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Permeability Enhancement of Methotrexate Transdermal Gel using Eucalyptus oil, Peppermint Oil and Olive Oil(Conference Paper)[#]

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Abstract

The idea of this study is to improve transdermal permeability of Methotrexate using eucalyptus oil, olive oil and peppermint oil as enhancers.

Eucalyptus oil (2% and 4%), peppermint oil (2% and 4%) and olive oil (2% and 4%) v/v all used as natural enhancers to improve transdermal permeability of Methotrexate via gel formulation. The gel was subjected to many physiochemical properties tests. *In-vitro* release and permeability studies for the drug were done by Franz cell diffusion across synthetic membrane, kinetic model was studied via korsmeyer-peppas equation.

The results demonstrate that safe, nonirritant or cause necrosis to rats' skin and stable till 60 days gel was successfully formulated. Methotrexate permeability without enhancer was only about 20%, while with enhancers, it reached to 85%, 99% and 90% with eucalyptus oil 4(v/v %), peppermint oil 4(v/v %) and olive oil 4(v/v %) respectively after 24 hours.

Methotrexate transdermal gel was prepared and evaluated fruitfully *in-vitro* with a good permeation across semipermeable membrane. The results indicated that using of peppermint oil as enhancer has superiority to enhance the transdermal permeation of the Methotrexate.

Keywords: Permeability, Methotrexate gel, Eucalyptus oil, Peppermint oil and olive oil.

تعزيز نفاذية جل الميثوتريكسات عبر الجلد باستخدام زيت الكافور وزيت النعناع وزيت الزيتون (بحث مؤتمر)[#]

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الخلاصة

فكرة هذه الدراسة لتحسين نفاذية دواء الميثوتريكسات جل عبر الجلد باستخدام زيت الأوكالبتوس وزيت الزيتون وزيت النعناع كمعززات للنفاذية.

زيت الأوكالبتوس (٢٪ و ٤٪) ، زيت النعناع (٢٪ و ٤٪) وزيت الزيتون (٢٪ و ٤٪) كسوائل مذيبة في سوائل (ح/ح) جميعها طبيعية تستخدم كمعززات لتحسين نفاذية دواء الميثوتريكسات عبر الجلد. خضع الجل للعديد من اختبارات الخصائص الفيزيائية والكيميائية. تم إجراء تجارب الحركية والنفاذية في المختبر للدواء عن طريق خلية فرانز عبر الغشاء الاصطناعي ، ودُرس النموذج الحركي عبر معادلة كورسمير-بيبباس. أظهرت النتائج أن المادة الجيلاتينية آمنة وغير مهيجة أو تسبب نخرًا لجلد الفئران ومستقرة حتى ٦٠ يومًا. كانت نفاذية دواء الميثوتريكسات بدون مُحسِّن للنفاذية حوالي ٢٠٪ فقط ، بينما وصلت مع المُحسِنات إلى ٨٥٪ و ٩٩٪ و ٩٠٪ بزيت الأوكالبتوس ٤ (ح/ح ٪ (وزيت النعناع ٤ (ح/ح ٪) وزيت الزيتون ٤ (ح/ح ٪) على التوالي بعد ٢٤ ساعة.

تم وضع جل الميثوتريكسات عبر الجلد وتقييمه في المختبر مع نفاذ جيد عبر الغشاء شبه القابل للنفاذ. أشارت النتائج إلى أن استخدام زيت النعناع كمحسن له تفوق في تعزيز نفاذ دواء الميثوتريكسات عبر الجلد.

الكلمات المفتاحية : نفاذية ، جل الميثوتريكسات ، زيت الأوكالبتوس ، زيت النعناع وزيت الزيتون.

Introduction

Methotrexate (MTX) is an anti-metabolite that is a synthetic chemical analogue of folic acid and is commonly prescribed to treat psoriasis. Although it is authorized for the treatment of mild / moderate psoriasis with oral and parenteral dosing, long-term usage causes diarrhea, mucosal ulcers, stomatitis, bone marrow suppression, hepatic fibrosis, and cirrhosis. As a result, patients might benefit from topical MTX treatment to prevent its serious side effects. Unfortunately , current commercial topical MTX formulations have limited penetration into the stratum corneum, which has been linked to its hydrophilicity and significant dissociation at physiological pH.[1, 2].

