Universal Journal of Pharmacy
Take Research to New Heights

Research Article
ISSN 2320-303X

DEVELOPMENT AND CLINICAL EVALUATION OF SOME TOPICAL PHARMACEUTICAL DOSAGE FORMS OF PHENYTOIN

El-Sayed AM[1], El-Mahdy MM[2*], Essam-El dien MM[3] and Saddik MS[4]

[1]Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[2]Department of Pharmacology, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[3]Department of Dermatology and Venerealogy, Faculty Medicine, Al-Azhar University, Assiut, Egypt
[4]Pharmacist in Al-Azhar, University Hospital, Assiut, Egypt

Received 30-11-2013; Revised 29-12-2013; Accepted 28-01-2014

ABSTRACT

Phenytoin (diphenlhydantoin), a hydantoin derivatives that has long been for its use in epilepsy, Clinical data indicate that systemic or topical phentoin enhances the healing of various types of wounds and chronic ulcers. This pushes us to formulate and optimize some topical dermatological formulations containing phentyoin (PHT) or its sodium salt for treatment of certain skin healing. Dermal films, creams and ointment of phenytoin sodium were designed and developed for the first time by in vitro and clinical evaluation. Dermal patches of phenytoin sodium were prepared by solvent evaporation technique using different concentration of Hydroxypropyl, methylcellulose (HPMC) and Hydroxypropyl cellulose (HPC). Characterization of phenytoin creams and ointment were evaluated from the point of view of drug content, viscosity measurements and drug release studies. Moreover all topical formulations under investigation were evaluated for their physicochemical characteristics; the possible drug-polymer interaction by DSC and IR studies and kinetic release studies. In vivo evaluation was to evaluate and compare dermal phenytoin patch with phenytoin powder and normal saline in healing of chronic skin ulcer in diabetic patients. Assessment of the ulcers was done statistically weekly interval up to 12 weeks. Patients with post deep acne scars were assigned to PHT cream, placebo cream and microdermabrasion alone. Photographs of ulcers and scars were taken at baseline and at the end of treatment.The results show that, the thickness as well as the weight of the patch increased with the increase in polymer concentration and there was no interaction between the polymers and the drug. In vitro release studies shows that, HPMC enhanced the release rate of phenytoin sodium from prepared films and the drug release followed Higuchi and first order kinetics. The ulcer volume reduction of the phenytoin patch group was greater and statistically significant comparing with phenytoin powder and control groups. Phenytoin patch appears to be the most stable and effective topical formula in the healing of chronic diabetic ulcer.The results of the phenytoin release from ointment bases showed that emulsion based ointment exhibited the higher phenytoin release as well as lower viscosity than other formulations. Therefore it was selected for further evaluation because of its lower viscosity, easy to remove from the skin, creamy in nature and higher release of phenytoin . On the other hand, the release of phenytoin from cold cream bases increase as the borax concentration increased. Kinetic studies revealed that the best fitted model for phenytoin selected formula was Higuchi kinetic model. The clinical investigation revealed a significant difference between phenytoin ointments treated group and other groups and has proven that, phenytoin ointment help in better and faster resolution of the acne lesion as well as in reducing inflammation.

Keywords: Phenytoin patch, Phenytoin cream, HPMC, HPC.

INTRODUCTION

Phenytoin has been investigated as a treatment for more than 100 diseases [1,2]. Phenytoin (Diphenylhydantoin), is best known for its clinical use in convulsive disorders and has been reported to enhance the healing of various types of cutaneous ulcers including venous stasis, decubitus, diabetic, and trophic ulcers in leprosy, as well as second-degree burns. The trials for phenytoin in the treatment of large abscess, stage II decubitus ulcers in the elderly,

*Corresponding author:
El-Mahdy MM
Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt , Mobile:0201006262088
Email: monamahdy2010@ yahoo.com

chronic trophic ulcers in leprosy, split-thickness skin autograft donor sites and other previous studies were performed using phenytoin or its mono sodium salt in the form of powder, suspension or solution [3-5]. The selection of formulation type for dermatological products is usually influenced by the nature of the skin lesion and the opinion of the medical practitioner. Kitson and Maddin[6]. Elegantly stated: ''It is idle to pretend that the therapy for skin diseases, as currently practiced, has its origins in science.'' To this day a practicing dermatologist would prefer to apply a ''wet'' formulation (ranging from simple tap water to complex emulsion formulations, with or without drug) to a wet lesion and a ''dry' formulation (e.g., petrolatum) to a dry lesion. The preparation of such formulations as poultices and pastes is extemporaneous, and it is unlikely that the industrial pharmaceutical formulator will be required to develop products of this type.

