Research Article

Formulation and *Ex-Vivo* Skin Permeation Study of *Mangifera indica* Ethosomal Gel

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ABSTRACT

The present study was designed to incorporate *Mangifera indica* leaf extract in to gel formulation and evaluate for ex-vivo permeation study. The ethosomes and developed gel formulation was characterized using various parameters. Different formulations of ethosomes using lecithin, cholesterol and ethanol were prepared using different doses of *Mangifera indica* herbal leaf extracts. Carbopol 940 was used to prepare ethosomal gel. The entrapment efficiency of ethosomes was 65.1%-96.54% and the average vesicle size was 920nm. Three formulations (different doses) were selected based on entrapment efficiency and drug release and used for further incorporation into gel formulations. Prepared gels were then evaluated for physicochemical characteristics and drug content. Ex-vivo skin permeation studies of the experimental formulations were performed. It was conducted across the depilated rat abdominal skin using Franz's diffusion cell. The pH of the gel formulations was found to be in the range of 5.4-6.2 with viscosities ranging between 2250- 2399 centipoises. The drug content of gels ranged between 74.67-82.31%. Ex-vivo studies were performed for the optimized gel and the drug release was found to be 78.5% in 8 hrs respectively. It can be concluded that the present study revealed that ethosomal gel was proved to be an efficient drug delivery system for herbal extract.

Keywords: Ethanol, Lecithin, transdermal, *Mangifera indica*.

INTRODUCTION

Mangifera indica is the species of mango which belongs to the family Anacardiaceae, grows in tropical and subtropical regions. Its parts are commonly used in folk medicine for a wide variety of remedies. Various parts of the plant are used to treat diarrhoea, asthma, hypertension and insomnia.[1] *Mangifera indica* have been meticulously studied for its chemical constituents and pharmacological activities. Mangiferin is a main constituent is a polyphenolic and a glucosylamine xanthone with strong antioxidant, wound healing, cardiotonic and antidiabetic activities.[2,3] According to World Health Organisation (WHO) 80% of the population in developed countries relies on plant based traditional medicines to maintain their primary health care needs. High treatment cost and side effects along with drug resistance are major problems associated with synthetic drugs. [4] The medicinal values of plants are due to the presence of chemically active substances that produce a definite physiological action on human and animal health. However, delivery of herbal drugs also requires modifications with the purpose of better cure for variety of diseases. Now-a-days novel drug delivery systems open the door towards the development of herbal drug delivery systems. Novel drug delivery system is advantageous in delivering the herbal drug at predetermined rate and delivery of drug at the site of action which minimises the toxic effects with increase in bioavailability of drugs. Incorporation of novel drug delivery technology to herbals reduces the drug degradation or pre-systemic metabolism and serious side effects by accumulation of drugs to the non-targeted areas. Skin is composed of three main layers such as subcutaneous tissue, dermis and epidermis layer. Stratum corneum decides the rate of permeation of compounds and it is the major obstacle in diffusing the drug across it. Enhanced drug delivery through skin can be achieved by novel lipid carriers called as Ethosomes.[5]

Ethosomes are soft malleable lipid vesicles composed mainly of phospholids, alcohol (10-40%) and water. The physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the skin in terms of quantity and depth when compared to conventional liposomes. Ethosomes play an important role in controlling the release rate of drug over an extended time keeping the drug shielded from immune response or other removal systems. In contrast to conventional liposomes, ethosomes shows smaller vesicle size, high entrapment efficiency as well as improved stability. The size of ethosomes may vary from nanometres to microns. Ethosomes has become an area of research interest in herbal formulation because of its enhanced skin permeation and improved entrapment efficiency. As plant drugs are considered safe because of their natural origin, they exhibit promising therapeutic effect. However most of the phytoconstituents fail to achieve bioavailability because of poor absorption. [6] The reasons may be the large molecular sizes and low lipid solubility which causes poor absorption of phytoconstituents resulting in reduced bioavailability. Incorporation of these plant actives or

extracts into vesicular carriers vastly improves their absorption and consequently bioavailability.

