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Effect of arachis and eucalyptus oils on insulin release from transdermal patches

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ABSTRACT

Study intended to demonstrate the effects of arachis and eucalyptus oils as release modifiers in insulin transdermal patches.Patches were prepared by solvent casting technique using hydroxypropyl methlycellulose solution as the aqueous phase and polysorbate 80 as emulsifying agent. Various batches (TD1-TD5) were prepared using varying proportions of the oils and aqueous phases and then subjected to physical evaluations such as; weight variation, thickness, percentage moisture sorption and content, drug content, bioadhesion and folding endurance. *In vitro* release studies was carried out using the USP paddle method prescribed for transdermal patches. *Ex vivo* permeation and *in vivo* release studies were also carried out. Patches containing eucalyptus oil showed highest percentage moisture sorption of 775 %, while those containing arachis oil gave highest percentage moisture content of 77.8 %. There was increase in the *in vitro* release of insulin with increasing concentrations of the oils with patches containing arachis oil giving a higher release than the eucalyptus oil patches. *Ex vivo* diffusion through treated rat skin gave similar diffusion rates as in the *in vitro* release. *In vivo* results obtained showed significant percentage blood glucose lowering activity with TD1 (36 %) and TD3 (23 %) after 6 h of patch administration as against the intraperitoneally administered insulin (control) which gave 82 % glucose lowering after 1 h. Arachis and eucalyptus oil improved and sustained the *in vitro* and *in vivo* release of insulin from transdermal insulin patches with arachis oil at 20 % showing the greatest improvement.

INTRODUCTION

Protein based drugs are very important for the treatment of several disease conditions such as metabolic diseases (diabetes), cancers and other hormonal disorders. Proteins are complex molecules with large molecular weights, acid-base side chains and are polar in nature. Stability issues, along with their complex nature, make proteins difficult drug candidates for delivery. Currently, proteins are predominantly administered by the parenteral route. However, since most proteins have short half-lives, this route has the disadvantage for the requirement of repeated administrations and consequently low patient compliance. Other routes such as oral, pulmonary and nasal routes have being investigated as alternatives [1,2]. However these routes have the limitations such as gastrointestinal degradation, low bioavailability, and local irritation [3].

A major breakthrough in the delivery of insulin in the last decade has seen the introduction of transdermal patches of insulin using both ultrasonic methods [4] and methods that employ the use of phospholipids [5,6]. The new invention, though still being developed has a great potential for the possibility of controlling blood sugar levels in insulin dependent diabetes mellitus (IDDM) patients in such a way that it is no encumbrance to daily activities.

Advantages of transdermal drug delivery (TDD) over oral administration include; avoidance of peak and valley levels in the serum, avoidance of first-pass metabolism, less frequent dosing regimens, longer sustainability of zero-order drug delivery and less inter-subject variability [7]. Other advantages include aspects such as the accessibility of the skin; a relatively large surface area for absorption and the fact that it is non-invasive making it more patients compliant [8]. Major challenge with TDD include the

ability for the drug to overcome the penetration barrier posed by the skin layer and in particular with large molecular drugs like insulin. A major polymer used in TDD formation is hydroxypropyl methylcellulose (HPMC). It has unique functional property of bioadhesiveness while drug penetration may be achieved by inclusion of other polymer or penetration enhancers such as chitosan, mucin, hyaluronan, polysorbates or PEG [9-11]. The objective of this study was to investigate the effects of eucalyptus and arachis oils on modulating insulin release from transdermal device.

MATERIALS AND METHOD

Materials

The following were the materials used in the course of this study; Hydroxypropyl methylcellulose and polysorbate 80 (Tween 80) were purchased from Sigma Aldrich, Germany. Eucalyptus oil B.P, a product of Bell Sons & Co, England, arachis oil and soluble human insulin (Actrapid®) were procured from a local Pharmacy in Benin City, Edo State, Nigeria. Other chemicals used were of reagent grade.