Solutions and powders lack staying power (retention time) on the skin and can afford only transient relief. In modern-day pharmaceutical practice, semisolid formulations are the preferred vehicles for dermatological therapy because they remain in situ and deliver the drug over extended time periods. In most cases, therefore, the developed formulation will be an ointment, cream, emulsion, or gel [7-9]. Many patients and physicians prefer creams to ointment. For one thing, they are generally easier to spread, in the case of creams the oil-in-water emulsion type easier to remove than many ointments, Pharmaceutical manufactures frequently market their topical preparation in both cream and ointment bases to satisfy the preference of the patient and physician [10].

Dermal therapeutic systems are dosage forms used for the topical treatment of skin disease. The typical of these formulations is a patch, in which a backing layer, an adhesive, a drug reservoir and a removable protection liner, are the constitutive elements[11-13].

Foot ulcers and amputations are a major cause of morbidity, disability and costs for people with diabetes[14]. Ulcer healing property of topical Phenytoin has been reported from several animal studies[15] and clinical trials [15- 19]. In most of the clinical trials, investigators have used Phenytoin powder from the capsules or phenytoin suspensions [1, since they are convenient to use. However, they contain numerous additional components (e.g. dyes, flavors) and it is not possible to differentiate the effect of phenytoin from other components of the preparation. In addition, finding an inert placebo powder for conducting a double-blind clinical trial is difficult. The validity of most of the previous studies is questionable as they were not conducted in randomized, double-blind design[14]. In the light of this, the aim of this part of preliminary study is to prepare and evaluate clinical efficacy of newly developed PHT patch in chronic diabetic.Acne is caused and characterized by multiple factors, including: Propionibacterium acnes activity; increased sebum production; androgenic stimulation; follicular hypercornification; lymphocyte, macrophage, and neutrophil inflammatory response; and cytokine activation[20]. Various types of scars have been described; these may be broadly categorized into two main groups, that involving tissue loss and those involving tissue excess[21]. Acne scars caused by loss of tissue are more common than hypertrophic scars and keloids. Hence, the clinical work in this study concerning with scars caused by loss of tissue. The absence of perfect treatment of such scars leading us to study the inclusion of phenytoin cream in addition to microdermabrasion in this preliminary research (to accelerates the formation and maturation of collagen fibers, stimulates fibroblast proliferation, and inhibits collagens activity) aiming to induce equal thickness and better appearance of the skin.

MATERIALS AND METHODS

Materials

Phenytoin sodium was a gift from El-Nile Pharm. Chem. Co., Cairo, Egypt, Hydroxypropyl methylcellulose (HPMC), Hydroxypropyl cellulose (HPC) and Propylene glycol (PG) from (Sigma Chemicals Co., USA). Polyethylene glycol 400 (PEG 400) and PEG 6000 (Merk CO.,Germany **).** Dimethylformamide DMF (C.P., Evans., CO., England), Absolute ethanol ,White petrolatum, beeswax, anhydrous lanolin, stearyl alcohol, cetyl alcohol, wool alcohol, hard paraffin , liquid paraffin, stearic acid, potassium hydroxide , glycerin, ethanol, methanol, tween 80.(EL-Naser Pharm. Chem.. Co., Cairo, Egypt). Borax (Ostrade Chem. CO). Span 65 (Flua Chem. Buch. Switzerland). All other chemicals used were of pharmaceutical grade .Teflon plates fabricated in the faculty of engineering, Assiut University, Cairo, Egypt.

Methods

HPLC determination of phenytoin

HPLC analyses were performed using a Water Associates Model 600 pump equipped with a Water 990 variable wavelength UV detector, a Waters 712 WISP autosampler, and a 20 µl loop injection valve (U6K). For analysis, a reversed phase symmetry c18 (25cm x 4.6 mm: 5µm particles) column in conjunction with a precolumn insert was eluted with mixtures of methanol and deionized water (65:35). A flow rate of 1 ml/min was maintained. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions. Standard curves were measured at a wavelength of 254 nm.