Topical drug delivery system (or) formulation can be evaluated by the assessment of percutaneous absorption of molecules which is considered as an important step. Skin of rodents (mice, rat and guinea pigs) is most commonly used in *in vitro* percutaneous permeation studies, due to its availability, their smaller size, and relatively low cost. Amongst the rodents, rat skin is more structurally similar to human skin and it is the most frequently used rodent model. Rat skin is generally more permeable than human skin. Transdermal drug delivery system delivers the drug across epidermis to achieve systemic effects. For any medications, it is important that the administration regime to be as simple and non-invasive in order to maintain a high level of compliance by a patient.[6] The aim of the present study was to develop and characterize the herbal ethosomal gel and evaluate the same for ex vivo skin permeation study.

MATERIALS AND METHODS

Authentication of Plant material

Mangifera indica leaves were collected from local market, Hyderabad, India and were further authenticated by Dr. Madhava Chetty, Botanist, Tirupati, Andhra Pradesh. All the other solvents and reagents were of analytical grade.

Collection of Plant material and Preparation of extract

Fresh leaves of the plant were washed with water immediately after collection. These were chopped into small pieces; air dried at room temperature for 10 days, grounded in to fine powder and stored in air tight containers. 650 grams of powder was macerated with 5 litres pure methanol for 7 days at room temperature. Later it was filtered and the extract was concentrated under reduced pressure below 50^0C in rotary vacuum evaporator. It was kept in petri dish for air drying to remove the traces of methanol and finally a concentrated extract is formed.[6,7]

Preliminary Phytochemical investigation

The test extract was screened for the presence of various phytoconstituents like alkaloids, carbohydrates, phenolics, flavonoids, glycosides and tannins.

Preparation of ethosomes

In this lipid and cholesterol were measured accurately and dispersed in water by stirring it on a magnetic stirrer for 30 minutes with heating at 40°C. Organic phase containing 100mg of extract was added to ethanol and to this propylene glycol was added and kept for stirring separately. Lipid solution was added drop by drop to the organic phase and kept for stirring on a magnetic stirrer for 1 hour. Total of 12 batches of ethosomal formulations were prepared using different concentrations of lipid (100-400 mg) and ethanol (10-40%). The optimized formulation was chosen and further ethosomal preparations of other doses (200mg, 300mg) were formulated. The formulations with high entrapment efficiency and drug release were selected to incorporate in to gel formulations.[8]

Preparation of ethosomal gel

The gels were prepared by dispersion method using carbopol 940.Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. To this gel solution optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8^0 C.[9]

Table 1: Optimization of concentration of lecithin

Formulation code	Drug concentration (mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol (ml)
F1	100	100	20	10	3
F2	100	200	20	10	3
F3	**100**	**300**	**20**	**10**	**3**
F4	100	400	20	10	3

Ethosomal dispersions F1-F4 were prepared by varying the lecithin concentration. The dispersions were evaluated and based on rate of drug release the lecithin concentration was optimized.

Table 2: Optimization of concentration of cholesterol

Formulation code	Drug concentration (mg)	Lecithin(mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol(ml)
F5	100	300	20	10	3
F6	100	300	30	10	3
F7	**100**	**300**	**40**	**10**	**3**
F8	100	300	50	10	3

Ethosomal dispersions F5-F8 were prepared by varying cholesterol concentration. Based on drug release the cholesterol concentration was optimized

Table 3: Optimization of ethanol concentration

Formulation code	Drug concentration (mg)	Lecithin(mg)	Cholesterol(mg)	Ethanol (ml)	Propylene Glycol (ml)
F9	100	300	40	10	3
F10	**100**	**300**	**40**	**20**	**3**
F11	100	300	40	30	3
F12	100	300	40	40	3

Ethosomal dispersions F9-F12 were prepared by varying the ethanol concentration and based on the drug release the ethanolconcentration were optimized.

