

Topical preparations from the Iraqi plant aloe vera and their efficacy in skin infections.

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الخلاصة

ان المستحضرات الجلدية عادة تتكون من مادة او عدة مواد مذابة او منتشرة في وسط معين . ان نبات الصير (Aloe vera) ينتشر بكثرة في العراق وهو علاوة على استعماله التقليدي في معالجة الامساك فقد استعمل مسبقا في معالجة الجروح و الحروق وداء الثعلبية . تمت في هذه الدراسة تحديد كمية الـ (aloin) الذي هو المادة الرئيسية الفعالة في النبات المذكور و اتضح ان كميته تساوي 15,4 % من العصير الجاف المستخلص من نبات الصير العراقي . كذلك تمت في هذه الدراسة استخدام قواعد دهنية مختلفة لتحضير مستحضرات جلدية من العصير الجاف للنبات وبتراكيز مختلفة ومقارنتها مع بعضها بطريقة النضوح من خلال الجلد لاختبار الافضل منها و اتضح ان قاعدة الهلام المحضرة باستخدام Sodium carboxy methyl cellulose وبنسبة 4% هي افضل صيغة تركيبية محضرة . وكذلك تم اجراء دراسة سريرية للمستحضرات الجلدية المختلفة المحضرة على 61 مريضا مصابا بالتهابات جلدية تسببها جراثيم Tinea corporis و Tinea cruris واثبتت النتائج ان قاعدة الهلام المحضرة من العصير الجاف لنبات الصير العراقي وبتراكيز 4% اعطت نسبة شفاء عالية مقارنة مع نفس الالتهابات الجلدية المعالجة باستخدام nystatin و hydrocortison وان هذا المستحضر له فعالية مضادة للحكة و مضادة للالتهابات و كذلك خالي من اية اعراض جانبية . واخيرا تم في هذا البحث تحديد فترة انتهاء المفعول للمستحضر المختار ووجد انها مساوية سنتان تقريبا.

ABSTRACT

The dermatological preparations consist of simple or compounded bases in which one or more active ingredients are dissolved or suspended .The plant Aloe vera is widely distributed in Iraq, in addition to it's traditional use in the treatment of occasional constipation , it is also used to assist healing of wounds ,burns and psoriasis. In this study; the amount of aloin (the major component of the plant) in the dried juice of the Iraqi plant Aloe vera is determined and was found to be 15.4% w/w and it is the major compound responsible for the antimicrobial activity of the plant.

Different ointment bases were used in this study to prepare topical preparations from the dried juice of the Iraqi plant with different concentrations and were found that 4%w/w sodium carboxy methyl cellulose gel base was the best formula using comparative diffusion study through the skin. The clinical study which is carried out in this research on 61 patients with skin infections (mainly Tinea corporis and Tinea cruris) showed that 4% w/w gel base formula of aloe gave significant improvement in comparison with those treated with nystatin and hydrocortison and proved the antipruritic and anti-inflammatory action of the dried juice of the Iraqi plant Aloe vera without any adverse effect. Finally the expiration date of aloin in the selected formula is determined and was found to be \approx 2 years.

INTRODUCTION:

The skin route of drug administration has many advantages over other routes of administration and can be used for local and systemic action^(1,2).

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The dermatological preparations consist of simple or compounded bases in which usually one or more active ingredients are dissolved or dispersed and may have hydrophilic or hydrophobic properties⁽³⁾. Aloe is the dried juice of the leaves of Aloe vera (L) Brum. F. of the family Liliaceae^(4,5). In addition to the medicinal oral use of the powdered dried juice preparations for short- term treatment of occasional constipation⁽⁶⁾, it is also used in ointment and creams to assist the healing of wounds, burns, eczema and psoriasis. Aloe vera exhibited an anesthetic action, antibacterial action and increased the local micro-circulation⁽⁷⁾. The major active principles of aloe are hydroxyanthrone derivatives mainly of the aloe- emodin-10-C-glucoside and known as barbaloin (aloin) which is in fact a mixture of the two isomers aloin A and B⁽⁸⁾.

Determining the amount of aloin in the dried juice of Iraqi Aloe vera and preliminary study of its antimicrobial activity is carried out in this study.

Also formulation and percutaneous absorption of some topical preparations of aloe using different types of ointment bases and different concentrations is done, in addition to clinical effectiveness study of the selected formula in the treatment of some skin infections mainly Tinea corporis and Tinea cruris and finally, the expiration date of the prepared formula is established. All these studies in a hope to prepare an effective and safe topical preparation from the dried juice of the Iraqi plant Aloe vera that can be used successfully in the treatment of some skin infections.

EXPERIMENTAL:

Methods:

1- Plant Material:

4.6 Kg of the leaves of Iraqi Aloe vera is collected and the dried juice is obtained by making incision in the rind of the leaves and the sap material was let to drop down, collected, dried and powdered to give 500gm residue.