I-Preparation and evaluation of phenytoin sodium patches topical formulations:

Dermal patches of phenytoin sodium were prepared by solvent evaporation technique[12] using varying

concentration of HPMC and HPC keeping drug concentration constant (30 mg of phenytoin /9 cm^2 patch). The drug: polymer ratios used were 1:3 (F1), 1:4 (F2) with HPMC, 1:6 (F3), 1:8 (F4) with HPC and 1:2:4 (F5) with mixture of HPMC: HPC. The required amount of drug and polymer were dispersed separately in casting solvent (ethanol: distilled water in 8:2 ratio). The polymeric dispersions were sonicated for 2 minutes to remove entrapped air bubbles.

The two preparations were then mixed and 15% propylene glycol was incorporated as plasticizer. This polymeric dispersion of the drug was poured into teflon plates (55 cm^2) and allowed to dry in oven at $40C^0$ till a flexible film was formed.

Dried films were carefully removed, checked for any imperfection or air bubbles .To prevent fast evaporation from the patches a funnel was placed inverted on the plate.

After ensuring the complete evaporation of the solvent, patches of 9 cm^2 were cut with a borer and packed in aluminum foil and stored in desiccator to maintain the integrity and elasticity of the patches.

Physicochemical Characterization

Thickness

The thickness of the films was assessed using an electronic digital Micrometer 0.25 mm (MIME Technology Europe Moastricht-Netherlands).

Ten randomly selected patches of each formulation were tested for their thickness. The thickness was measured at 5 separate points on each patch in order to ensure uniform thickness.

Weight Variation

The patches were subjected to weight variation by individually weighing ten randomly selected patches. Such determinations were carried out for each formulation.

Film Folding Endurance

This was determined by repeated folding of the patch at the same place until it shows any crack or break. The number of times the film could be folded without breaking/cracking gave the value of folding endurance[7]. Five randomly selected patches (3×3 cm) of each formulation were tested.

Content Uniformity

The phenytoin content in the patch was determined by HPLC analysis. The patch (area 9 cm^2) was dissolved in 10 ml of the casting solvent (ethanol: water in ratio 8:2) and diluted further with methanol before estimation by HPLC.

In vitro Release Studies

The drug release studies from phenytoin patches were performed using dissolution rate test apparatus (USP-II) [22]. The patch of $9cm^2$ fixed to circular glass slide with the help of cyanoacrylate adhesive.Then it was placed in dissolution vessel containing 500ml of normal saline as release media. The study was conducted at 37 ± 0.20 C^0 and paddle speed was kept at 50 rpm. Samples (5 ml) were collected up to 6hrs at suitable intervals. The analysis was carried out using HPLC.

Drug Polymer Compatibility Study

To study the possible interaction between phenytoin and polymeric materials of the patches. Infrared (IR) spectroscopy & Differantial scanning calorimetry (DSC) was carried out on pure substances and their physical mixtures containing drug: polymer in ratio of 1:1.

Kinetic Studies

Kinetic study was carried out to determine the release model which describes the release pattern of the drug. The formulations were analyzed according to zero order, first order and Higuchi diffusion model.

Clinical Evaluation

The aim of this preliminary study is to evaluate clinical efficacy of newly developed phenytoin patch in chronic diabetic ulcers. The clinical study was conducted at the outpatient dermatology clinic, Al-Azhar University Hospital (Assiut). The ethical committee. The ethical committee of Al-Azhar University has approved the study protocol.

At base line (day 1), a detailed relevant history and ulcer examination was done for each patient. The wound swab was sent for bacterial culture and if there were any signs of systemic infection, the patient received appropriate systemic antibiotics. Prior to initiation of any therapy, all necrotic tissue and slough was removed. The patients were assigned to a phenytoin patch group (thirty six patients), phenytoin powder group (forty four patients) and saline group (thirty seven patients). In the phenytoin patch group, a patch of 30 mg of phenytoin per 9 cm^2 placed over the wound, followed by a layer of dry sterile gauze as a backing layer. Phenytoin powder was applied in a uniform, thin layer to the ulcer surface, and a sterile dry dressing was applied. In the saline group, sterile gauze soaked in normal saline was placed over the wound followed by a layer of dry sterile gauze. Assessment of the ulcer was performed weekly. The primary efficacy parameter was the percentage reduction in surface area of the ulcers. Ulcer volume was determined by filling the ulcer drop by drop with distilled water using a graduated syringe.