Evaluation of Prepared Ethosomes

Amongst all the formulations, F10 formulation was optimized based on % entrapment efficiency and drug release.[10,11]

Morphology

The samples are visualised by scanning electron microscopy (Hitachi S-3700N), SEM gives a three-dimensional image of the globules. One drop of ethosomal suspension was mounted on a stub covered with a clean glass. It was then air dried and gold coated using sodium aurothiomalate to visualise under scanning electron microscope 10,000 magnifications.[12]

Zeta Potential

Zeta potential was determined using Zetasizer (HORIBA SZ-100). Measurements were performed on the same samples prepared for size analysis. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

Entrapment efficiency (EE)

Entrapment efficiency of *Mangifera indica* ethosomal vesicles was determined by centrifugation. The vesicles were separated in a high-speed cooling centrifuge at 20,000 rpm for 90 minutes. The sediment and supernatant liquids were separated, amount of drug in the sediment was determined by laying the vesicles using methanol. It was then diluted appropriately and estimated using UV visible spectrophotometer at 214 nm. From this, the entrapment efficiency (EE) was determined by the following equation -

$$EE\% = \frac{(\text{Total drug}) - (\text{free drug})}{\text{Total drug}} \times 100$$

Formulation of Gels

Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. To this gel solution optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8^0 C.[13]

Table 4: Gels prepared by dispersion method using Carbopol 940 in different ratios

S NO	Formulation	Carbopol 940(%w/v)	Amount of extract
1	EG1	1	100
2	EG2	1	200
3	EG2	1	300

Evaluation of prepared gels [14]

Physicochemical properties

Appearance

The appearance was checked visually. They are light greenish in colour.

pH measurement

The pH was checked using pH meter (Systronics digital pH meter). The electrode was submersed in to the formulation at room temperature and the readings were noted.

Spreading diameter

The spread ability of gel formulation was determined by measuring the spreading diameter of 1g of gel between two horizontal plates (20cmx 20cm) after 1 min. The standard weight applied on upper plate was 125 gm.

Viscosity

Viscosity of prepared formulations was prepared carried out by Brookfield Synchro Electric Viscometer (LVDV Pro

II), spindle S64 (small sample adaptor) and the angular velocity increased from 5,10,50,100 rpm and values were noted.

Drug content of the formulated gels

Drug content was estimated spectrophotometrically, 100mg of the formulation was taken and dissolved in methanol and filtered. The volume was made up to 100ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 212nm.

***In-vitro* drug release**

The franz diffusion cell consisted of two compartments (cells). Upper one is donor cell, consisting of two open ends and lower one is receptor cell, with one open end capacity of 15 ml. one end of the donor compartment was covered with Himedia dialysis membrane(cut off molecular weight 12000-14000), which was previously soaked in warm water and placed on the receptor compartment. The receptor cell contained a small magnetic bead and was rotated at a constant speed. The temperature in the donor and receptor cells was maintained at 37^0C, with the help of a thermostat. Phosphate buffer 7.4 was placed in the receptor cell. A 5ml of sample of each formulation was transferred to the diffusion cell. 3ml samples were withdrawn from the receptor cell at specified time intervals. Each time immediately after the removal of the sample, the medium was compensated with the fresh media. The samples were analysed for drug content using a UV-Visible spectrophotometer at 212nm.[15]

Ex vivo drug release studies

The experimental protocol was duly approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for purpose of control and supervision of Experimentation on animals) through its reference no: IAEC/SVCP/2016/008, Dated: 27/2/16.

Ex vivo studies were carried out using skin of albino rat. Rats (male albino) 6 to 8 weeks old, weighing 120 to 150g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was separated from the underlying connective tissue with scalpel. The excised skin was placed on aluminum foil and the dermal side of the skin was gently teared off for any adhering fat and / or subcutaneous tissue. The skin was checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or crevices in the portion that is used for transdermal permeation studies. The skin was mounted between donor and receptor compartment with the stratum corneum side facing upward towards the donor compartment. Phosphate buffer 7.4 was taken in the receptor compartment. Temperature was maintained at 37± 0.5^0c. Optimized gel formulation was placed in the donor compartment. Samples were withdrawn at predetermined time intervals over 8 hrs and replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed using UV-Visible spectrophotometer at 212 nm.[16,17]

RESULTS AND DISCUSSION

The microscopic evaluation showed the surface morphology of ethosomes. It was observed that most of the vesicles were spherical in shape and its smooth surface was further confirmed by SEM. The vesicular size of the ethosomes significantly increased with increase in phospholipid concentration and decreased with increased concentration of ethanol.

