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Original Article

Enhancing Transdermal Delivery of Glimepiride Via Entrapment in Proniosomal Gel

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ABSTRACT

Objective: The aim of this study is to formulate and evaluate proniosomal gel formulations as transdermal delivery systems of glimepiride (GM) to improve its therapeutic efficacy. **Methods:** Proniosomal formulations have been prepared using different types of non-ionic surfactants with cholesterol in different molar ratios. Proniosomal gel; PN_{16} (Span 60, Tween 60, Cholesterol; 35:35:30 molar ratio) that exhibits maximum EE % (94.01 ± 0.88) and most prolonged release was chosen for *ex-vivo* skin permeation and *in-vivo* hypoglycemic activity studies. **Results:** Proniosomal gel produced better permeation through rabbit skin than HPMC niosomal gel and HPMC gel. The pharmacokinetic parameters; time of maximum response (T_{max}), % reduction in blood glucose concentration and area above the blood glucose levels-time curve (AAC) of proniosomal gel were studied. Proniosomal gel showed a controlled release behavior and a significantly higher hypoglycemic activity (65.34 ± 6.54%). **Conclusion:** It is evident from this study that proniosomal gel can act as an alternative approach for enhancing transdermal delivery of GM.

Key words: Glimepiride, Hypoglycemic activity, Nonionic surfactants, Proniosomal gel, Area above the blood glucose levels-time curve.

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INTRODUCTION

Glimepiride (GM) is used for the treatment of type 2 diabetes. It is one of the third generation sulfonylurea drug having a poor aqueous solubility, slow dissolution rate.[1] Due to its short elimination half-life (2-3 h) repeated doses are required which may cause different side effects such as headache and gastrointestinal disorders.[2] There are a number of formulation approaches to resolve the problems of low solubility and low bioavailability. Proniosomes technology offers novel solution for poorly soluble drugs to enhance its bioavailability hence, avoid repeated dosing. A novel transdermal GM loaded proniosomal gel was developed to improve its therapeutic efficacy.

Proniosomes are dry formulation of water soluble carrier particles that are coated with non-ionic surfactant. They are rehydrated to form niosomal dispersion immediately before use on agitation in hot aqueous media within minutes.[3] For the transdermal delivery of the drug proniosomes were formulated as gel like concentrated niosomes suitable for topical application.[4] Regarding the applications of transdermal proniosomes in diabetes: Gupta *et al.* concluded that metformin proniosomal gel is promising prolonged drug delivery system.[5]

The Aim of the present study was to prepare and evaluate transdermal GM loaded proniosomal gel. Drug entrapment efficiency, release profiles, *ex-vivo* skin permeation and *in-vivo* hypoglycemic activity were evaluated.

MATERIALS AND METHODS

Glimepiride (GM) was a gift sample kindly supplied by Sigma Pharmaceutical Industries (S.P.I) SAE, Mubarak 1st Industrial Zone, Monufia, Egypt. Span 20, Span 60, cholesterol, poloxamer 407 and sodium azide were purchased from Sigma Chemical Co., St. Louis, MO, USA. HPMC was obtained from El-Nile pharmaceutical company, Cairo, Egypt. Tween 20, Tween 60, ethanol and propylene glycol were purchased from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt.

Preparation of Glimepiride loaded proniosomal gel

Proniosomal gels were prepared by co-acervation phase separation method.[6] Surfactant and cholesterol were mixed in a dry, clean and wide mouthed glass vials in different molar ratios to make 1000 µmol total lipids. About 400 mg propylene glycol and absolute ethanol mixture (1:1) was added then all ingredients were mixed well and open end of the glass vials were tightly closed to prevent any loss of solvent. Then, vials were warmed in water bath at 55-60°C for about 5 min while shaking until complete dissolution of lipids. The drug (10 mg/ml) was then added to the lipid mixture while warming in a water bath. Then, the aqueous phase (PBS, pH 7.4) containing 2% sodium lauryl sulphate (SLS) as solubilizer warmed to 60°C was then added. The obtained mixtures were allowed to cool down at room temperature. The composition of proniosomal gel formulations is listed in Table 1.

