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Research Article

STUDY ON IMPROVING BIOAVAILABILITY RATIO OF ANTI-INFLAMMATORY COMPOUND FROM GINGER THROUGH NANO TRANSDERMAL DELIVERY

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ABSTRACT

Medicinal plants form the basis for health care across the world. Discovering a single efficient bioactive compound from ginger which possesses anti-inflammatory property with better pharmacokinetics values, in turn will create a good impact on pursuing the suitable drug delivery mode with enhanced ratio of bioavailability. Many routes of drug delivery have been in practice for number of decades, which are affected by poor drug absorption into the circulatory system due to hepatic metabolism (first pass effect) and solubility of the compound. These factors affect the large scale setup of the drug discovery. Overcoming strategies are in need to identify these problems through the development of novel drug delivery (NDD) carrier system. The studies focus about the improvement of bioavailability of the bioactive compound through transdermal mode and are substantially improved by nano vesicular system to surpass the barriers like stratum corneum layer of the skin. Enhanced *in-silico* method of identification of suitable bioactive compound called Gingerol from ginger plant with effective pharmacokinetics scope from other available compounds. Simple compound extraction procedure was followed to extract the gingerol (drug) from the crude ginger and purified by flash column chromatography. Gingerol was quantified by Folin Cio-calteau assay with Gallic acid as standard. The permeation studies for drug and nanovesicle encapsulated drug were performed using *in-vitro* dermal membrane system involving ear skin of *Capra aegagrus hircus*. The permeated drug samples were collected and absorbance value was measured using UV-Vis Spectrophotometer at 282 nm. Results concluded that the vesicular mediated drug release is relatively higher than the drug alone permeation. The ultra-flexible or ultra-deformable vesicle system is much more efficient in delivering a low/high molecular weight, hydrophobic/hydrophilic drugs deep into to the skin with better entrapment efficiency in carrying the drug molecule of 85%. Thus, the ratio of bioavailability of the Gingerol was substantially increased in transdermal mode of delivery when compared to the other delivery methods.

Keywords: novel drug delivery, ultra-deformable vesicle, bioavailability, first pass effect, Pharmacokinetics

INTRODUCTION

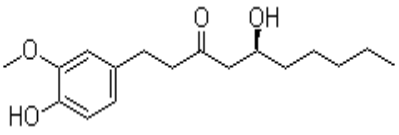
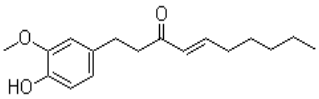
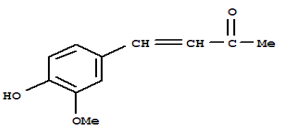
Treatment and care of a patient for the purpose of both preventing and combating diseases is a major objective of the pharmaceutical sector. Several drugs are available in market as natural drugs, synthetic drugs, and semi-synthetic drugs. Since natural drugs are still untouched by the big pharmaceutical industry, because of poor study of its pharmacokinetics. All the existing synthetic drugs which are taken through oral form lack certain parameters like poor absorption, less % of bioavailability. Several other route of drug delivery like intravenous, intramuscular are not supported because of poor patience compliances [1]. Based on this entire phenomenon, pure natural drug was taken in consideration for transdermal route of delivery with better patience compliances.

In Transdermal route of delivering a drug molecule are absorbed through the skin (percutaneous absorption) to reach blood circulation and transported to target tissue to achieve therapeutic effect. Penetration of drug into the horny skin layer of Epidermis to reach the dermis layer for absorption into the blood vessels had been a major challenge in administering the drug through transdermal mode. To overcome these difficulties there is a need for the development of novel drug delivery system, which will improve the therapeutic efficacy and safety of drugs with more precise, spatial and temporal placement with in the body, thereby reducing the size and number of dosages given to the patients [2].

