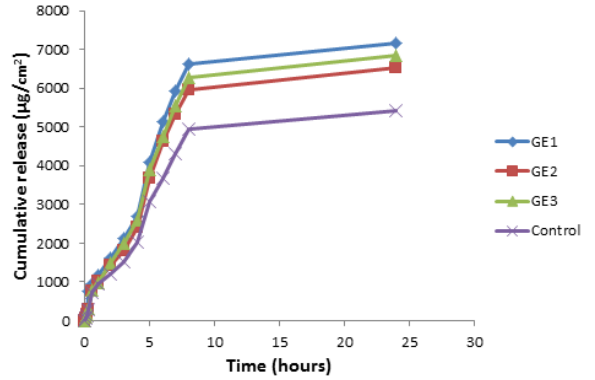

Fig. 4: Permeation profiles of three ME formulations in comparison with control

RESULTS

Pseudoternary Phase Diagram

The pseudo-ternary phase diagram is presented in fig. 1. ME formulations were made according to the ratios given in table 1. All formulations were found to be transparent. After incorporation of ginger extract, the color of ME formulations turned to light brown.

In-vitro release studies

The *in-vitro* release profiles of ME formulations containing ginger extract were evaluated and cumulative drug release per unit time is demonstrated graphically in fig. 2. It was evaluated that maximum drug was release by formulation GE-1 and GE-5 showed lowest drug permeation. The order of release rate was GE1>GE3>GE2>GE4>GE5>control.

The permeation rate (flux) values (fig. 3.) and permeation coefficient values show significantly higher values ($p<0.05$) as compared to control. Lag time values were also calculated. GE1, GE2, GE3 showed greatest release and flux values and shortest lag time as compared to GE4 and GE5 due to low viscosity and small globular size. The results of flux, kP and lag time are given in table 2.

Ex-vivo drug permeation studies

For *ex-vivo* permeation studies, the formulations GE1, GE2, and GE3 were selected from *in-vitro* data based on higher release rates. The release order was GE1>GE3>GE2>Control as indicated in fig. 4. Permeation rate (flux) of three ME formulations in comparison with control is shown in fig. 5. There was significantly higher ($p<0.05$) permeation rate values of all formulations in comparison to control which is simple solution of ginger extract in tween 80. Various permeation factors are given in table 3.

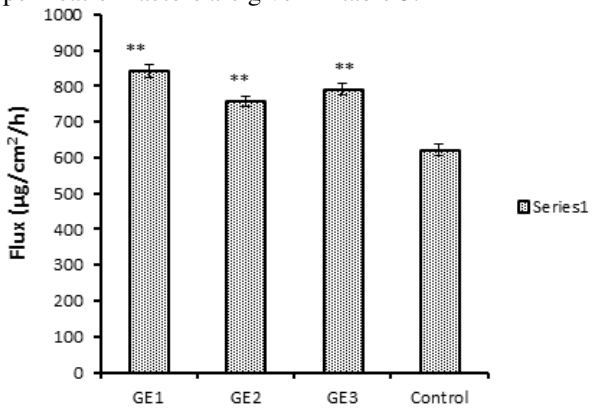

Fig. 5: Permeation rate (flux) of three ME formulations in comparison with control. (*) represents significant difference of ME formulations flux from control

DISCUSSION

Pseudoternary Phase Diagram

Based on the findings of solubility study, pseudo-ternary phase diagram was created to identify the ME region. For this purpose, components selected were Tween-80, IPM and PEG 400 as ginger extract showed maximum solubility is these components. Surfactant and co-surfactant were used at 1:1 ratio. From the phase diagram, microemulsion region was determined. From this region, five different compositions of oil, surfactant and co-surfactant were selected to make five ME formulations.

In-vitro release studies

The maximum drug was released by GE-1 while GE-5 showed minimum drug release. As the internal phase ratio is increased, there is gradual increase in viscosity of formulations due to which release rate was decreased (Tsai *et al*., 2010, Abd-Allah *et al*., 2010). Another possible reason is that, as the globular size increases the release rate decreases. It was seen that drug release is decreased on increasing surfactant concentration. The possible reason is the increase in thermodynamic activity of the drug on increasing surfactant level. As

thermodynamic activity is a driving force for the penetration of drug into the skin (Kajbafvala *et al*., 2016). The flux and permeation constant values were also higher as compared to control. This is because the formulations contain IPM as oil which served as potent skin penetration enhancer and act by partitioning itself with lipid domains of stratum corneum (Malakar *et al*., 2012). Non-ionic surfactants like tween 80 also served as strong penetration enhancers. ME formulations also contain PEG 400 having glycol part which also act as sorption enhancer. It acts as driving force for drug and takes it through skin by partitioning phenomena.

Ex-vivo drug permeation studies

Formulation GE1 showed maximum release profile, flux and lowest lag time because of smallest particle size and lowest viscosity. Thus it was considered as ideal formulation for further anti-inflammatory activity.

