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TRANSDERMAL PERMEATION OF SALICYLIC ACID EMBEDDED IN AN ARRAY OF FORMULATIONS BASED ON MUCINATED HONEY ACROSS EXCISED EXPERIMENTAL RAT SKIN

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ABSTRACT

This work focuses on the skin permeation properties of drug formulations such as ointments, creams and gels produced using various quantities of mucin, honey and their admixtures (mucinated –honey (MH) containing salicylic acid as the model drug. Permeation studies involved the assessment of the transdermal migration of salicylic acid across excised rat skin at room temperature using phosphate buffered saline as the release medium. Results obtained indicate that formulations containing mucinated honey (MH) showed higher salicylic acid release than those containing only mucin or honey alone. Furthermore, the apparent permeability (P_{app}) values obtained for ointments varied between 2.036×10^{-4} to 2.372×10^{-4} cm^2S^{-1} while those obtained for creams ranged from $2.726 - 3.027 \times 10^{-4} cm^2S^{-1}$. Additionally, the P_{app} values for the gels formulated were between 2.5174 and 2.9368×10^{-4} $cm^2 S^{-1}$. Results for the steady state flux study of the formulations showed that MH formulations have much higher values than those obtained for formulations containing mucin or honey alone. Findings suggest that the materials handled could be possible candidates for the formulation of ointments, creams and gels for topical drug delivery applications to wounds.

Keywords: *Skin permeation; mucinated honey; salicylic acid; apparent permeability.*

INTRODUCTION

The skin by virtue of its extension is easily the largest organ in the body and as a consequence, it has been the focal point of intensive research aimed at its utilization as a portal for systemic drug delivery seen as a corollary of its millennium long use for topical local drug delivery. The skin as an access point for drugs is deemed to offer several advantages over other conventional routes of drug administration[1]. Normally; the extent of skin permeation by a xenobiotic is greatly influenced by the physicochemical properties of the medicinal agent and its accompanying excipients in the particular formulation. Research shows that the crossing of the stratum corneum remains the most significant obstacle to the free transport of drug across the skin surface.

This work focuses on the percutaneous transport of salicylic acid across the surface of excised rat skin. Emphasis is placed on the effect of the accompanying excipients in the formulation especially honey, mucin and their combinations which is referred to here as mucinated honey. Honey is known to have potent antibacterial activity. It is very effective in clearing infection in wounds and protecting wounds from becoming infected. It also has a debriding action, an anti-inflammatory action, and a stimulatory effect on granulation and epithelialization [2]. Parameters evaluated included the percentage of percutaneous penetration of the model drug salicylic acid as well as the estimation of the total quantity of drug released over a test period of 5 hours. The apparent permeability of the drug was also investigated.

MATERIALS AND METHODS

Materials

Purified mucin type 111 (Sigma Aldrich, UK). Honey procured from Nsukka Market, Gelatin A powder(Fairlawn,New Jersey,USA),Mueller Hinton Agar(Lancashire,UK),Emulsifying wax, white soft paraffin,liquid paraffin (BDH Chemicals,England, Flamazine cream (Hoechst, Germany), salicylic acid BP(Lewes,Sussex,UK),Sodium hydroxide, monobasic potassium phosphate (Bergoyne, India), disodium hydrogen phosphate anhydrous,sorbic acid, chlorocreosol (BDH, England). Albino rats were obtained from the Animal house of the Department of Pharmacology, University of Nigeria,Nsukka.

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Methods

Standardization of honey

Honey used for this investigation was standardized using the method described in the Pharmaceutical codex. This was done by diluting the honey with a known quantity of sterile distilled water to a weight of 1.36g/ml at room temperature (29±1^{0}C).

Determination of the antimicrobial activity of honey

Briefly, this was ascertained by the following process: Various dilutions of the standardized honey(1:1, 1:2, 1:3 and 1:4), were prepared using sterile distilled water.Next, the antibacterial activity of each dilution obtained against three species of bacteria usually implicated in wound contamination namely *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli,* were determined. Some cultures of the bacteria were collected by means of an inoculating loop and seeded in 1 ml of sterile distilled water after which the resulting suspension was transferred into 9 ml of sterile double strength nutrient broth and the set up then incubated at 37^{0}C for 24 hours in a standard incubator(Gallenkamp, Model 111-150). The bacterial suspension was constituted by transferring 1 ml of the seeded nutrient broth to 99 ml of sterile molten Mueller Hinton agar. Additionally, 38g of Mueller Hinton Agar powder was soaked in 0,5 litre of deionized water for 10 minutes, mixed with a vertex mixer and sterilized by autoclaving (Haereus Type KKB500) for 15 min at 121^{o}C. The set up was allowed to cool to 47^{o}C and

20ml was poured into a petri dish and allowed to solidify at room temperature. Then, a sterile glass borer was utilized to excavate 6mm holes in the solidified agar. Two drops of each dilution of honey was then placed in each of the holes and the petri dish incubated at 37^{o}C for 48 h.The zone of inhibition was measured in mm using a calibrated ruler. This procedure was repeated for each of the test microorganisms.