BIOACTIVE COMPOUND AS A DRUG

Ginger plant (*Zingiber officinale*) belong to family Zingiberace has been widely used spice and flavouring agent, Common spice throughout the world and used in traditional oriental medicine. Several active components are present in ginger among them major active ingredients are gingerol, shogaol, gingeberin and paradol. These components are used for treatment of inflammation, rheumatism, and bronchitis [3].The ginger extracts have been extensively studied for a broad range of biological activities including antibacterial, analgesic, anti-inflammatory, anti-tumor [4-7].

Table 1: Bioactive Compounds Structure.

Molecular structure of gingerol ($C_{17}H_{26}O_4$)	Molecular structure of shogaol ($Co_{17}H_{24}O_3$)	Molecular structure of paradol ($C_{17}H_{26}O_3$)
		

Novel Drug Delivery (NDD) Carrier System

Vesicular mediated delivery of drug compounds has attracted the pharmaceutical sector in recent times. Vesicles having the tendency to encapsulate the drug compounds with both hydrophobic and hydrophilic nature to carry it to site of action, which in turn improves the viable way to cross the delivering barriers in transdermal route [8].

Vesicular structures are having lipid bilayer composed of surfactant and phospholipids which enables more flexibility and permeabilityfor its drug carrying system. Such vesicle is called ultra-deformable vesicle which are bio-compatible and bio-degradable with increased entrapment efficiency of a drug molecule [9].

Vesicular mediated drug delivery through transdermal route delivers the drug intact skin in controlled releasing form into the systemic circulation with more drug bioavailability ratio [10].

MATERIALS AND METHODS

Pharmacokinetics of the active compound was analyzed by Molinspiration and PubChem values. Ginger plant purchased from local market and kept under sterilized condition. Solvents used Methanol, hexane, chloroform were purchased from MERCK. Lecithin (Phospholipids) were purchased from MERCK, Sodium Deoxycholate (Surfactants) were purchased from Titan Biotech Ltd. Gallic acid, Folin cio-calteau, Sodium Carbonate for phenolic compound estimation were purchased from MERCK. Silica gel 230 – 400 mesh was purchased from MERCK.

Screening of Active Compound

Active compounds from ginger plant were screened using Molinspiration and PubChem values. Statistical representation were plotted between each compounds for various parameters like logP, Melting Point (M.P), Molecular Weight (M.W) to identify the drug like active compound [11] which possess good pharmacokinetics for enhancing the therapeutic role of the constituents.

Extraction, Purification and Estimation of drug like compound

Screened active compound is meant for extraction using methanolic extraction in room temperature. 10g of ginger were crushed and suspended in 200 ml of methanol. Mixture was kept for stirring for the period of 30 min. Total extract were centrifuged and supernatant was taken for purification to separate the Gingerol from the crude extract using Flash Column Chromatography packed with silica gel 230 – 400 mesh. Six fractions were analyzed for determining the high concentrated fraction by absorbing at 282nm in UV- Vis Spectrophotometer [12]. High concentrated fraction sample was estimated for total amount of phenolic compound by Folin cio-calteau assay and quantified by comparing with Gallic acid standard curve.

Identification of Gingerol by Thin layer chromatography

Thin layer chromatography is performed on a Pre-coated Silica gel sheet with thin layer absorbent material. The layer absorbent is known as stationary phase. The samples spotted on the plate, and then plates are dibbed in solvents (Hexane: di ethyl ether) with the ratio of 12:18. The samples move with solvents via capillary action, once reach the marker point, the plates are allowed to dried and sprinkling with Vanillin sulphuric acid on the plate, it forms color. With the distance moved by the solvent and distance moved by the sample, R.F value is calculated to identify the Gingerol presence.

Nano Vesicle Encapsulation of Drug like compound

10mg of lecithin and 10mg of sodium deoxycholate 1:1 ratio dissolved in 10ml of Chloroform taken in round bottom flask. To this 5ml of purified drug was added and mixed thoroughly and kept in water bath for evaporation at 70 degree centigrade [13]. A thin film was formed after evaporation. This film was hydrated with 5ml of phosphate to form nanovesicle by Hand-Shaking method [14-15]. Synthesized vesicle was microscopically visualized under SEM.