Preparation of niosomes

Niosomes were prepared by hydration of the prepared proniosomal gels.[7] Glass vials containing GM loaded proniosomal gels were heated to melting and certain volume of PBS heated to about 60°C was added into each vial to obtain niosomal dispersion. The vials were vortexed for 2 min. The final volume was adjusted to 10 ml by the same buffer.

Determination of Glimepiride entrapment efficiency percentage

One milliliter niosomal samples obtained after hydration of proniosomal gels were centrifuged at 14,000 rpm for 1 h at 4°C using Centrifuge.[8] The supernatant containing the free drug was decanted. Niosomal pellets were re-suspended in PBS containing 2% SLS and re-centrifuged again. This washing procedure was repeated twice, then the collected superna-

tant was assayed spectrophotometrically at 230 nm using PBS containing 2% SLS as a blank by UV-Vis Spectrophotometer. The EE was calculated according to the following equation:

$$\text{Glimepiride EE \%} = \frac{\text{Amount of GM entrapped}}{\text{Total amount of GM}} \times 100$$

Formulation of Glimepiride gels

GM gels 0.5% w/w were formulated using two different gelling agents; HPMC (2 %) and poloxamer 407 (15 %). The weighed amount of gelling agent powder was sprinkled gently on 100 ml PBS containing 500 mg of the drug and stirred at 500 rpm using magnetic stirrer. Stirring was continued until a thin dispersion was formed.[9] The prepared dispersion was kept overnight in the refrigerator until homogenous gel was obtained.

Formulation of Glimepiride niosomal gels

Niosomal gels loaded with GM were prepared by replacing a portion of the PBS by concentrated niosomal dispersion loaded with the required quantity of drug and the procedure was completed as previously mentioned.[10] The final lipid concentration per 20 g of the gel formulation was 50 µmol/g.

Viscosity measurement

The viscosity of the prepared gel formulations was determined at room temperature using Viscostar viscometer using spindle R5 at 2 rpm.[11]

Microscopic examination

Small amounts of the proniosomal gel and its hydrated form were spread on a glass slide as a thin layer and examined for the structure of the prepared gel using an ordinary light microscope with magnification power of 40 x.[12]

Backing membrane reservoirs

Circular plastic holders with a 2.9 cm inner diameter were used as backing membrane reservoirs for proniosomal gels.

In-vitro release study through cellophane membrane

The *in-vitro* release of GM from different formulations was determined by using a diffusion cell fabricated in our laboratory. The cell consisted of a hollow glass tube of internal diameter of 2.9 cm. The backing membrane reservoirs (donor) containing one gram of each formulation (containing 5 mg of GM) were attached to the glass tubes at one end and covered with semi-permeable membranes with the aid of rubber bands. The tubes were attached to the stem of the dissolution apparatus by using parafilm to avoid water evaporation. The tubes were allowed to stir at 100 rpm in 250 ml of PBS containing 2% SLS maintained at 37 ± 0.5°C.[13] In order to determine the release of GM from niosomal dispersion, the cellophane membranes were attached directly to one end of the tubes and certain volumes of niosomal dispersion containing 5 mg of GM were used. Three ml samples were withdrawn at specified time intervals for 24 h and replaced with fresh buffer solution. The samples were analyzed spectrophotometrically at 230 nm.

Ex-vivo drug permeation studies through rabbit skin

Abdominal full-thickness skins of white Albino male rabbits were used. The skin was carefully removed from animals and prepared for experiment. The prepared skin samples were mounted on the receptor compartment with the stratum corneum facing upward towards the donor compartment and the dermal facing downward. The receptor compartment was then filled with 250 ml of PBS containing 2% SLS and 0.02% sodium azide as preservatives and maintained at 37 ± 0.5°C. The procedure was completed as previously mentioned. GM steady state transdermal flux (J_{ss}) and lag time for each formulation were calculated. Permeability coefficient (K_p) was calculated using the following equation: $k_p = J_{SS}/C_o$ where, C_o is the initial drug concentration in the donor.[9]

In-vivo studies

Selection of animals

Healthy albino-Wistar male rats, weighing about 200-250 g were used. Rats were obtained from Faculty of Veterinary Medicine, Zagazig University, animal breeding center, Egypt and treated according to Ethical committee of animal handling in Zagazig University "ECAHZU". The animals were housed under standard conditions, maintained on a 12-h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1 h before the experiments. The blood glucose level was determined for all rats and the fasting blood glucose level (BGL) for rats was (50-80 mg/dl).