Determination of the antimicrobial activity of mucin

The procedure followed with respect to honey was followed in the case of mucin. Briefly, various dilutions of mucin (1:1, 1:2, 1:3 and 1:4), were prepared with sterile distilled water giving concentrations of 200 mg/ml, 100mg/ml, 66.7mg/ml, 50 mg/ml and 40 mg/ml respectively.

Determination of the antimicrobial activity of mucin-honey mixtures

A similar procedure was followed. Various mixtures of mucin and honey in the ratios of 1:1,1:2, 1:3, 1:4, 2:1, 2:3, 3:1 and 4:1 by weight were prepared. Next, the minimum inhibitory concentration (MIC) was calculated from the x-intercept of the plot of the inhibition zone diameter squared against the log concentration.

Preparation of ointments, creams and gels

These preparations were prepared using the standard procedures.

Evaluation of the ointment

The absolute drug content of the ointments was determined after allowing the product to stand for 30 minutes. The quantity of salicylic acid which served as the model drug was assayed in each batch of the ointment at 37^{o}C in phosphate buffer pH 7.6 serving as the dissolution medium. In each batch, a 0.1g quantity of the ointment was weighted out and placed in phosphate buffer solution in a suitable vessel. The volume was made up to 100ml with the buffer solution and the resulting solution allowed to stand for 30 minutes before the assay for the salicylic acid content.The salicylic acid content was ascertained by assessing the quantity of the drug released in the dissolution medium by means of a simple spectrophotometric assay at a wavelength of 298 nm. The spectrophotometer used (Coleman,U.V. Model 6/20 A,UK).The procedure was repeated five times for each batch and the average taken.The absolute content of salicylic acid for each batch was determined .

The absolute drug content of the creams and the gels were determined using the aforementioned procedure.

In vitro skin permeability studies for ointments

The skin permeability of salicylic acid from the ointments formulated was determined in vitro using excised rat skin as the membrane .A diffusion cell was maintained at constant temperature (37±1 ^{o}C) using a magnetic stirrer. The rat skin was then placed on the vessel containing the phosphate buffer. A 0.1g quantity of the ointment to be tested was placed on the skin.The set up was allowed to stand and aliquots of the medium were withdrawn from the sampling port at fixed intervals of time allowing for the replacement of the dissolution medium after the removal of the desired sample. Samples were assayed in a spectrophotometer (Coleman, U.V. Model 6/20 A, UK)

RESULTS AND DISCUSSION

Standardization of honey

Honey used in the study was standardized in line with the criteria established in the Pharmaceutical codex. Upon storage for over 14 months, there were no observed changes in its organoleptic properties.

Antibacterial activity of mucin and honey

The outcome of the antibacterial properties of mucin and honey are presented in Table 1. The results indicate that mucin had the highest inhibition zone diameter (IZD) against *Staphylococcus aureus* followed by *pseudomonas aeruginosa* and *Escherichia coli*.The various concentrations of honey did not exhibit antibacterial activity against *staphylococcus*

aureus but showed activity against *pseudomonas aeruginosa* and *Escherichia coli.* The inhibition zone diameter decreased as the concentration of the mucin or honey decreased. It has been reported that various strains of *Staphylococcus aureus, is* inhibited by 5-10% honey in an infected wound[3]. Also, Obasieki-Ebor and co-workers have reported that the distillate of undiluted honey exhibited antimicrobial activity *against Escherichia coli, staphylococcus aureus* and *pseudomonas aeruginosa*. Honey has been used to treat neonatal post-operative wound infections [4].

Table 1: *Comparison of ointment formulations and SSD in terms of % permeation, steady state flux(SSF) and apparent permeability coefficient (Papp) from in vitro permeation studies using excised pig skin*

S/No	Batches	Drug permeation in 5hrs(g)	% drug permeation	SSF (µg/min/cm²)	P_{app} × 10⁻⁴
1	23.5% honey	1.32±0.159	13.98	11.0092 ± 1.16	4.4606± 0.005
2	11.75% honey	1.48 ±0.046	15.89	12.325 ± 385	4.8006± 0.15
3	11.75% honey + 23.5% honey	1.67±0.196	17.32	13.9475 ± 1.64	4.8533±0.57
4	Emulsifying ointment base	1.22 ± 0.208	12.95	10. 1317 ± 1.73	4.238 ±0.77
	SSD	1.4 ±0.127	14.6	11.6667 ± 994	4.5755±0. 46

± is standard deviation . SSD stands for 1% silver sulphadiazine cream

Antimicrobial activity of mucin-honey mixtures

The various combinations of mucin and honey evaluated showed enhanced activity against the three strains of bacteria tested.it is posited that mucin exerts its antimicrobial action by surface activity of bacterial adhesion and surfactant activity [5]. Honey exerts its antibacterial action by hydrogen peroxide, gluconic acid and denaturation of bacterial cell wall.