Drug Entrapment analysis of Nano Vesicle

Previously prepared drug encapsulated vesicle sample was taken and centrifuged at 4000 rpm for 30 min to separate the un-entrapped drug from the entrapped drug into the vesicles. The supernatant (un-entrapped drug) is discarded and 50% of 5ml of iso-propanol [9] is added to the pellet and kept in vortexing to dissolve the pellet. Dissolved pellet was centrifuged at 4000 rpm to separate the drug from lysed vesicles. Supernatant was meant for absorbance at 282nm in UV-Vis Spectrophotometer to measure the concentration of drug entrapped into the vesicles.

R – Concentration of drug released from Vesicle
O – Original concentration of drug taken

Entrapment efficiency (E) = R / O *100

In-vitro Drug Releasing Kinetics of Nano Vesicle drug encapsulated

2ml of prepared nano vesicle drug encapsulated sample was taken on donor compartment of Franz-diffusion Cell placed above the goat skin material between donor compartment and receptor compartment. Circulation rotation is maintained in receptor compartment by having the magnetic bead and whole experimental setup placed on the magnetic stirrer for rotation of the buffer solution. Releasing kinetics of drug from the vesicles was measured timely for the drug transmitted from donor compartment to the receptor compartment across the skin material. For every 30 min 3ml of sample collected via capillary tube which is attached with the receptor compartment using the pistol syringe [16]. Each 3ml of sample taken in separate test tube for further analysis to measure the maximum concentration of drug released from vesicle in particular time interval at 282nm in UV-Vis Spectrophotometer.

RESULTS AND DISCUSSION

Table 2: *In-Silico* Drug Pharmacokinetics.

Drug	6G	8G	10G	6S	6P	Zingerone
M.P	158.94	174.29	189.64	140.83	140.53	91.71
log P	2.49	3.55	4.61	3.85	3.83	0.64
Mol.wt	294.385	322.439	350.492	276.37	278.386	194.227

6G – 6 gingerol;8G – 8 gingerol;10G – 10 gingerol;6S – 6 shogaol;6P – 6 paradol

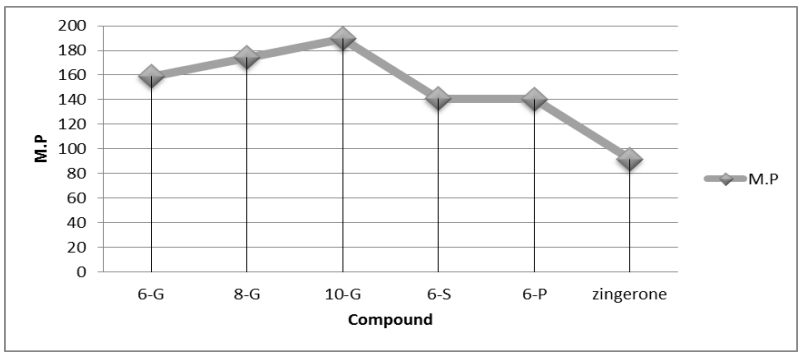


Fig 1: Pharmacokinetics- M.P (Melting Point)

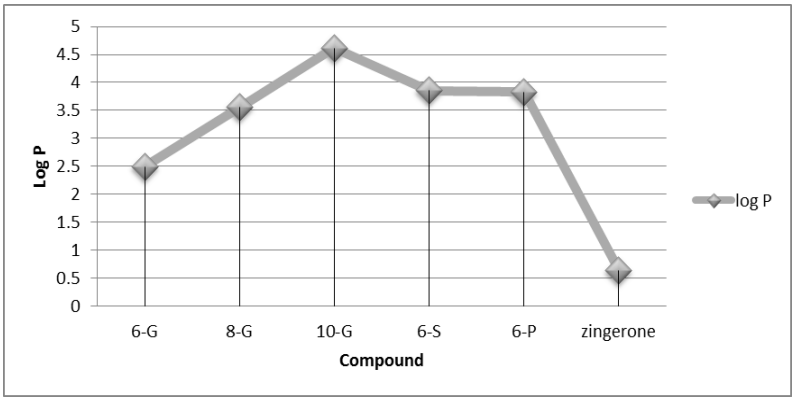


Fig 2: Pharmacokinetics- log p.