Experimental induction of diabetes

Induction of diabetes was done according to the method employed by Rajaram, 2013.[14] The rats were injected with alloxan monohydrate freshly dissolved in saline at a dose of 120 mg/kg intraperitoneally. Rats with fasting glucose ranging from 180-220 mg/dl were considered diabetic and were used in the experiment.[15]

Experimental Design

About 6.61 cm^2 of skin on the dorsal side of the rat was shaved and then washed with distilled water on the previous day of the experiment. Four groups of wistar rats (5 in each group) fasted overnight prior to the experiments were used for the study. After collecting zero hour blood samples, rats were divided into four groups (5 in each group):

Group I: Diabetic rats treated with 2% HPMC gel base without drug. **Group II:** Diabetic rats treated with GM suspension prepared in distilled water with the aid of gum acacia orally in a dose of 4 mg/ kg body weight.[16] **Group III:** Diabetic rats transdermally treated with 2% HPMC gel loaded with GM. **Group IV:** Diabetic rats transdermally treated with proniosomal gel loaded with GM.

Blood sample were collected from tail vein for glucose estimation at 0, 1, 2, 3, 4, 6, 8, and 24 h intervals. BLG was determined by using blood glucose measuring instrument One Touch ltra (Lifescan, Inc. Milpitas, CA 95035 U.S.A.) and percentage reduction in blood glucose level was calculated.

Statistical analysis

Data were expressed as mean ± SD (standard deviation). The significance was determined by ANOVA. $P<0.05$ is considered statistically significant.

RESULTS AND DISCUSSION

Formulation of proniosomal gel

Span 20 is liquid at room temperature as it has low phase transition temperature[17] (Tc=16°C) and cannot form gels at lower concentrations of Chol, Table 1. Proniosomal gel containing Span 20 will start to be formed at Chol molar percent of 30%. Since Span 20 is less hydrophobic (HLB=8.6), it produced proniosomal liquids of one phase at Chol concentrations below 30%. On the other hand, Span 60 produced white creamy gels like appearance in the presence or absence of Chol as it has high (Tc=53°C) and are solids at room temperature.[17] No gel was

Table 1: Composition of proniosomal gel formulations

Batch Code	Surfactant type	Chol Molar %	Surfactant: Chol weight (g)	Appearance	EE%± SD
PN_1	Span 20	0	0.347:0.00	One phase liquid	55.68±1.08
PN_2	Span 20	10	0.312:0.039	One phase liquid	56.84±1.52
PN_3	Span 20	20	0.277:0.077	One phase liquid	58.14±1.27
PN_4	Span 20	30	0.243:0.116	Transparent gel	60.15±1.79
PN_5	Span 20	40	0.208:0.155	Translucent gel	62.74±1.23
PN_6	Span 20	50	0.173:0.193	Translucent gel	64.39±0.89
PN_7	Span 60	0	0.431:0.00	White creamy gel	65.16±0.68
PN_8	Span 60	10	0.388:0.039	White creamy gel	71.01±1.11
PN_9	Span 60	20	0.345:0.077	White creamy gel	72.89±1.40
PN_{10}	Span 60	30	0.302:0.116	White creamy gel	75.29±1.36
PN_{11}	Span 60	40	0.259:0.155	White creamy gel	73.75±2.12
PN_{12}	Span 60	50	0.216:0.193	White creamy gel	64.80±1.25
PN_{13}	Tween 20	30	0.366:0.116	One phase viscous liquid	36.89±1.65
PN_{14}	Tween 60	30	0.454:0.116	One phase viscous liquid	42.57±1.53
PN_{15}	Span60:Tween 20 (1:1)	30	0.151:0.183:0.116	White creamy gel	85.82±1.91
PN_{16}	Span60:Tween 60 (1:1)	30	0.151:0.227:0.116	White creamy gel	94.01±0.88

Each result is the mean of 3 determinations ± standard deviation (S.D).