Methods

Preparation of insulin patches

Insulin transdermal patches were prepared according to the formula in Table 1. One milliliter of 100 IU/ml insulin was mixed intimately with 16 ml of distilled water containing 1.6 g of HPMC. Then 0.5 ml of Tween 80/arachis oil mixture containing approximately 0.1 ml of Tween 80 and 0.4 ml of arachis oil was then added and stirred for about 5 min. The mixture was poured into a petri-dish and air-dried to give TD1 batch of patches. The air dried patches were sectioned into 1×1 cm^2 patches and stored in-between foils to retain their flatness in an airtight container. Other batches of transdermal patches were similarly prepared such that two batches with different concentrations were prepared for each oil while a fifth batch was prepared without oils as the control.

Evaluation of transdermal patches

Dimensions

Ten patches of 1×1 cm^2 from each batch were weighed individually using a digital balance and the average weight of the 10 patches were calculated and recorded. The thickness of the various batches of patches was measured using a micrometer screw gauge at different spots on the surface of the patch and the average thickness was documented.

Folding endurance

This was determined by repeated folding and opening of the patches at the same point until it cracked or broke. The results were expressed as number of repeated folds.

Moisture content and uptake

The patches from the various batches were weighted individually and placed in a desiccator containing activated silica gel as desiccant. They were then withdrawn at different time intervals and weighed again to check for moisture loss. The process continued till no further loss in weight was observed. The moisture content was then calculated as a difference between initial and final weight with respect to the initial weight and expressed as a parentage.

To determine the moisture uptake, a patch from each batch was weighed and placed on a soaked mass of cotton wool in a petri dish. Using Amaranth as an indicator, the patches were observed until they were soaked up to the top surface. The patches were then reweighed and the moisture uptake for each of the patches was calculated as the difference between the final and initial weights with respect to the initial weight and expressed as a percentage.

Bioadhesion test

This test was carried out for each batch of patches by using a modified version of the method of Attama *et al*. [12]. The apparatus used consists of a burette clamped to a retort stand. A wooden support was used to position a glass slide at an angle of 30°. Excised and treated rat skin attached to the glass slide and the patch was placed on the exposed surface of the skin for a period of 15 min, to allow for polymer interaction and hydration. The burette was filled with water and then allowed to flow over the patch on the skin using a lamina flow rate of 2 ml/sec until the patch detached from the excised rat skin. The mass flow rate of water (g/sec) was then used as a measure of bioadhesion [13].

Drug content

A patch from each batch was cut into small pieces and placed in a 50 ml beaker containing 10 ml phosphate buffer solution (pH 6.8). The beaker contents was shaken intermittently until complete dissolution. One milliliter of this solution was then further diluted in 9 ml of the phosphate buffer solution and the insulin content of the resultant solution determined spectrophotometrically at max of 275 nm (T70 PG Instrument Ltd, USA).

In vitro release studies

Insulin release from the transdermal system was evaluated using the USP paddle over disc dissolution apparatus prescribed for transdermal drug delivery systems [14]. The dissolution test apparatus thermostated at 32 ± 0.5 °C and stirred at 50 rpm. The patch was fixed on an inverted glass petri-dish using cyanoacrylate adhesive allowing drug release from the upper surface and was placed at the bottom of the vessel containing 500 ml of phosphate buffer pH 6.8. At various time intervals, aliquots of 5 ml of sample were withdrawn and replaced with equal volume of the dissolution medium each time to maintain sink condition. The samples withdrawn were then filtered and analyzed spectrophotometrically at 275 nm against a blank using UV/Visible spectrophotometer Triplicate determination were carried out for all samples.

Ex vivo studies

This study was carried out using a highly vascularized dorsal section of full thickness skin of an adult albino rat. The section was soaked in 5 % NaOH for 30 min to remove the hair from the skin and thereafter defatted by soaking in acetone for 1 h. After defatting, it was soaked in pH 6.8 phosphate buffer overnight to equilibrate. The patches were pressed firmly to the rat skin and tied to ensure adhesion throughout the experiment, forming the donor unit. The donor unit was introduced into the basket unit of a dissolution apparatus acting as the receptor compartment containing 500 ml phosphate buffer solution pH 6.8 maintained at 32 ± 0.5 ºC and stirred at 50 rpm. Aliquots of 5 ml of sample were withdrawn from the receptor compartment at hourly intervals up to 6 h, while replacing with equal volume of the receptor medium. Withdrawn samples were then analyzed spectrophotometrically at 275 nm.