***In vitro* skin permeability studies**

The results of the permeation studies in terms of percentage permeation, steady state flux (SSF) and apparent permeability coefficient (P_{app}), are presented in Tables 1-3.The results indicate that the MH formulations had a higher skin permeation rate compared to the other agents with the order being MH>Mucin>Honey>SSD. With respect to the ointment batches, a similar pattern was observed .In the case of the cream formulations as shown in Table 2, MH showed the highest SSF with P_{app} followed by mucin, honey, SSD and aqueous cream base. The gel formulations, shown in Table 3 exhibit the same pattern as the ointments and creams with higher values than ointment batches. The permeation results indicated that MH preparations showed a more enhanced permeation of salicylic acid across excised rat skin than the standard SSD (1% silver sulphadiazine cream). It has been shown that the two parameter Fickian diffusion model and the developed skin porous –pathway theory[6] , have shown that hydration culminates in the induction of new pores /reduction of the tortuosity of the existing pores within the excised rat skin. The permeability of drugs across rat skin may be due to

Table 2: *Comparison of cream formulations and the SSD in terms of % permeation, steady state flux (SSF) and apparent permeability coefficient (Papp) from in vitro permeation studies using excised pig skin*

S/No	Batches	Drug permeation in 5hrs(g)	% drug permeation	SSF (µg/min/cm²)	P_{app} × 10⁻⁴
5	23.5% honey	1.32±0.104	13.76	11.0092 ± 1.16	4.7447 ± 0.37
6	11.75% honey	1.7 ±0.346	17.37	14.1667 ±346	5.0195± 1.02
7	11.75% honey + 23.5% honey	1.83±0.11	18.44	15.2500 ± 3.11	5.2963± 0.46
8	Aqueous cream base	1.26 ± 0.104	13.25	10.5010 ± 3.78	4.4498± 1.86
	SSD	1.4±0.127	14.6	11.6667 ±994	4.5755±0.46

Table 3: *Comparison of gel formulations and SSD in terms of % permeation, steady state flux (SSF) and apparent permeability coefficient (Papp) from in vitro permeation studies using excised pig skin*

S/No	Batches	Drug permeation in 5hrs(g)	% drug permeation	SSF (µg/min/cm²)	P_{app} × 10⁻⁴
9	23.5% honey	1.49 ±0.208	15.43	12.4125 ± 1.73	4.7447 ± 0.37
10	11.75% honey	1.65 ±0.15	17.01	13.7288 ± 1.25	5.0195 ± 1.02
11	11.75% honey + 23.5% honey	1.69 ±0.058	17. 17	14.07 92 ± 0.048	5. 1089 ± 0.18
12	(15% gelatin gel)	1.25 ± 0.069	13.07	10.4167 ± 0.577	4.382±0.3
	SSD	1.4 ± 0.127	14.6	11.6667 ± 994	4.5755±0.46

the structural changes in the skin although the exact mechanism is unclear. The apparent permeability coefficients of formulations that contained MH compared well with that obtained for SSD in the various formulations.it has also been observed that drug release from collagen matrices is in most cases governed by diffusion from swollen matrices but may also involve enzymatic matrix degradation or hydrophobic drug-matrix or polymer interactions. Thus, when a hydrophilic polymer takes up some quantity of aqueous liquid when in contact with physiological fluid, it swells. Furthermore, drug release appears to occur by countercurrent diffusion through a penetrating solvent with the release rate being determined by the diffusion rate of the solvent in the polymer. An adequate dissolution rate is important to maintain a steady concentration of the formulation. This is because dissolution behavior is an important parameter affecting the drug permeation flux through the stratum corneum from a suspension. As drug dissolution rate is lower than permeation flux, drug concentration in the suspension decreases causing a decrease in permeation itself. *In vitro* permeation studies of the various formulations show that the increase in salicylic acid permeation can be primarily be attributed to the increase in salicylic acid solubility in the formulation containing MH. Nakano *et al* [7] have shown that there should be a balance between lipid and aqueous solubility of drug to optimize permeation. Findings suggest that MH has such characteristics.it can be inferred that conventional pharmaceutical bases used in the formulation of ointments, creams and gels did not act as enhancers capable of modifying the permeation coefficient of the drug in the stratum corneum. The increase in skin permeability observed may be also attributed to the hydrophilic nature of MH combinations. The higher permeation flux may also be attributed to the presence of a diffusion layer at the

skin surface where the MH acts as a carrier, carrying the drug from the donor phase to the lipophilic part of the skin[8]. Cross and co-workers stated that the concentration gradient over the diffusion layer is the main driving force for the drug molecules to be delivered from the base to the surface of the skin barrier. The dissolved drug availability is crucial for effective drug delivery. Also, Ceschel *et al* [9] further observed that the complexation of two polymers could increase the flux of drug in percutaneous permeation. The formulations containing MH improved the permeation flux of the salicylic acid across the rat skin.

CONCLUSION

In conclusion, it may be posited that on the basis of the findings in this course of investigation, MH preparations appear to be more optimal candidates for the formulation of ointments, creams and gels for topic drug delivery than either mucin alone or honey alone.

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