Table 2: Viscosity measurements of the prepared formulations

Formulation	HPMC Gel	Poloxamer Gel	HPMC niosomal gel	Poloxamer niosomal gel
Viscosity	3108±151 cPs	5687±209 cPs	3232±175 cPs	5765±195 cPs

Each result is the mean of 3 determinations ± standard deviation (S.D).

Table 3: Permeation parameters of GM across abdominal rabbit skin

Formulation.	Steady-state transdermal flux (J_{ss}) (µg/cm². h) ± S.D.	Permeability coefficient (k_p) (cm/h *10^{-3}) ± S.D.	Lag time (h)
HPMC gel	25.13±2.96	5.03±2.37	0.801
HPMC niosomal gel	29.67±1.75	5.93±0.59	0.423
Proniosomal gel	36.54±3.67	7.31±3.73	0.245

Each result is the mean value ± S.D. (n=3).

obtained from Tweens (20, 60), but when Tween 20 or Tween 60 mixed with Span 60, it produced white creamy proniosomal gels.

Morphology of the prepared formulations

Figure 1 represents the photomicrograph of proniosomal gel (PN_{10}) and its niosomal dispersion. Niosomes were spherical in shape. On the other hand, proniosomal gel is formed of floccules of small vesculating particles which have a creamy opaque appearance.[18]

Entrapment efficiency

The EE % of different formulations followed the order Span 60> Span 20> Tween 60> Tween 20 as shown in Table 1. The results are dissimilar to those reported by Mohawed *et al*. who found that the clomipramine EE of different surfactants could be ranked as follows: Tween 60> Span 60> Tween 20> Span 20.[19] The significant increase ($P<0.05$) in EE% of Span 60 proniosomes may be due to the solid nature, hydrophobicity and high Tc of Span 60 compared to Span 20. This could also be attributed to the structure of Span 60 which has the longest saturated chain length (C_{18}) among all Span series.[20] Table 1 shows that, the EE of proniosomes formed from Span 20 and Span 60 was found high compared with proniosomes prepared from Tween. Most of surfactants used to make niosomal vesicles such as Spans have a low aqueous solubility. However, freely soluble non-ionic surfactants such as Tweens can form the micelles on hydration in the presence of Chol.[21]

For Span 20 proniosomes EE% of GM was increased significantly ($P<0.05$) with increasing Chol concentration from 0% to 50%. Above the phase transition temperature, Chol had the ability to make the membrane more ordered and abolished the gel to liquid phase transition of niosomal system producing niosomes that are less leaky and less permeable.[22]

Also, Results show that GM EE% increased by Chol addition in case of Span 60 proniosomes when Chol concentration increased to 30%. Then,

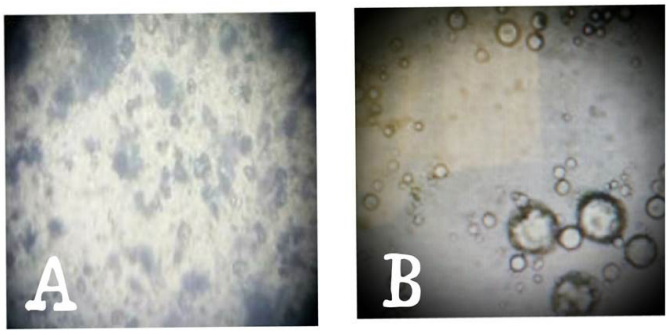

Figure 1: Micrographs (magnification power is 40X) of (A) Proniosomal gel (B) Corresponding niosomal dispersion.