Table 1. : Formula for the different batches of the insulin transdermal patches

Batch	Insulin (100 IU/ml)	Tween 80 (ml)	HPMC (g)	Arachis oil (ml)	Eucalyptus oil (ml)
TD1	1.0	0.10	1.6 (80%)	0.4 (20%)	-
TD2	1.0	0.10	1.8 (90%)	0.2 (10%)	-
TD3	1.0	0.10	1.6 (80%)	-	0.4 (20%)
TD4	1.0	0.10	1.8 (90%)	-	0.2 (10%)
TD5	1.0	0.10	2.0 (100%)	-	-

In vivo studies

Three batches of patches (TD1, TD3 and TD5) were used for this study. Twenty five rats were weighed and separated into five groups with five rats per group. The rats were allowed to acclimatize with free exposure to food and water for two weeks and then were fasted overnight. Their basal glucose levels were checked and recorded then diabetes was induced using alloxan at a dose of 150 mg/kg body weight. After 48 h, experimental diabetes was confirmed in the rats using the Accu-Chek Active glucometer (Roche, USA) and fasting blood glucose level greater than 200 mg/dl (11.1 mmol/l) was taken as being diabetic [15].

The animals were anesthetized 15 min prior to drug administration using chloroform, their hair were shaved off and the patches placed on their bare skin and taped to prevent dislodging. Groups A, B and C rats received insulin patches of TD1, TD3 and TD5, respectively, equivalent to 5.0 IU/kg body weight while groups D and E rats were given insulin intraperitoneally at a dose of 5.0 IU/kg body weight and normal saline at a dose of 2 ml/kg body weight, respectively. Insulin absorption was monitored based on the effects on blood glucose level. Blood samples were obtained from all groups of animals by the tail snipping method at hourly intervals for a period of 6 h and then after 24 h. Blood glucose level was determined immediately after sample collection using Accu-Chek Active glucometer and expressed as a percentage of the initial level, prior to drug administration.

Statistical analysis

Statistical analysis was performed utilizing Student's t-test (GraphPad InStat software version 3.10). All the results obtained were analyzed using one-way analysis of variance (ANOVA) at a confidence interval of 95 %.

RESULTS

The results of the physicochemical tests carried out on the transdermal patches are shown in Table 2. Weights of the patches varied from 0.10 - 0.16 g among the batches. This variation was also seen in the thickness of the patches which ranged from 0.79 - 1.53 mm. However, these variations were not significant within the various batches. The folding endurances of the patches both within and among the batches were not significantly different ($p > 0.05$). The patches with eucalyptus oil (TD3 and TD4) had the highest percentage moisture uptake of 540-775 %, followed by the HPMC-only (TD5) patches with 425 % and arachis oil patches (TD1 and TD2) with 233 375 %. The reverse was the case with the moisture content of patches with those of arachis oil having a higher percentage moisture content than the eucalyptus oil and HPMC-only patches. The bioadhesion values of the patches ranged from 2.30 1.33 g/sec, with TD2 and TD5 showing significantly highest values ($p < 0.05$) when compared to others.

In vitro release result

The *in vitro* release profiles of the various batches of the insulin transdermal patches is shown in Figure 1. From the plots obtained, insulin release was prompt but steady from all the patches until after 30 min when drug release from patches then appeared steady. TD1 with 20 % arachis oil gave the highest release of 45 % after 30 min followed by TD2 and TD5 with 19 and 17 %, respectively. TD3 patches gave the lowest insulin release of 4.6 %.

Ex vivo studies

Figure 2 shows the percentage insulin diffusion from the transdermal patches through a treated rat skin. Results obtained show that patches with higher concentration of the oils (TD1 and

Fig 2. : Properties of insulin transdermal patches

Parameter	TD 1	TD 2	TD 3	TD 4	TD 5
Weight (g±SD)	0.16 ± 0.024	0.14 ± 0.033	0.11 ± 0.013	0.15 ± 0.037	0.10 ± 0.013
Thickness (mm±SD)	1.53 ± 0.17	1.23 ± 0.16	0.98 ± 0.16	0.79 ± 0.4	1.11 ± 0.35
Folding Endurance (n)	25	25	26	25	26
Moisture Content (%)	77.8	72.6	60.2	67.3	66.7
Moisture Uptake (%)	233.3	375	775	540	425
Drug Content (%)	97.3	96.4	68.53	62.87	83.60
Bioadhesion (g/sec)	1.57	2.3	1.33	1.33	2.15