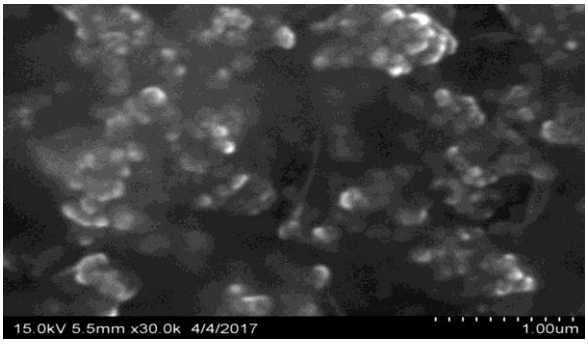

Figure 1: Showing the average size of ethosomes as 926nm.

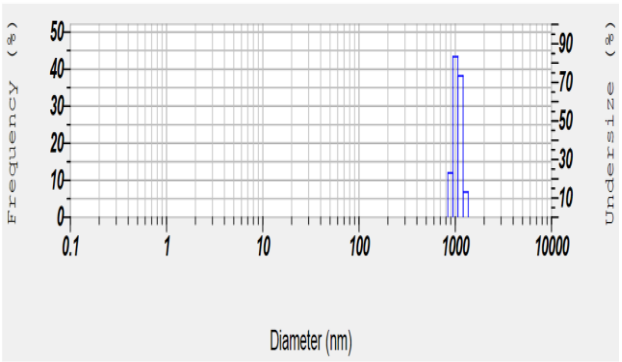

Figure 2: Showing particle size of ethosomes

The zeta potential of the ethosomes was determined using zeta sizer. From the fig 3 the value of the optimized ethosomal formulation – was found to be -8.8mv which indicated that ethosomes were stable.

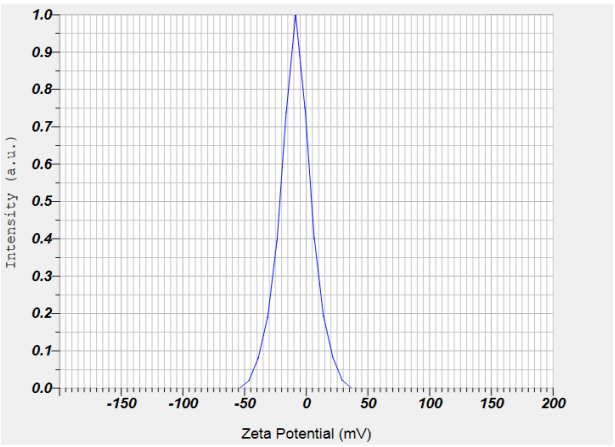

Figure 3: Showing the zeta potential of ethosomes

The entrapment efficiency of ethosomes was found to be in the range of 65.31-89.38%. The entrapment efficiency was found to be higher for the formulation F10. The entrapment efficiency was influenced by amounts of ethanol, lecithin and cholesterol which were used for preparation. Of all the factors examined the concentration of ethanol was found to influence the entrapment efficiency to a significant increased level due to the formation of thinner membrane.

Table 5: Entrapment efficiency and % drug release of different formulations

S. No	Formulation Code	Entrapment Efficiency	% Drug Release
1	F1	65.31±0.22	63.98±0.37
2	F2	68.42±0.5	72.2±0.54
3	F3	70.88±0.31	74.75±0.2
4	F4	71.5±0.66	72.85±0.72
5	F5	68.65±0.26	73.53±0.24
6	F6	72.73±0.9	77.06±0.14
7	F7	70.82±0.67	80.58±0.21
8	F8	75.2±0.36	76.8±0.12
9	F9	82.4±0.44	82.62±0.73
10	**F10**	**89.58±0.26**	**87.79±0.50**
11	F11	84.33±0.45	86.5±0.42
12	F12	86.21±0.33	87.88±0.5

In the *in vitro* drug release, the cumulative percentage drug release from various ethosomal formulations was done. The formulation F10 showed higher drug release of 87.79% in 8hrs. Therefore, F10 has been selected for formulating the ethosomal gel and based on this; different doses of 100, 200 and 300 mg drug extract were also formulated.