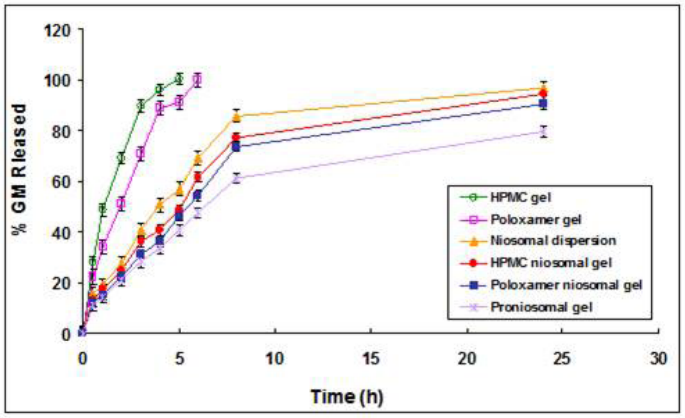

Figure 4: *In-vitro* release of GM from different formulations across cellophane membrane compared to proniosomal gel.

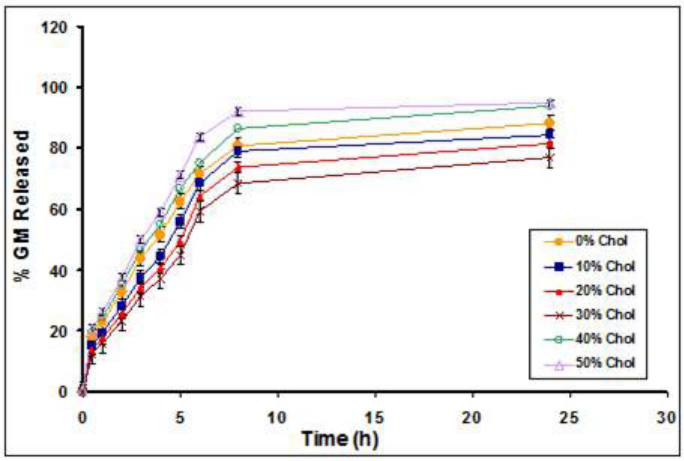

Figure 2: Effect of Chol concentration on *in-vitro* release of GM from Span 60 proniosomes derived niosomes.

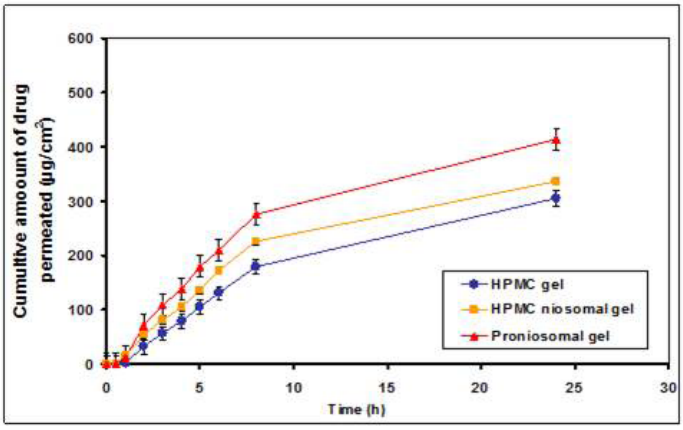

Figure 5: Permeability studies of GM from HPMC gel, HPMC niosomal gel and proniosomal gel across abdominal rabbit skin.

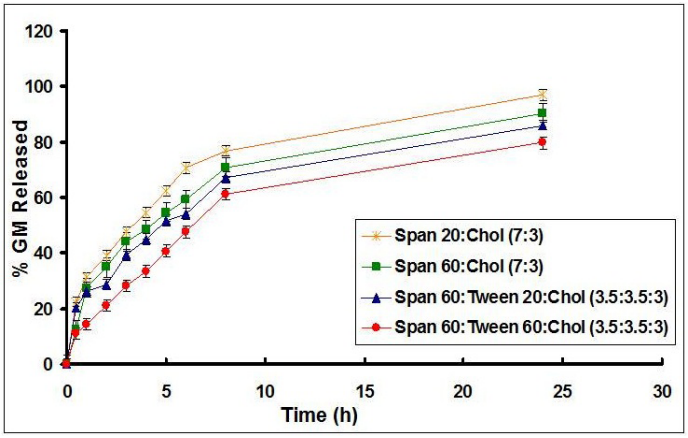

Figure 3: Effect of lipid composition on *in-vitro* release of GM from different formulations.