Preparation and evaluation of phenytoin topical formulations (ointments and creams):

Methodology

The composition of certain phenytoin ointment formulation is shown in table (1&2).Oleaginous and absorption bases were prepared by fusion or incorporation method [23], phenytoin was sieved through 150 µg sieve and the calculated amount was incorporated by the aid of magnetic stirrer. Emulsion bases were prepared by placing all the aqueous- phase and the oil- phase ingredients into separate beakers and heated to 70 C° ±2 C° at hot plate, and then the two phases were mixed and stirred until cold[24]. The fine powdered phenytoin can be added to the base and

stirred continuously using mechanical stirrer. PEG ointment was prepared by first melting PEG 6000 by heating to 50 C^0 and then mixed thoroughly with PEG 400 to homogeneity until room temperature was reached [25] then phenytoin as fine powder (less than 150 μg size range) could be incorporated into the base.

Drug Content Studies

Drug content was determined by dissolving an accurately weighed quantity of the formulation (100 mg) in 10 ml of DMF. Then filtration using 0, 45 mm membrane filters then diluted with methanol before estimation by HPLC.

In-Vitro Release of Phenytoin from the Prepared Formulations PHT.

One gram sample of the formulation under investigation was placed on a circular area (6cm diameter) of cellophane membrane previously moistened with the receptor phase. The loaded membrane was firmly stretched over one end of a glass tube with fixed cross area the tube was then immersed in a 100 ml beaker containing 50 ml of the release media (30% v/v DMF in phosphate buffer pH 6.8) and placed in thermostatically controlled water bath at 37±1 ^{0}C at 50 rpm. An aliquot of 5ml sample was withdrawn at different time intervals, and then replaced by equal volume of the release medium maintained at the same temperature. The amount of phenytoin released at time intervals was determined by HPLC.

Viscosity Measurements:

Whenever possible the viscosities of formulations were measured using a Model-RVIII + Brookfield Viscometer (Brookfield ENG Labs Inc., Stoughton, MA, USA). The viscometer was operated at 100 rpm using a T-F (code 96) spindle. In order to obtain stable display readings, all measurements were recorded 60 sec after the commencement of spindle rotation and a maximum of three (3) readings were taken to obtain an average viscosity value.

Kinetic Studies

Kinetic study was carried out to determine the release model which describes the release pattern of the drug. The selected formulations were analyzed according to zero order[26], first order, and Higuchi diffusion model[27].

Clinical evaluation of phenytoin ointment in the treatment of deep post acne scar:

Patients with post deep acne scars were recruited from the outpatient clinic of the department of dermatology, Al-Azhar University hospital. The ethical committee of Al-Azhar University hospital has approved the study protocol.

At base line (day 1), a detailed relevant history and scar examination was done for each patient. The study was performed using microdermabrasion on the face and 12 sessation weekly intervals. The patients were assigned to a PHT cream (35 patients), placebo cream (26 patients) and microdermabrasion group (27 patients). In the phenytoin cream group, after microdermabrasion the cream spread over the scar twice a day (morning and evening) along the period of treatment.

All patient self-administered treatment for 12 weeks. Assessment of the scar was performed weekly. Photographs of the scars were taken at baseline and at the end of treatment. Two phenytoin plasma concentrations were obtained on each patient in the phenytoin ointment group. The first plasma concentration was obtained within four week after treatment had begun, and the second at the end of treatment. Concentrations were determined within four hours of the last phenytoin cream application. Phenytoin analysis was done by HPLC. Only three of the treatment patients had blood drawn for two phenytoin concentrations, two had blood drawn only once and others had no blood drawn. The other patient refused due to health problem or other reason model.

Statistical Analysis

Values were expressed as (excellent, good and poor). The differences in the three groups were analyzed using the chi-square test. P-values < 0.05 were considered statistically significant. The analysis was carried out using Statistical Package for Social Sciences (SPSS version 14 Inc., Chicago, IL).

RESULTS AND DISCUSSION

Dermal patches of phenytoin sodium

Dermal films of phenytoin sodium were prepared using varying ratio of drug: polymer (HPMC and HPC). The thickness and weight of the patches were found to be uniform among different batches Table 1. Irrespective of the concentration of polymer used, the drug content per patch (9cm^2) was found to be within 29.943 to 29.986 mg but the thickness and weight of the patch increased linearly with the increase in polymer content table (3).