In the evaluation of ethosomal topical gel, all the formulations were found to be opaque, light greenish in colour, odourless, semi solid in nature and had smooth appearance.

Table 6: Evaluation of physicochemical properties of gel formulations

Formulation	Colour	Appearance	Spread ability (g.cm/sec)	pH	Viscosity (cps)	Drug content %
G1	Greenish	Homogenous	35.07+0.86	5.6	2399	74.67
G2	Greenish	Homogenous	33.72+0.52	5.8	2574	78.92
G3	Greenish	Homogenous	34.62+0.67	5.5	2250	82.31

The pH for all the formulations exhibited in the range of 5.4 - 6.2. The formulations were analysed spectro photometrically at 212 nm. All the formulations were found to possess uniform drug content.

The viscosity of all the gel formulations ranged from 2250 - 2574 cps. The viscosity of the formulations decreased on increasing the shear rate i.e. pseudo plastic behaviour was noted. In the *in vitro* drug release, the cumulative percentage drug release after for 8 hrs was highest for all the three doses of extracts using 1% carbopol. The drug content of the gels ranged between 74.67 - 82.31 %.

Ex vivo studies

Ex vivo drug permeation study was performed for optimized gel formulation.

In the *Ex vivo* drug permeation studies, it was found that the drug release from the skin was higher than the drug release from the cellophane membrane. The mechanism underlying might be due to the effect of ethanol and ethosomes, where ethanol increases the lipid fluidity and decreases the density of lipid multilayer and ethosomes enable inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids.

Table 7: Permeation of drug through the skin

Time (hrs)	Drug Permeated (µg/cm^2/hr)
0	0
1	713.5
2	1372.5
3	1936
4	2510.5
6	3072
8	3816

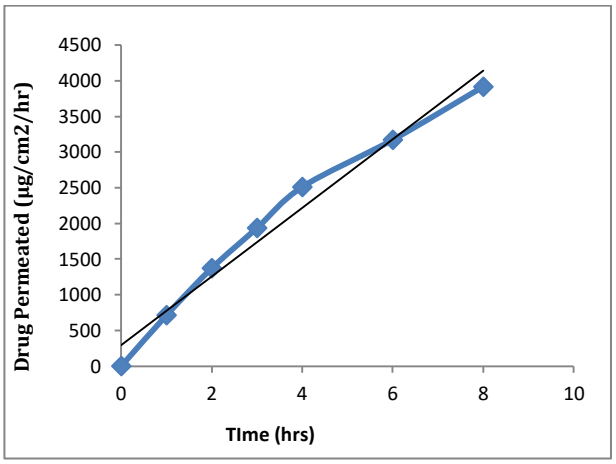

Figure 4: Drug permeation profile of ethosomal gel

Permeability parameters such as Steady-state flux (J_{SS}) and Permeability coefficient (K_p) were determined. The results were depicted in the table below.

Table 8: Permeation data analysis

Formulation	Jss ($\mu g/cm^2/h$)	Kp ($cm/h) *10^{-2}$
Ethosomal gel	491.7	K_p = 3.5 * 10^{-2} cm/hr

CONCLUSION

Based on the observations of present study, it can be concluded that a combination of 20 ml of ethanol, 300 mg of lecithin and 40 mg of cholesterol were used for preparation of ethosomes of *Mangifera indica* leaf extract for formulating three different doses (100 mg, 200 mg and 300 mg). The incorporation of ethosomal systems in gel represented an important step to get better skin-permeation and therapeutic results. Thus ethosomes can become a promising drug carrier in future for local and systemic disorders.

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