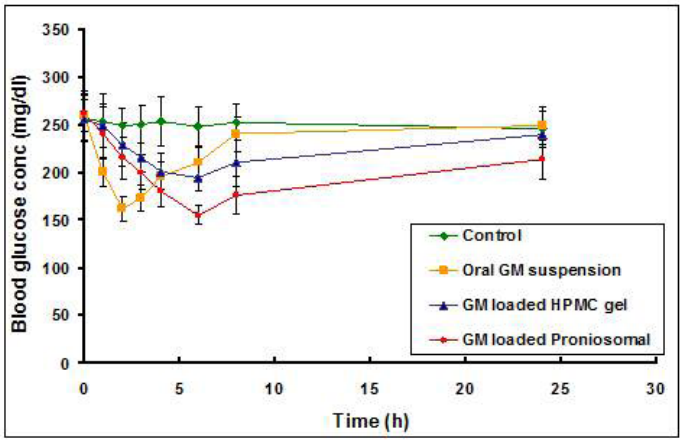

Figure 6: Blood glucose concentration after administration of different GM formulations.

further increase in Chol concentration led to decreased EE% of GM. This result could be attributed to competition between drug and Chol for packing in the limited sites in the bilayer structure of niosomes.[23]

Results indicated that proniosomes prepared from a mixture of Span 60 with Tween (1:1) resulted in a significant increase in the EE % ($P<0.05$). This could be attributed to the larger vesicles size which resulted from the lower hydrophobicity of the mixture of Span 60 and Tween. [22] This effect may be also related to the increase in the membrane rigidity and formation of less leaky niosomal vesicles upon using mixed surfactants.[24] Proniosomes prepared from a mixture of Span 60 and Tween 60 had the highest EE % followed by those prepared from a mixture of Span 60 and Tween 20.

In-vitro release studies

Figure 2 showed that, % drug released at 6 h was significantly decreased ($P<0.05$) as Chol concentration increased from 0% to 30%, which is due to membrane stabilizing ability of Chol. However, further increase in Chol molar ratio starts disrupting the regular linear bilayerd structure of the vesicular membrane leading to increase the drug release rate.[25]

Figure 3 showed that the rate of release of GM from the proniosomal gel prepared from Span 60 (PN_{10}) was lower than Span 20 proniosomal gel (PN_4). This was attributed to higher Tc of Span 60 than that of Span 20.[22] Moreover, proniosomes prepared from a mixture of Span 60 and Tweens showed a greater decrease in the *in-vitro* release of GM. The proniosomal gel formulation (PN_{16}) which prepared from Span 60, Tween 60 and Chol (35:35:30) molar ratio showed the highest EE% and *in-vitro* prolonged release of drug was chosen for further studies.

Figure 4 showed that, the percentage drug released from HPMC gel was significantly ($P=0.003$) higher than that released from poloxamer gel. This may be due to lower viscosities of HPMC compared to poloxamer gels as shown in Table 2. Results also showed that, the drug release from different vehicles showed the following order: polymeric gels> niosomal dispersion> niosomal gel> proniosomal gel. It was demonstrated that incorporation of drug into niosomes resulted in retardation in drug release in case of niosomal dispersion which was further delayed in case of niosomal gel due to presences of gelling agents which form an additional diffusion barrier to drug release.[19] The slower release of drug from niosomal gel formulations may be due to drug encapsulation into vesicles (micro-reservoirs) providing prolonged drug release rate.[10] The proniosomal gel (PN_{16}) gave the lowest drug release among the tested formulations (about 47.5 ± 1.25% after 6 h). This could be related to the condensed vesicular structure composed of Span 60, Tween 60 and Chol (35:35:30), which is considered a great barrier to drug diffusion and a retardant to its release. The delayed release from proniosomal gel formulation is due to slow release of drug from proniosomes and this may be attributed to the need of proniosomes for a time to be hydrated to form niosomal vesicles before starting release of drug across the cellophane membrane.