Folding endurance values of matrix films found more than 300 indicating good strength and elasticity, which is explained by the linear nature of the cellulose structure. Formulations were selected on the basis of their drug release content and release pattern. The release of phenytoin sodium from prepared films are illustrated in figure 1. The drug released rates from films were in descending order: F1 > F2 > F5 > F3 > F4. The higher release rate of the formulations can be attributed to the hydrophilic nature of the polymers.

The compatibility between a drug and polymer is a factor in determining the effectiveness of polymeric delivery systems [28]. It was found that there is no interaction between phenytoin sodium and polymeric materials of the patches, appeared in the infrared (IR) figures 2.On the other hand, the DSC curves of phenytoin sodium (figures 3 to 5) showed a single exothermic peak at about 328.7C^0 corresponding to melting of the drug. Such peak remains in the physical

mixture indicating that presences of polymer increased the exothermic characters which in turn mean increasing the stability of the drug (salt) by retarding its conversion to the insoluble form (which it's DSC endothermic).

The data of the in-vitro release of the formulations were analyzed according to different kinetic mechanisms table 4.The results revealed that the release of phenytoin sodium from F1, F2 and F5 follow Higuchi kinetic model while from F3 and F4 follow first order model. However F1 was selected for in vivo evaluation on the basis of both its drug release content and release pattern.

As shown in table 5, the mean percentage reduction in ulcer's volume at the end of 1 week was higher in phenytoin patch and powder treated groups (19.1±1.02 and 16.6±0.89 respectively) than saline group (10.9 ± 1.08) (P< 0.001 for each). Compared with powder, Phenytoin patch showed marked effect and increase in ulcer's volume reduction

At the end of the second, third and fourth weeks, the mean percentage reduction in ulcer's volume in the PHT patch group were (49.9 ± 3.63, 73.4±4.04 and 90.1±2.51), respectively which is significantly higher than in phenytoin powder-treated patients (38.8±2.29, 62.7±2.51 and81.1 ± 2.8). P <0.01 for each and the control group (25.6 ±1.78, 45.8 ±2.59 and 64.3 ±3.21). All patients treated with Phenytoin powder reported irritation after application of the powder, whereas no irritation reported in the other groups

Phenytoin ointments and creams

Drug Content Studies:

Phenytoin content of the prepared formulations was determined and found to be 99.876 % ± 0.1 of the claimed amount (2%w/w).

In vitro Release Studies:

Phenytoin release from different ointments and creams are illustrated in figures (7-10). It was observed that the amount released increases with the time of release. It is also observed that oleaginous base formed of white petrolatum and beeswax or anhydrous lanoline (F1 & F2) exhibits the lowest phenytoin release than other formulations. This may due to the stiffening property of beeswax [29] and the higher affinity of phenytoin towards these vehicles. Ezzedeen et al. [30] have studied the release of scorbic acid from an oleaginous ointment base, they observed a lower release of the drug from the base and they attributed this finding to the higher affinity of scorbic acid to the oleaginous base.

Absorption base containing cetyl alcohol exhibited higher phenytoin release than that containing stearyl alcohol (F4 > F3) .This may be due to the reason that stearyl alcohol increases the stiffness of the prepared formulation. However, a lower release rate of both oleaginous and absorption bases may be attributed to the hydrophobic nature of those bases, which render them immiscible with diffusion medium and thus attaining poor release rate of the medicament [31]. The release of phenytoin from water-soluble ointment base F5 was found to be higher than from other formulations figure (8) this may be due to the hydrophilic nature of the base.

On the other hand, formulation ns denoted F8, F9 and F10 are cold cream bases containing beeswax-borax system as emulsifying agent. Figure 9 shows the effect of different concentrations of borax (1.2, 1 and 0.8 % w/w) on the release pattern of phenytoin cream .it is obvious that release of phenytoin increases as the borax concentration increases.

On comparing the release of phenytoin from emulsion-based ointment as shown in figure 10, it could be ranked in the following descending order: F11 > F13 > F12 respectively. However, emulsion base containing low stearyl alcohol content (F11) has higher phenytoin release rate than that of high stearyl alcohol content (F6). Among ointment bases, it is evident from the results that, the emulsified ointment bases exhibited higher release values than either the absorption or the oleaginous base. The difference in the release behavior from ointment bases may be attributed to the type and composition of the base rather than the viscosity of the base.

Viscosity Measurements:

Table 5 , depicts the value of the viscosity of the formulations. The result revealed that F11 has the lowest value of viscosity than other formulations.