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Transdermal medication delivery is a method of delivering a drug formula via the skin for systemic absorption.

It is completely painless technique that begins with drug penetration from the surface of the skin (stratum coronium) to the deeper layers: epidermis and dermis, to be available for systemic absorption. [3]

Transdermal drug delivery system offers several advantages include self-administered and consistent, sustained release of drug, and with steady plasma levels throughout the entire time of administration, hepatic first pass effect and enzymatic degradation of gastrointestinal tract (GIT) is avoided without GIT side effects, and it can be used as an alternate route for patients who are unable to take their pills orally, furthermore, the medication administration terminates with the formula being removed from the surface of skin. [4, 5]

Gel is a semi-rigid substance made up of at least two components: a base, which is a smoothly flexible liquid in which the dispersion medium (water) is entirely absorbed. The combination of these two components is what gives the gel its unique properties. [6] Penetration enhancers can improve the drug's penetration through the skin. Permeation enhancers come in a variety of forms, the majority of which are surfactants that can harm cells. Natural enhancers, such as oils and fatty acids, are favored since they are not dangerous to cells like surface active agents and may not interfere with calibration techniques.[7]

Several physical and chemical techniques have been employed to improve MTX skin permeability, like iontophoresis, liposomes, chemical enhancers as well as microneedles. [8] These methods had limited success and some imperfections remain.

In this work, MTX transdermal permeability was enhanced by adding and increasing the content of eucalyptus, peppermint and olive oils by forming the product as a gel using (Carbopol 940) as gelling agent.

Materials and Methods

Materials

MTX was bought from YIBAI biotechnology (China), Carbopol 940 was purchased from Sigma ch. (USA), Eucalyptus, Peppermint and Olive oils were provided from BAR-SUR-LOUP (France), Triethanolamine (TEA) was bought from Merck (Germany). Franz cell was purchased from SES GmbH (Germany).

Methods

Preparation of methotrexate gel

The gel was prepared by dissolving the gelling agent 2 (w/v) % of Carbopol 940 in deionized water by stirring until a homogeneous mixture was obtained. Then, the MTX was dissolved in mixture of (1 ml dimethyl sulfoxide and 4ml acetone) and this mixture was left for about 10 min to ensure that completely dissolved in the solution. The MTX solution was added step by step to the homogenous mixture of Carbopol 940 [9] .

An enhancer (Eucalyptus, Peppermint and Olive oils) with different concentration was added to six different formulas, for pH adjustment [10] a solution of triethanolamine (TEA) was added in drops and mixed separately with each formula. Eventually, the final volume was made up to 25 ml by adding the sufficient quantity of deionized water, with stirring until a homogeneous gel was made. Different concentration of each component explained in Table 1.

Table 1. Formulation of MTX 0.1% (w/v) transdermal gel.

Formulas	MTX (w/v) %	Carbopol 940 (w/v) %	TEA (v/v) %	Enhancer			Deionized water
				Eucalyptus oil(v/v) %	Peppermint oil(v/v) %	Olive oil(v/v) %	
F1	0.1	2	1	0	0	0	Up to 25ml
F2	0.1	2	1	2	0	0	Up to 25ml
F3	0.1	2	1	4	0	0	Up to 25ml
F4	0.1	2	1	0	2	0	Up to 25ml
F5	0.1	2	1	0	4	0	Up to 25ml
F6	0.1	2	1	0	0	2	Up to 25ml
F7	0.1	2	1	0	0	4	Up to 25ml

Evaluations of methotrexate gel

Organoleptic characteristics

The organoleptic properties of the MTX gel including liquefaction, color, phase separation and homogeneity were investigated by visual inspection at different intervals of 1st, 7th, 14th,21th, 28th, and 60th days. [9]

Skin irritation test

This test was done by applying sufficient quantity of the gel to small region of rats' skin under supervision (at time of application and after one hour) for any hypersensitivity reaction in the skin (redness, irritation) or any necrosis could be made

by the gel, the number of rats has been used are seven. [10]