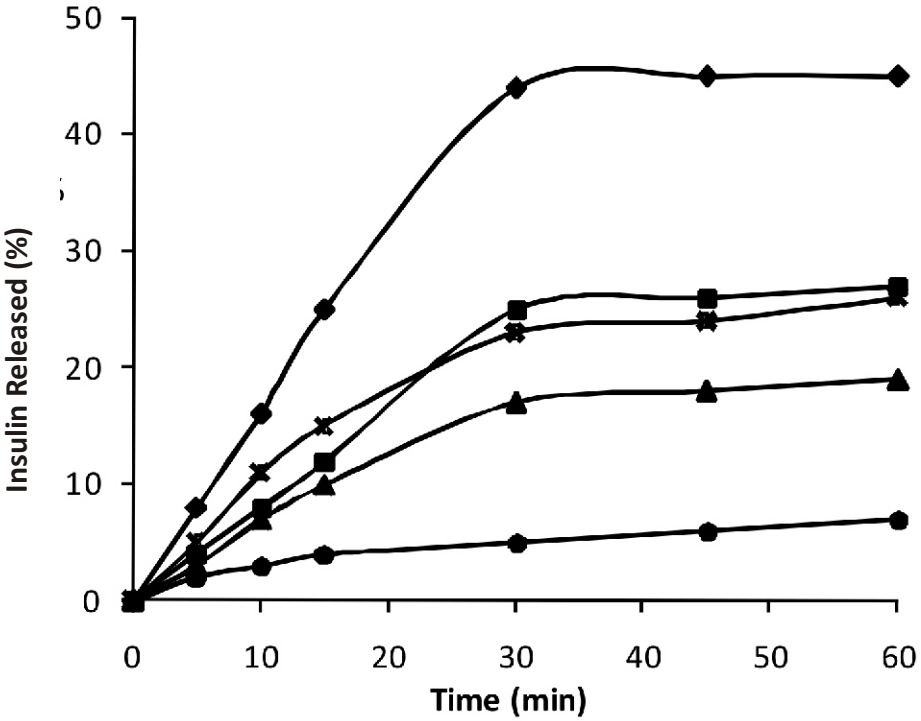

Fig 1. : *In vitro* insulin release profile of the transdermal patch formulations TD1 (◆), TD2 (■), TD3 (▲), TD4 (●) and TD5 (✱)