In the light of the pervious results F11 was selected for further evaluation since it has low viscosity, easy to remove from the skin, creamy in nature and higher release rate of the tested drug..

Kinetic Study:

The data of the in-vitro release of the selected formula F11 was analyzed according to different kinetic mechanisms and the results listed in table (6).The result revealed that the release of phenytoin from selected formula F11 follow Higuchi kinetic

Clinical evaluations

The final study cohort was made up of 77 patients only undergoing microdermabrasion for treatment of post acne scar, 30 patients were received PHT cream plus microdermabrasion, 22 patients were received placebo cream plus microdermabrasion and 25 patients were treated with microdermabrasion alone.

The characteristics of the patients (age, sex) and the scar duration are summarized in tables 7.

The result percentage at the end of treatment had shown in table 8 and figures 11 & 12.

The results revealed that, an excellent percentage was observed in phenytoin ointment treated group (40% &26.67 respectively) than microdermabrasion group (32% &16 % respectively) and placebo treated group (27.27% &18.18 respectively). The poor percentage was found in microdermabrasion and placebo group (52%

&54.55 % respectively) than phenytoin treated group (33.33%) figure (11).

From the statistical point of view it is clear that, the difference between phenytoin treated group and other groups (Microdermabrasion alone p = 0.01 and Placebo cream plus microdermabrasion p =0.01) was statistically significant (P < 0.05) while the difference between microdermabrasion and placebo group was statistically not significant p = 0.7 (P > 0.05). There is no any detectable phenytoin found in all blood drawn samples which mean no notable systemic absorption occurred.

Figure (14) showed photographs of scars at base line and at the end of treatment. The faster resolution of acne lesions and decreasing inflammations by phenytoin ointment can be attributed to the ability of phenytoin to inhibiting collagenase activity, promoting collagen disposition, enhancing granulation tissue formation and decreasing bacterial contamination [32-34].

No adverse effects, either local or systemic, observed in our patients along the study period.

CONCLUSION

From this study we conclude that introduction of dermal patch of phenytoin is valuable in achieving prominent healing of chronic diabetic ulcers. Phenytoin patch PHT is more effective, safe, easy to use and inexpensive comparison with other traditional treatment used. Moreover, the clinical investigation proved that therapy of acne with phenytoin ointment was successful and produced high efficacy in improving acne lesion of the patients, in addition of reducing inflammation.