Changes in pH

The pH of different formulas was measured using pH meter device at 1st, 7th, 14th, 21th, 28th, and 60th days after formulation. [11]

Drug content

A gel amount of 50 mg from each produced formula was taken, then dissolved with 50 ml of pH 7.4 phosphate buffer with stirring until a completely soluble mixture obtained. The mixture then filtered with syringe filter. The concentration of MTX was measured spectrophotometrically by using equation of the drug calibration at 303nm. [12]

Viscosity

The viscosity of the formulated gel was determined by using Brookfield . Viscometer at 37 °C. The apparatus spindle was rotated at 10 rpm. [13]

***In vitro* drug permeability and release kinetic study**

All formulas permeation were studied by using Franz diffusion cell. The donor compartment was supplied by 1gm of MTX gel and the receptor compartment with 100ml of phosphate buffer PH 7.4. The donor and receptor parts separated by synthetic semipermeable membrane (MW 8000 Da). The media was stirred at 100 rpm and the temperature kept at 37 C. 5ml sample of MTX gel was withdrawn from the receptor which was replaced by the same volume of buffer to maintain the sink condition. The quantity of MTX was determined spectrophotometrically at 303nm by using the phosphate buffer as the blank. The release of the drug determined by applying the result data on korsmeyer peppas model kinetic. [14] In vitro the release mechanism of MTX determined by using the following model equation

$Mt/ M\infty = K*t^n$

Where $Mt/M\infty$ is the fractional MTX re
lease from the gel into the receptor media, K is a drug delivery constant whereas (n) is diffusion coefficient and its value indicates the mechanism of the drug release in the solvent. The (n) value of 0.5 that indicates Quasi- Fickian diffusion mechanism, while if (n>0.5) then anomalous or non-Fickian diffusion mechanism exists and if it is (=1) then the Zero order release one exists. [15]

Statistical analysis

One-way analysis of variance (ANOVA) was applied in this research and if $p<0.05$ the difference was considered statistically significant.

Results and Discussion

The general properties of all prepared formulas were good from preparation till two months, so the homogeneity of all formulas was acceptable with no particles was made. The color of all formulas was yellow (attributed to MTX color) and without change in color till two months. [16] The formulas were stable without liquefaction at 2% of Carbopol 940 so this indicates a good percentage of gelling agent. No phase separation was made at all time to the all formulas.

The more important character which was skin irritation (Irritancy test), redness and necrosis not observed at rats' skin after apply sufficient quantity of MTX gel [17]. The drug content data obtained was shown in Table 2. The drug content was between 95.2 % to 97.1%. This indicates the good content of the drug formulas and good uniformity of the preparation.

The viscosity of the preparations was decreased with increasing the concentration of the enhancer, that are between 17112 cp to 18655 cp. as shown in the Table 2 and Figure 1 [18].

Table 2. Organoleptic properties and skin irritation test of MTX transdermal gel

No.	Parameters	F1	F2	F3	F4	F5	F6	F7
1	Color	yellow	yellow	yellow	yellow	yellow	yellow	yellow
2	liquefaction	No	No	No	No	No	No	No
3	Phase separation	No	No	No	No	No	No	No
4	Homogeneity	Good	Good	Good	Good	Good	Good	Good
5	Skin irritation test	No	No	No	No	No	No	No
6	Drug content	99.1% ±1.2	95.6% ±1	96.1% ±2.1	96.3% ±2.1	97.1% ±1.3	95.2% ±1.5	95.6% ±1.9
7	Viscosity	19775 ±43	18655 ±36	17323 ±22	17745 ±45	17112 ±18	18221 ±29	17342 ±32