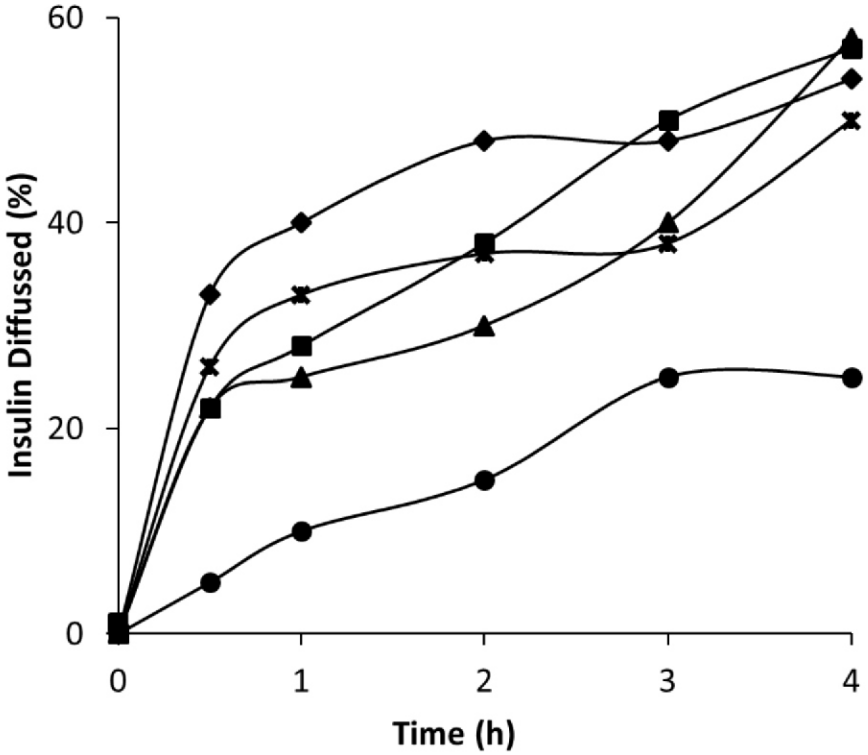

Fig 2. : *Ex vivo* diffusion of insulin from the transdermal patches across treated rat skin TD1 (◆), TD2 (■), TD3 (▲), TD4 (●) and TD5 (✱)

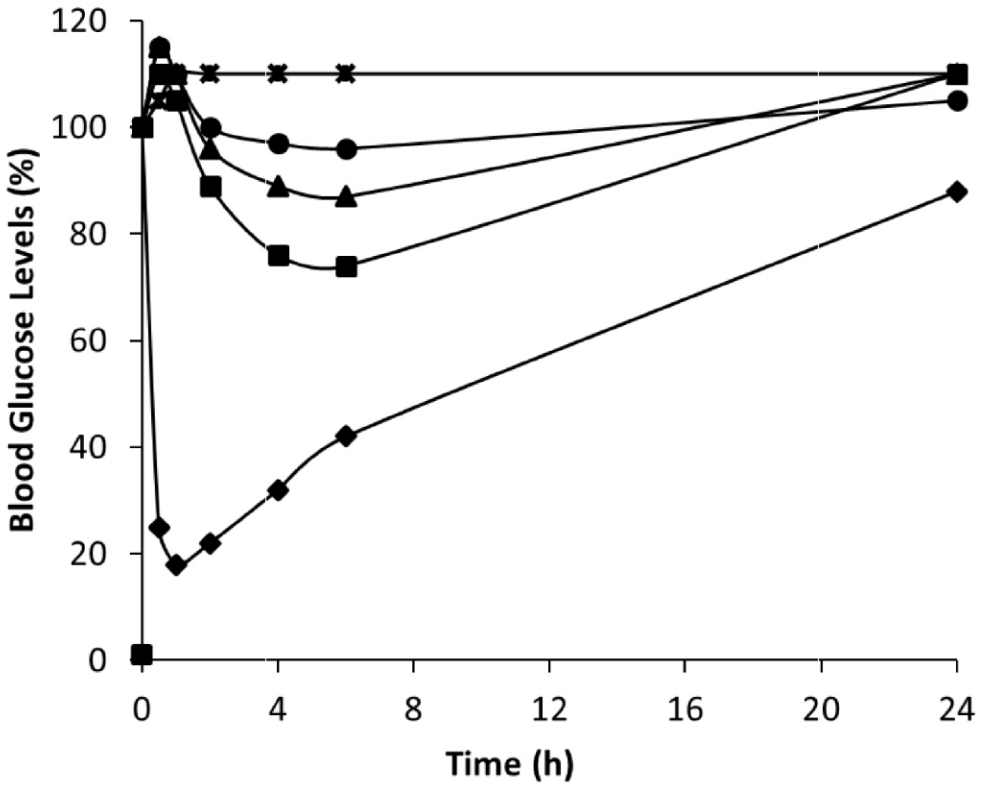

Fig 3. : Percentage blood glucose level in alloxan induced diabetic rats after transdermal administration of insulin (IP Insulin) TD1 (◆), TD2 (■), TD3 (▲), TD4 (●) and TD5 (✱) Data are expressed as the mean ± SD (n= 2)

TD3) gave a quicker rate of diffusion through the skin as can be seen in the differences of percentage insulin diffusion within 2 h of experiment. Generally, all the patches except TD4 perform better than TD5 (HPMC alone) in facilitating insulin diffusion through the rat skin.

In vivo studies

Changes in blood glucose level after administration of the insulin transdermal patch and the two controls are shown in Figure 3. From the results obtained, TD1 patches showed the greatest mean blood glucose level reduction of 36 % as compared with TD3 patches 23 % while TD5 gave an insignificant blood glucose reduction of 4 % within 6 h and then a gradual rise in the blood glucose levels of the animals to 100 % at 24 h. Comparing the test patches with the positive control, it was observed that insulin given intraperitoneally (IP) gave the highest percentage reduction in blood glucose level of 82 % within 1 h and followed by a steep rise in blood glucose towards 100 % at 24 h.