Ex-vivo permeability studies

Figure 5 showed that HPMC niosomal gel resulted in a significant increase in the amount of drug permeated through the skin ($P=0.04$), compared to that permeated from HPMC gel. This increase may be related to the action of non-ionic surfactant as penetration enhancer for the drug and the solubilization of lipids within the stratum corneum.[10] Also, the amount of drug permeated from proniosomal gel was significantly higher than that permeated from niosomal gel ($P=0.03$). The superiority of proniosomal gel can be explained on the basis that, the former contained high ethanol content which was diluted in case of niosomes.[25]

It was concluded that, amount of drug permeated through the skin was completely different from that obtained from release experiments through cellophane membranes. The lower permeability of GM across rabbit skin in comparison to cellophane membrane may be related to the barrier properties of skin which hinder drug diffusion rates.[10]

Results demonstrated that, the obtained transdermal flux values were significantly higher in the case of vesicular formulations ($P=0.003$). The increase in transdermal flux was associated with a reduction in the lag time, Table 3. The J_{ss} of GM from proniosomal gel and niosomal gel was 1.45 and 1.18 fold greater than that from HPMC gel, respectively. Also, the reduction in the lag time after application of vesicular gels indicates increased diffusivity and supports skin penetration enhancement of components which suggested a reduction in the barrier nature of skin.[26] So, Proniosomal gel (PN_{16}) formulation was selected for further *in-vivo* hypoglycemic study due to its higher EE% and the enhanced *ex-vivo* permeation through the skin.

Hypoglycemic activity study

In-vivo evaluation of the selected formulation was carried out in alloxan induced diabetic wistar rats by measuring hypoglycemic effect produced after transdermal administration of GM loaded proniosomal gel in comparison to GM (pure drug) at same dose either orally or transdermally.

Figure 6 revealed that oral administration of GM suspension produced rapid and significant lowering in the BGL ($P = 0.003$). The maximum hypoglycemic activity of about 36.75 ± 3.19% (94.71 ± 15.59 mg/dl, reduction in BGL) was observed after 2 h (*Tmax*). On the other hand, group 4 which treated transdermally with GM loaded proniosomal gel, showed slow hypoglycemic effect and the maximum reduction in the BGL appeared after 6 h (*Tmax*). The hypoglycemic activity was extended to 24 h by transdermal administration of proniosomal gel loaded with GM compared with the oral administration of GM suspension which terminated after 6 h.[27]

Proniosomal GM gel application resulted in 65.34 ± 6.54% (101.68 ± 15.88 mg/dl, reduction in BGL) after 6 h while the inhibition in the BGL produced by the application of GM loaded HPMC gel was 31.71 ± 5.15% after 6 h. The obtained hypoglycemic activity difference between GM loaded proniosomal gel and GM loaded HPMC gel was around two folds. The results confirm the fact that a significant amount of GM was delivered from the proniosomal gel through rat skin to induce the hypoglycemic effect, so it was concluded that proniosomes enhanced drug delivery through the skin, thereby enhance the pharmacological effect.[28]

The area above the BGL-time curve over 24 h (AAC_{0-24}) after transdermal administration of GM loaded proniosomal gel was found to be (~1599.2 mg.h/dl), ~2 fold higher than transdermal administration of GM loaded HPMC gel (~825.9 mg.h/dl) and oral administration of GM suspension (~686.98 mg.h/dl).

CONCLUSION

This paper has shown that Glimepiride can be entrapped in proniosomal gel with high efficiency providing a means to deliver GM transdermally. The experimental findings show that either fusion of the vesicles with the intercellular lipid of the stratum corneum and direct transfer of drug from vesicles to the skin and/or the penetration enhancement effect of the non-ionic surfactants may contribute to the mechanism of drug permeation enhancement by proniosomal formulations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

GM: Glimepiride; **EE:** Entrapment efficiency; **HPMC:** Hydroxypropyl methylcellulose; **Tmax:** Time of maximum response; **AAC:** Area above the blood glucose levels-time curve.

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