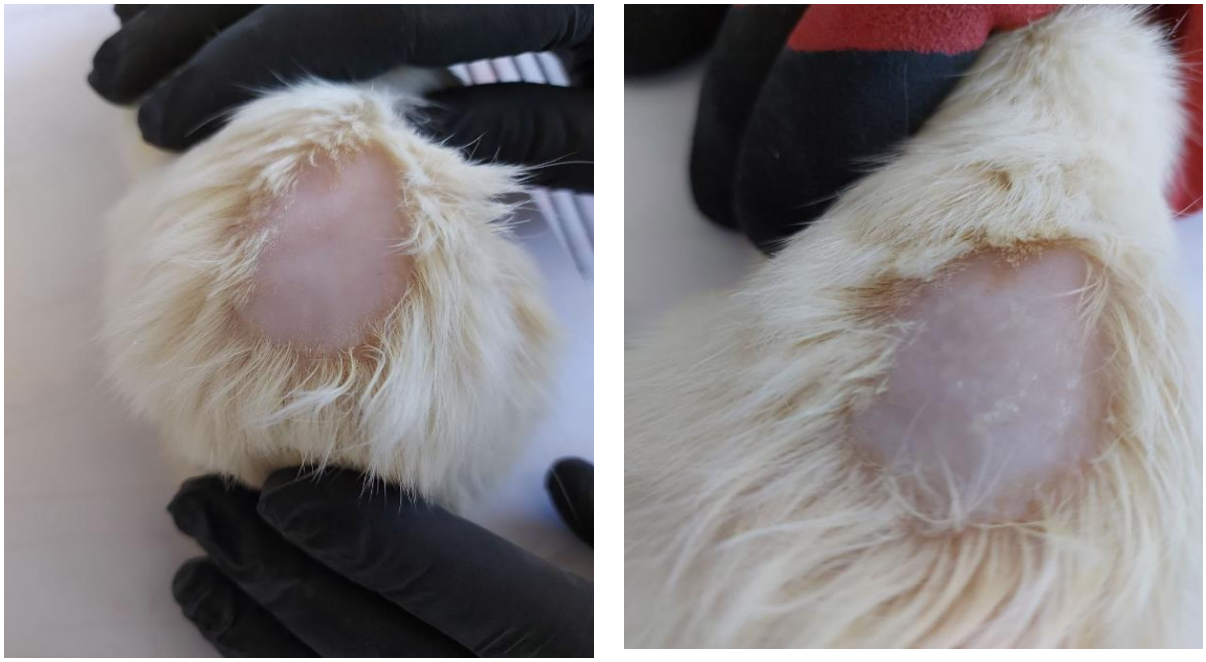

Figure. 1 rats' skin at time of application (left) and after one hour (right) no redness, irritation or any necrosis could be seen after application of MTX gel.

Changes in pH

The pH of all formulas are within the normal range of skin till to 60 days, so no irritation or burning sensation to the skin could be occurred. Figure 2 shows pH as Y axis and duration of storage as X axis. [19]

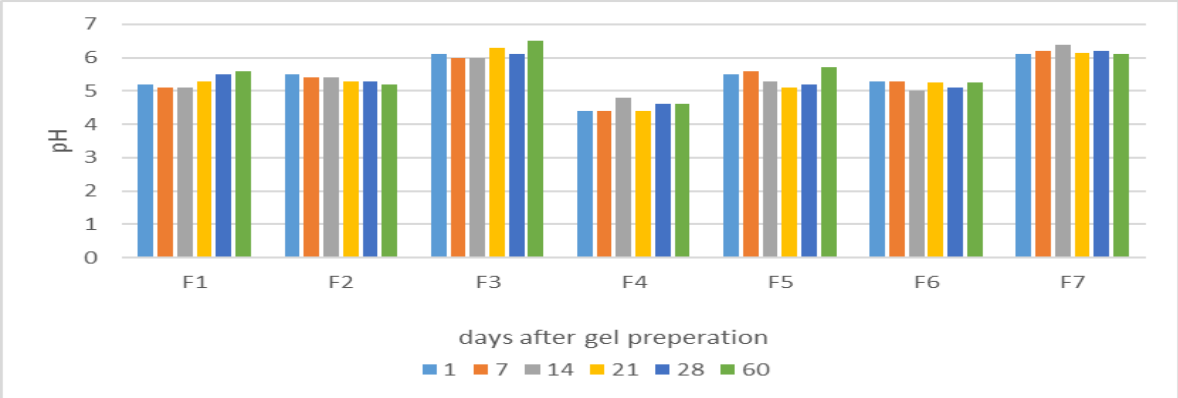

Figure.2 pH change during 60 days after formulation. All formulas represented by pH±SD, where the F1 gel without enhancer, where other formulas with enhancer.