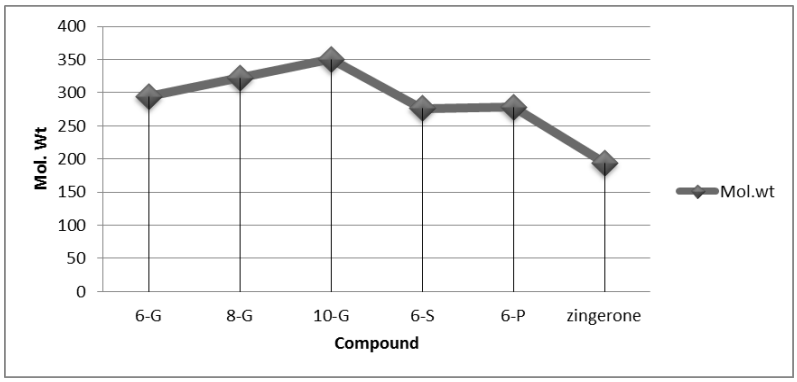


Fig 3: Pharmacokinetics- M.w (Molecular weight)

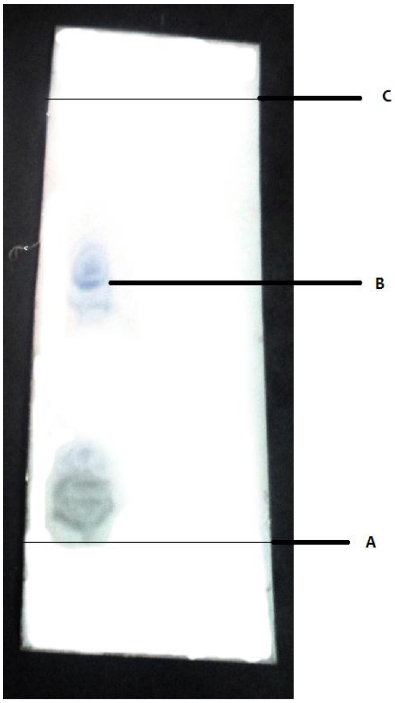


Fig 4: Conformation of gingerol present in the methanol extract by thin layer chromatography method.

A – Initial level of sample; B – Sample distance Moved; C- Solvent Front Run
R.F – B / C
R.F – 3.5 / 7.5
R.F – 0.466

Table 3:Gallic Acid Standard for Gingerol Quantification

Gallic acid absorbance at 725nm		Gingerol absorbance at 725nm	
Standards	O.D	Sample	O.D
0	0	T_1	0.8
10µg/ml	0.13		
20µg/ml	0.26		
30µg/ml	0.39		
40µg/ml	0.52		
50µg/ml	0.65		
60µg/ml	0.78		
70µg/ml	0.91		