CONCLUSION

In this study, five ME formulations were prepared and tested for physicochemical properties. The results indicate that formulation GE1 containing 4% IPM, 40% of S/Co ratio and 56% water showed best physicochemical properties. *In-vitro* and *ex-vivo* release profiles suggest that the permeation rates were significantly influenced by nanostructured domain of ME system. The results suggest that there is significant effect of nature and concentration of surfactant and oil on the rheology and other properties of ME system. The solubility and stability of ginger extract is significantly improved by incorporating in microemulsion system.

REFERENCES

Abd-Allah FI, Dawaba HM and Ahmed A (2010). Development of a microemulsion-based formulation to improve the availability of poorly water-soluble drug. *Drug Discov. Ther.,* **4** (4): 257-66.

Chan EWC and Wong SK (2015). Phytochemistry and pharmacology of ornamental gingers, Hedychium coronarium and Alpinia purpurata: A review. *Journal of Integrative Medicine,* **13**(6): 368-379.

Frederiksen K, Guy RH and Petersson K (2015). Formulation considerations in the design of topical, polymeric film-forming systems for sustained drug delivery to the skin. *Eur. J. Pharm. Biopharm.,* **91**: 9-15. https://doi.org/10.1016/j.ejpb.2015.01.002

Habib SHM, Makpol S, Hamid NAA, Das S, Ngah WZ W and Yusof YAM (2008). Ginger extract (Zingiber officinale) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. *Clinics,* **63**(6): 807-813.

Kajbafvala A, Salabat A and Salimi A (2016). Formulation, characterization and *in vitro/ex vivo* evaluation of quercetin-loaded microemulsion for topical application. *Pharm. Dev. Technol.,* **23**(8): 1-30.

Kawakami K, Yoshikawa T, Moroto Y, Kanaoka E, Takahashi K, Nishihara Y and Masuda K (2002). Microemulsion formulation for enhanced absorption of poorly soluble drugs I. Prescription design. *J Control Release,* **81**(1-2): 65-74.

Kogan A and Garti N (2006). Microemulsions as transdermal drug delivery vehicles. *Adv Colloid Interface Sci,* **123**, 369-385. https://doi.org/10.1016/j.cis.2006.05.014

Lucia A, Argudo PG., Guzman E, Rubio RG and Ortega F (2017). Formation of surfactant free microemulsions in the ternary system water/eugenol/ethanol. *Colloids and Surfaces A*, **521**(5): 133-140.

Lv X, Cong Z, Liu Z, Ma X, Xu M, Tian Y, Zhang X, Xu B, Zhang J and Tang Z (2018). Improvement of the solubility, photostability, antioxidant activity and UVB photoprotection of trans-resveratrol by essential oil based microemulsions for topical application. *J. Drug Deliv. Sci. Tec*., **48**: 346-354. https://doi.org/10.1016/j.jddst.2018.10.017

Malakar J, Nayak AK and Basu A (2012). Ondansetron HCl microemulsions for transdermal delivery: Formulation and *in vitro* skin permeation. *ISRN Pharm.,* 1-6 doi 10.5402/2012/428396

Okur NU, Apaydın S, Yavasoglu NUK, Yavasoglu A and Karasulu HY (2011). Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. *Int J Pharm,* **416** (1): 136-144.

Puri A, Kaur A, Raza K, Goindi S and Katare OP (2016). Development and evaluation of topical microemulsion of dibenzoylmethane for treatment of UV induced photoaging. *J. Drug Deliv. Sci. Tec*., **37**: 1-12. https://doi.org/10.1016/j.jddst.2016.09.010

Salerno C, Carlucci AM and Bregni C (2010). Study of in vitro drug release and percutaneous absorption of fluconazole from topical dosage forms. *AAPS Pharm. Sci. Tech.,* **11** (2): 986-993.

Sintov AC and Botner S (2006). Transdermal drug delivery using microemulsion and aqueous systems: influence of skin storage conditions on the in vitro permeability of diclofenac from aqueous vehicle systems. *Int. J. Pharm.,* **311** (1-2): 55-62.

Sunitha S, Jitendra W, Sujatha D and Santhosh Kumar M (2013). Design and Evaluation of Hydrogel-Thickened Microemulsion for Topical Delivery of Minoxidil. *Iran. J. Pharm. Sci.,* **9** (4): 1-14.

Tsai YH, Lee KF, Huang YB, Huang CT and Wu PC (2010). *Int. J. Pharm.,* **388** (1-2): 257-262.

Vlaia L, Olariu I, Coneac G, Mut AM, Popoiu C, Corina S, Anghel DF, Maxim ME, Kalas S and Vlaia V (2016). Development of microemulsion-loaded hydrogel formulations for topical delivery of metoprolol tartrate: Physicochemical characterization and *ex vivo* evaluation. *FARMACIA,* **64** (6): 901-913