In vitro MTX permeability and release kinetic study

The permeability and release of the MTX study by using Franz cell membrane explained in figure 3, according to the results; using the gel without enhancer had poor (20%) permeability after 24 hours, the addition of eucalyptus oil as enhancer at 2% and 4% (F2, F3) respectively, result in significantly ($p<0.05$) increasing the permeability to 79% and 85% respectively after 24hr, the increasing in permeability due to increasing in enhancer concentration. In formulas with peppermint oil (F4 and F5) the permeability is 92% and 99% with increasing in concentration of enhancer from 2% to 4% respectively. In the F6 and F7 which had olive oil as enhancer with concentration 2% and 4% the permeability reaches 80% and 90% respectively. So, from the data, the permeability of the MTX gel was maximum with peppermint oil due to the solubility of the MTX is higher than in eucalyptus oil and in olive oil. The suggested mechanism for the oils to work as penetration enhancers is either disintegration of the intracellular highly ordered lipid structure or induce conformational modification in the intracellular proteins or might be due to increasing the partitioning of MTX into stratum cornium. [20]

Carbopol 940 was used to control the release of MTX up to 24hr as it acts as a controlling rate polymer. So, according to krosmeyer-peppas model, the kinetic release of drug was obtained. And the mechanism of release was non-Fickain and super case II diffusion which is demonstrated in figure 3. (21)

Table 3. Korsmmeyer-peppas kinetic model for MTX transdermal gel through Synthetic membrane

formulas	R^2	n-value	Type of release
F1	0.9594	0.74	Non-fickain diffusion
F2	0.876	2.75	Super case-II transports
F3	0.8906	2.97	Super case-II transports
F4	0.9023	3.57	Super case-II transports
F5	0.8879	3.98	Super case-II transports
F6	0.8945	2.89	Super case-II transports
F7	0.9159	3.26	Super case-II transports

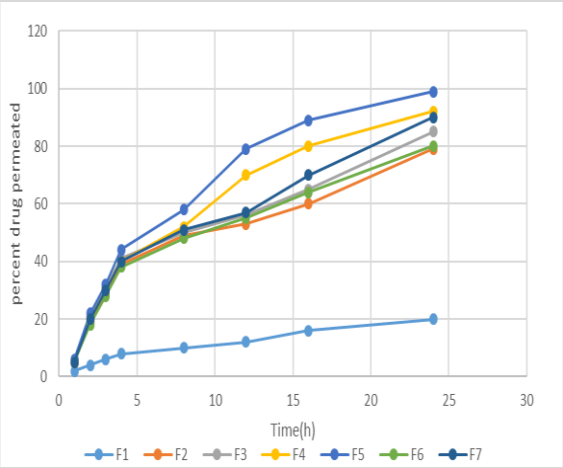

Figure3.Percent of MTX permeated across synthetic semipermeable membrane at pH 7.4 buffer solution, where n=3.

Conclusion

Methotrexate gel was successfully prepared and evaluated *in-vitro* with a good permeation across the synthetic membrane. The previous results showed the using of the peppermint oil as permeation enhancer at 4(v/v) % concentration give worthy transdermal gel of MTX with respectable permeation across semipermeable membrane consequently for stratum corneum. Moreover, the formula had good drug content, viscosity, pH as an end result suitable for the skin.