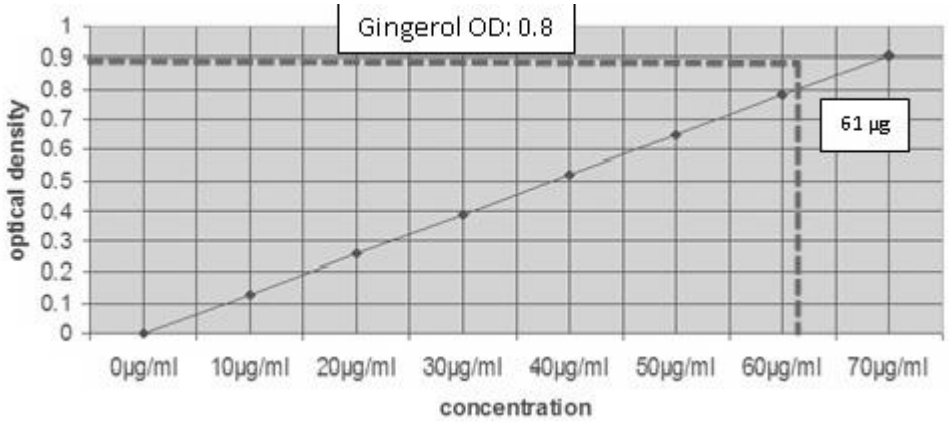


Fig 5: Quantification Of Gingerol Compare With Gallic Acid Standard

PURIFICATION OF GINGEROL

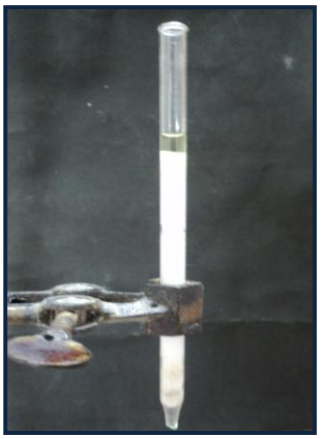


Fig 6: Flash Colum Chromatography.

Table 4: Flash Chromatography Fractions Quantification Of Gingerol At 725 Nm

Fraction (Fr) number	Optical density at 725nm	Concentration of gingerol in 20µl of each Fraction
Fr 1	0.74	57µg
Fr 2	0.64	49µg
Fr 3	0.49	37µg
Fr 4	0.37	28µg
Fr 5	0.29	22µg
Fr 6	0.16	12µg

DRUG ENTRAPMENT EFFICIENCY OF ULTRA DEFORMABLE VESICLES

Initial concentration of gingerol taken for vesicle drug encapsulation

20µl sample corresponds to 0.74 OD
0.36 OD corresponds to **57µg** when compared to standard graph.

Amount of drug released from the vesicle

20µl of released sample corresponds to 0.63
0.30 corresponds to **48.5µg** when compared to standard graph.

Entrapment efficiency (EE) = amount of sample extracted / total amount of sample * 100

EE = 48.5µg / 57µg * 100 = 85%

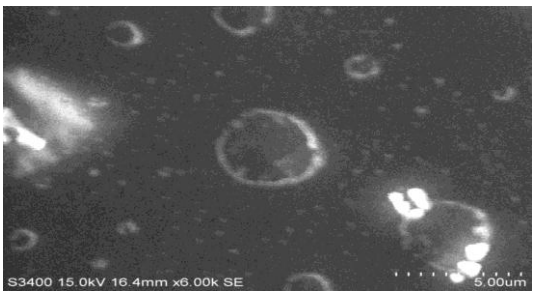


Fig 7: Scanning Electron Microscopy (SEM) View Of Ultra-Deformable Vesicle Encapsulated With Gingerol.

Fig 8: Drug release under diffusion cell method.

Table 5: Releasing Kinetics of Nano Vesicles Encapsulated Drug

No of samples	Time intervals	OD value at 282 nm
1.	60 min	0.028
2.	30 min	0.011
3.	30 min	0.002
4.	30 min	0.002

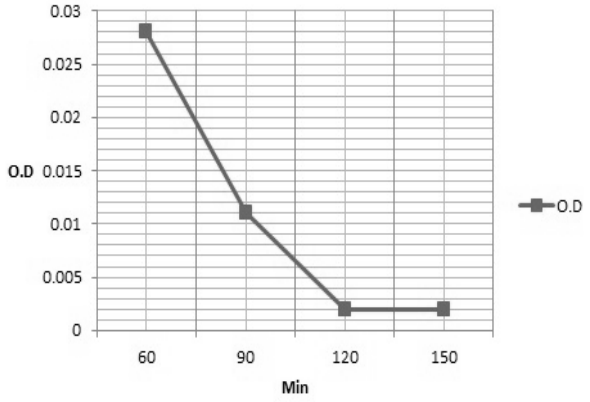


Fig 9: Drug Releasing Kinetics with Nano Vesicles

DISCUSSION

Our proposed hypothesis of the present study is the enhancement of bioavailability of the active compound called Gingerol from ginger, by carrier mediated drug delivery method. Different kinds of formulation are applied to check the increased bioavailability and permeation property of the active compound across the skin barrier. In the present study Ultra-deformable nano vesicle used for Anti-inflammatory disorder by transdermal medication and to improve the bioavailability of the drug compound compared with other drug delivery methods