DISCUSSION

Oils are neutral and non-polar chemical substances. They are usually viscous liquid at ambient temperatures and generally hydrophobic (immiscible with water) or lipophilic (miscible with other oils). Chemically, they consist of carbon and hydrogen chains with oxygen endings resulting in organic acids forming esters with glycerols. They are usually highly inflammable and slippery. Arachis oil, obtained from peanuts [16] and eucalyptus oil obtained from the leaf of eucalyptus [17] are both natural oils which are used in preparations for foods and flavours. They are also use as components in dosage form designs such as in emulsions. The concept of formulating transdermal patches with oils was to create a micro emulsion environment within the patches that will permit the drug to diffuse from the aqueous phase through the oily phase which is expected to form a bridge between the aqueous drug component and the organic skin barrier surface. It is also hoped that the oils being more lipophilic will in the course of diffusing across the skin barrier, facilitate drug carriage along with it.

The weights and thickness of the patches formed varied significantly ($p < 0.05$) due to the fact that arachis oil being more viscous and less volatile resulted in the formation of thicker patches. This attribute also resulted in the retention of more insulin in the patches. Eucalytus oil is a less viscous oil with lesser density. The bioadhesion is a measure of the ability of the patch to stay on the skin. Patches with eucalyptus (TD3 and TD4) had the lowest bioadhesion while the patch with 10 % arachis oil (TD2) gave the highest adhesion ability. HPMC patches usually possess strong bioadhesion properties which was significantly reduced ($p < 0.05$) by the presence of the oils. The oils appear to interfere negatively with the forces associated with surface bonding of bioadhesive molecules because of their lubricating effect. This reduction was however not directly proportional (in the case of eucalyptus oil) to the concentration of oil as increase in the concentration of the oils did not show a proportionate decrease in the bioadhesion strength of the patches.

In vitro and *ex vivo* release profiles of the patches were similar. TD1 patches had the highest release while TD4 patches had the poorest release profiles. The higher diffusion rate observed with TD1 at the early part of the experiment was due to the fact that because of the lower HLB value of arachis oil when compared eucalyptus oil, it is more lipophilic, hence allowing easier

diffusion through the highly lipophilic epidermis of the skin than the other preparations. The overall diffusion enhancing ability through the skin by TD3 and TD4 was observed to be considerably lower than expected. This can be attributed to the fact that since eucalyptus oil is a volatile oil, it is more likely prone to evaporation than penetration into the skin layer, this will further retard the possibility for any drug in the patch to penetrate as there will be a pull back by the eucalyptus oil components of the patch.

In vivo release study showed that IP administered insulin gave a steep reduction attaining lowest blood glucose level in less than an hour when compared with the test preparations. For test patches using TD1 as a case study, it was observed that blood glucose levels continued to increase even after patch attachment from 100 % at 0 h to 120 % at 1 h until about 3 h into the experiment when significant reduction in blood glucose levels started to occur. This can be attributed to the fact that some time will be required for patch hydration and drug diffusion out of the patch and subsequently across the animal skin into blood circulation. Furthermore, rats to which insulin was administered IP had their blood glucose levels returned faster than the TD1 administered rats. Hence the TD patches gave sustained release of the insulin over a prolonged period when compared to the IP administered. For the group given normal saline which served as the negative control, there was no reduction in blood glucose level. The implication of this study is that an initial IP administered insulin can be followed with the administration of an insulin transdermal patch which is expected to sustain insulin release into the blood for a prolonged period of time.

CONCLUSION

A combination of arachis oil up to 20 % and HPMC as carrier in insulin transdermal formulation gave the best release profile and percentage reduction of blood glucose levels, achieving significant reduction in blood glucose levels. As such, it is a potential new system for the transdermal delivery of insulin. This formulation could allow for the achievement of insulin administration over a long period of time while circumventing the inconveniences associated with the parenteral route and the high cost of other transdermal insulin formulations.

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