References

1. Murtadha S. Jabur, Mohammed R. I. Ghazal, Hayder R.Al-Hamamy. Topical Methotrexate for Treatment of Psoriasis: Formulation and Clinical Implications. Fac Med Baghdad 2010; Vol. 52, No. 1
2. Gillian S. Leslie Singka, Nor Abu Samah, Mohd H. Zulfakar, Aysu Yurdasiper, Charles M. Heard. Enhanced topical delivery and anti-inflammatory activity of methotrexate from an activated nanogel. European Journal of Pharmaceutics and Biopharmaceutics 76 (2010) 275–281
3. Nawaz A, Jan SU, Khan NR, Hussain A, Khan GM. Formulation and in vitro evaluation of clotrimazole gel containing almond oil and Tween 80 as penetration enhancer for topical application. Pak J Pharm Sci. 2013;26(3):617-22.
4. M A-H. Enhancement effect of almond oil on transdermal penetration of flurbiprofen topical gel. Journal of Kerbala University. 2015;13:160.
5. Jawad A AT, Jiyauddin k, S.Budiasih, M kaleemullah, samer A.D, Eddy Y, and fadi A. fabrication and evaluation of a stable flurbiprofen hydrogel. IJPAR journal. 2014;3(3):284-90.
6. Corbo M, Schultz T, Wong G, Van Buskirk G. Development and validation of in vitro release testing methods for semisolid formulations. Pharmaceutical Technology. 1993;17(9):112-.
7. Finnin BC, Morgan TM. Transdermal penetration enhancers: applications, limitations and potential. J Pharm Sci 1999;88:955-8.
8. Zhao YC, Zheng HL, Wang XR, Zheng XL, Chen Y, Fei WD, Zheng YQ, Wang WX, Zheng CH. Enhanced Percutaneous Delivery of Methotrexate Using Micelles Prepared with Novel Cationic Amphipathic Material. Int J Nanomedicine. 2020;15:3539-3550
9. Helal DA, El-Rhman DA, Abdel-Halim SA, El-Nabarawi MA. Formulation and evaluation of fluconazole topical gel. Int J Pharm Pharm Sci. 2012;4(5):176-83.
10. Alaayedi M, Mahmod, Hasanian, Saeed, Ashti. The enhancement effect of castor oil on the permeability of flurbiprofen as transdermal gel. International Journal of Applied Pharmaceutics. 2018;10(1):140-4.
11. Maryam H. Alaayedi, et al. (2017). "Effect of Hydroxy Propyl Methyl Cellulose (HPMC) on Amoxicillin Floating Tablet." karbala journal of pharmaceutical sciences(13): 311-319.
12. Güngör S, Bergişadi N. Effect of penetration enhancers on in vitro percutaneous penetration of nimesulide through rat skin. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2004;59(1):39-41.

13. Hasanain Sh. Mahmood et al / The Enhancement Effect of Olive and Almond oils on Permeability of Nimesulide as Transdermal Gel. International Journal of Pharmaceutical Research Oct - Dec 2019 Vol 11.
14. Hirva S, Jenisha P. Bicelle: a lipid nanostructure for transdermal delivery. J Crit Rev 2016;3:17-22.
15. Keleb E, Sharma RK, Mosa EB, Aljahwi AZ. Transdermal drug delivery system-designand evaluation. Int J Adv Pharm Sci 2010;1:201-11.
16. Li DX, Han MJ, Balakrishnan P, Yan YD, Oh DH. Enhanced oral bioavailability of flurbiprofen by combined use of micelle solution and inclusion compound. Arch Pharm Rev 2010;33:95-101.
17. Syed NHS, Mahboob R, Muhammad FA. Study of percutaneous absorption of diclofenac diethylamine in the presence of cetrimide through hairless rabbit skin. J Res Sci 2006;17:45-51.
18. Rambharose S, Kalhapure RS, Jadhav M, Govender T. Exploring unsaturated fatty acid cholesteryl esters as transdermal permeation enhancers. Drug Delivery Transl Res 2017;7:333–45.
19. Hussain A, Khan GM, Shah SU, Rahim N. Development of a novel ketoprofen transdermal patch: effect of almond oil as penetration enhancer on *in vivo* and ex-vivo penetration of ketoprofen through rabbit skin. Pak J Pharm Sci 2012;25:227-32.
20. Qiudong Jiang, Yeming Wu, Hui Zhang, Pei Liu, Junhong Yao, PeijunYao, Jun Chen & Jinao Duan .Development of essential oils as skin permeation enhancers: penetration enhancement effect and mechanism of action, Pharmaceutical Biology. 2017; 55:1, 1592-1600.
21. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-23.22.