Since Inflammation is a common problem which people are suffering these days, so our experimental study is more concentrated on anti-inflammatory compound from ginger, particularly out of several anti-inflammatory present in ***Zingiber officinale*** (Common name: Ginger) , one specific active compound is screened based on the physiochemical and pharmacokinetics properties of all the compound by *in-silico* method. It suggest that compound with log P around 1 to 3, Molecular weight < 500 da, Melting Point to closer to the human body temperature, will definitely play as crucial role as a drug [17]. One such compound identified through *in-silico* analysis is Gingerol. Each compound in the ginger is compared with its individual log P, Molecular Weight, Melting Point (Fig 1, 2, 3; Table 2) to understand it pharmacokinetics nature. Graphical illustrations show that Gingerol is the one active compound which satisfies the entire above mentioned drug-likeness parameters. When this active compound taken as a drug through transdermal mode, this might have better pharmacokinetics to permeate through the barriers like Stratum corneum and may bring about better anti-inflammatory activity.

Different types of extraction procedures were followed to extract our selected active compound Gingerol from Ginger. Presence of Gingerol is confirmed by Thin layer chromatography method, with R.F value 0.466 (Fig 4) Exact quantity of Gingerol present in the extract were estimated using Folin ciocalteau assay which produces blue color and O.D was observed in UV-Visible spectrophotometer at 725 nm were compared with Gallic acid standard O.D values to estimate the amount of Gingerol present(Fig 5; Table 3). Estimated quantity was observed as 61µg in the taken standard sample and converted to whole amount of sample in 1ml was 0.61mg. The extract was purified in Flash column chromatography (Fig 6), and purified sample was collected and estimated for presence of Gingerol by Folin ciocalteau assay. Samples were collected in different fractions and were quantified that maximum amount of gingerol obtained in first fraction 57µg (Table 4) of gingerol which was very high when compared to the other fractions (Table 5). Estimated amount of Gingerol present in first fraction was encapsulated into the nano vesicle.

Ultradeformable nano vesicle was synthesized with composition of cholesterol and surfactant (Sodium deoxycholate), when suspended with drug sample, ultradeformable nanovesicle encapsulates the drug molecule and vesicular shape has been obtained which is observed under SEM (Fig 7). The result was shown that when nanovesicle synthesized with correct composition of lecithin and surfactant 1:1 ratio, the drug entrapment efficiency was shown to be highly improved to 85%.

Further drug releasing kinetics of ultra-deformable nanovesicles are studied under diffusion cell with artificial skin contruct of goat skin (Fig 8) , which shows predominant release of drug in first 60 and 30 minutes the O.D value obtained at 282 nm is 0.028 (Table 6) and later it gradually decreased due to prolong releasing kinetics is not sufficiently suitable for transdermal delivery (Fig 9).

CONCLUSION

Medicines play a major role in human life. Drugs are introduced into the body via several routes; they are taken in consideration based on patience compliance and better bioavailability. Delivering the drug compound via transdermal route has been a problem for long time in pharmaceutical industry. Transdermal route is chosen for its better patient compliance, which is the major problem have been ideally faced in clinical practice. Other drug delivery modes haven't played great in delivering the compound into the human system, with most unfavorable pharmacokinetics of the drug. The inconveniences of the standard form of drug application and side effects due to the administration route are the reason for studying the improvement strategies of bioavailability. The chosen drug compound for our study is Gingerol that can be efficiently used for anti-inflammatory treatment, which is carried to reach the blood circulation by specially optimized nano vesicle called ultra-deformable vesicle, can thus be used to penetrate drugs across the biological permeability barriers, such as Stratum corneum with much better releasing kinetics. Thus we conclude that our findings will be a substituent for other mode of drug delivery. Finally we discuss that Ultra-deformable vesicle releasing the drug across the artificial skin membrane in first 90 minutes when its application over the skin membrane.

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