REFERENCES

1. Bhatia A. and Prakash S **:** Topical phenytoin for wound healing,. Dermatol. Online J. 04; 10: 5.
2. Jensen TS. "Anticonvulsants in neuropathic pain: rationale and clinical evidence." Eur. J. Pain.,6 Suppl .2002; A: 61-8.
3. Rhodes R, Heyneman C, Wilson S and Phatak H. Topical phenytoin treatment of stage II decubitus ulcers in the elderly., Ann. Pharm. 2001;35:675-681.
4. Malhotra Y. and Atnin S :Role of topical phenytoin in trophic ulcers of leprosy in India., Int. J. Lepr. 1991; 59: 337-338.
5. Bhatia A. and Prakash **S.** "Topical phenytoin for wound healing". Dermatol. Online J. 10: 5 (2004).
6. Kitson N and MaddinS: Drugs used for skin diseases, In: Roberts MS,Walters KA, eds. Dermal absorption and toxicity assessment, Marcel Dekker, New York, USA;1998: PP313-326.
7. Wallters k. "Dermatological and transdermal formulation." Chapter 7: Dermatological formulation and transdermal systems. Marcel Dekker New York, USA. 2002: pp. 3-4.
8. Yutaka I, Kayoko F, Rikimaru M, Isamu M, Ikuo K: Assessment of the physical properties and stability of mixtures of tetracycline hydrochloride ointment and acyclovir cream. Int J. Pharm., 2013; 447:158-164.
9. Vijay V, Rajesh K, Kavitha Ka and Satyavani K: Cream of ulcers., Indian. J. Med. Res., 2011;134 (2): 168-173.
10. Ansel C, Popovich G and Allen **V:**"Pharmaceutical dosage forms and drug delivery systems ". Sixth Ed. William and Wilkins, London, England, 1995:p.379.
11. Padula C, Colombo G, Nicoli S, Catellani L, Massimo G and Santi P: Bioadhesive film for the transdermal delivery of lidocaine: in vitro and in vivo behavior, J. Cont. R. 2003;88 : 277–285.
12. Desai S, Simonelli A and Higuchi W :Investigation of factors influencing release of solid drug dispersed in inert matrices., J. pharm. Sci. 1965;54: 1459-1564.
13. Gannu R, Vishnu Y, Kishan V and Madhusudan Y**:** Development of nitrendipine patches: in vitro and ex vivo characterization. Current D. Delivery. 2007; 4: 69-76.
14. Sweetman S: Martindale the complete drug references. 36th Edition, Pharmaceutical press, London, England .2009: p.495.
15. International Working Group on the Diabetic Foot. "International Consensus on the Diabetic Foot." Amsterdam, 1999.
16. Dumville JC, O'Meara S, Deshpande S and Speak K: Hydrogel dressings for healing diabetic foot ulcers (review)., The Cobrane Library. 2013: Issue 7.
17. EL-Sayed AM, Essam EL-din M, EL-Mady MM and Saddik M Sh: Clinical efficacy of novel phentoin patches for treating chronic diabetic ulcers., Egypt. J. Derm. and Venereol.2010;30(2):26-30
18. Yuan-Sung K, Hiung-Fei and Willium L: Plectranthus amboinicus and centella asiatica cream for the treatment of diabetic foot ulcers. Complementary and alternative medicine. 2012: page 9.
19. Kucharzewski M,Kozka M and Urbanek T: Topical treatment of nonhealing venous leg ulcers with propolis ointment.,Complementary and alternative medicine. 2012: page 9.
20. Rivera A. and Kirksville D: Missouri- Acne scarring: A review and current treatment modalities., J. Amr. Acad. Dermatol.2008; 59:659-76.
21. Bayat A., McGrother D and Ferguson J: Skin scarring., BM J. 2003; 32: 6:88.
22. Gannu R., Vishnu Y., Kishan V. and Madhusudan Y: Development of nitrendipine patches: in

vitro and ex vivo characterization., Current D. Delivery.2007; 4: 69-76.
23. The united states pharmacopeia: the National formulary", the United states pharmacopeial convention., Rockville, USA. 1995; p: 2283.
24. Özsoy Y, Güngör S. and Cevher E: Vehicle effects on in vitro release of tiaprofenic acid from different topical formulations II farmaco.2004; 59:563-566.
25. Mura P, Corti P and Manderioli A: Influence of vehicle composition variations on the in vitro and ex vivo clonazepam diffusion from hydrophilic ointment bases., Pharm. Acta. Helv. 1996 ;71:147-154.
26. Martin A, Swarbrick J, and Cammarata A:Physical pharmacy" 4th ed. Lea Febiger, Philadelphia, USA. 1993; 224:358 ,403 and 584.
27. Higuchi W :Analysis of data on the medicament release from ointments" J. Pharm. Sci.1962; 51:802.
28. Borodkin S, Flourence A. and Tucker F : Drug release from hydroxypropyl cellulose-polyvinyl acetate films." J. Pharm. Sci.1974; 63: 1359-1364.
29. Gennaro A: Remington's Pharmaceutical Sciences" 18th ed. Mack publishing Co., Easton, USA.1990; p.1399.
30. Ezedeen F, Shihab F and Stohs S: Release of scorbic acid from ointment bases. Int. J. Pharm.1986; 28:113-117.
31. Ezedeen F, Shihab F and Hussain E: Percutaneous diffusion of cefalexin, sulfamethoxazole and diphenhydramine from ointments. Pharmazie 1990; 45:512-514.
32. Kuru L, Yilmaz S and Kose K: Expression of growth factors in the gingival crevice fluid of patients with phenytoin-induced gingival enlargement., Arch. Oral Biol. 2004; 49: 945-950.
33. DaCosta M, Regan M, Al Sader M, Leader M. and Bouchier-Hayes D. :Diphenylhydantoin sodium promotes early and marked angiogenesis and results in increased collagen deposition and tensile strength in healing wounds: Surgery 1998;123(3): 87-93.
34. Kato T, Okahashi N, Kawai S, Kato T, Inaba H, Morisaki I. and Amano A. :Impaired degradation of matrix collagen in human gingival fibroblasts by the antiepileptic drug phenytoin.,J. Periodontol. 2005;76(6):941-50.

Source of support: Nil, Conflict of interest: None Declared