2- Assay of Aloin in the Dried Juice:

The presence of aloin in the dried juice is determined by TLC using silica gel G and solvent system (ethyl acetate : methanol: water (5 :0.85 :0.65) and detected by U.V. at 365nm after spraying with 10% methanolic potassium hydroxide⁽⁹⁾, using reference sample of aloin. Quantitative analysis of total anthracene calculated as aloin is done by dissolving 100mg of the dried juice in 80% aqueous methanol and then analyzing aloin spectrophotometrically using U.V. spectrophotometer at 365nm by comparison with previously prepared calibration curve of reference pure aloin in 80% aqueous methanol⁽¹⁰⁾.

3- Preparation of Ointments:

Fusion method was the general method employed for the preparation of the ointment bases. The drug was incorporated by trituration. Three types of ointment bases were selected to study their effect on the diffusion of aloin using four different concentrations 1,2,3 and 4% w/w of aloe (dried juice)^(14,15). The bases were:

- a- Water-in-oil emulsion base (wool fat and water).
- b- Oil-in-water emulsion base (white bees wax, cetylalcohol, propylene glycol, sodium lauryl sulphate and water).
- c- Sodium carboxy methyl cellulose gel base (sodium carboxy methyl cellulose, glycerol and water).

4- Preparation of Mouse Skin:

The full thickness skin from the abdominal surface of male mouse 8–10 weeks old was taken, prepared, defatted and frozen until use⁽¹⁶⁾.

5- In Vitro Membrane Diffusion:

Two grams of each ointment base prepared above containing 1,2,3 and 4% w/w of aloe each was introduced into test tube with 1.4cm in diameter (used as diffusion cell). The excised mouse skin was stretched over the mouth of the diffusion cell with the stratum corneum facing the mouth of the ointment and ligated with a cotton thread. The diffusion cell was then inverted and immersed to about 1 cm of its surface in 300ml of phosphate buffer PH 7.4 (collecting medium) contained in a flask of the dissolution apparatus and maintained at 37°C and the collecting medium was stirred at 100r.p.m. The diffusion of aloin was followed by monitoring the concentration of the drug in the collecting medium for 6 hours. 3ml samples were pipetted from the collecting medium at one hour intervals and replaced each time by equal volume of fresh phosphate buffer PH 7.4 at 37°C⁽¹⁷⁾. The samples were analyzed spectrophotometrically at a wave length of 365nm for aloin.

6- Irritancy Test:

For the estimation of irritancy of substances applied repeatedly to the skin of human, the following test has given a good correlation between the results obtained with human volunteers⁽¹⁸⁾. A group of three male albino rabbits were used to do irritancy test on the selected formula. The ventral sides of the animal were carefully shaved, and four circular areas of 2.5 cm in diameter were pointed with 20% aqueous solution of formal-dehyde. The solution was allowed to evaporate and this process was repeated three times. The selected formula, dried juice (aloe) powder and the gel base without drug were applied on three of the circular areas and substance of known irritancy (histamine) was injected intradermally into the fourth of the inscribed circular area for purpose of calibration. The back of the animal's ear was shaved carefully, xylene was used to dilate the superficial ear veins. Approximately 1ml of (0.5%) trypan blue solution was slowly injected through the selected veins of the animal. The degree of irritancy of the substance is estimated by the accumulation of trypan blue at the treated site, the degree of blueness at the treated site is visually ranked to provide relative order of irritancy of the substances tested. The test site are observed after 1hrs, 6 hrs and 24 hrs⁽¹⁸⁾.

7-Clinical Study:

A total of 61 patients with Tinea corporis and Tinea cruris skin lesions presenting at the department of dermatology and venereology, Baghdad teaching hospital were enrolled in this study, their ages range between 12-63 years. A detailed history from each patient was recorded and the lesions in all the patients were diagnosed depending on clinical physical examination (including morphology, itching, colour, border activity, scale, site and size of the lesion) in addition to laboratory examination (using potassium hydroxide test) and culture^(19,20). The patients were divided into groups, each group was given the selected formula of certain concentration of aloe to be applied topically.

These groups were:

- 10 patients given 2% aloe gel formula
- 10 patients given 3% aloe gel formula
- 21 patients given 4% aloe gel formula
- 10 patients given placebo (only gel base with no drug).
- 5 patients given nystatin ointment.
- 5 patients given hydrocortisone ointment.

No patients given 1% aloe gel formula since it gave low diffusion through the skin. The patients were instructed to rub the lesion with the given preparations gently three times daily and to visit the clinic every week for four weeks to follow up the treatment.

8-Expiration Date Determination:

The concentration of aloin in the aloe gel formula (4% w/w) (to which preservatives 0.18% methyl paraben and 0.02% propyl paraben is added) stored at three different temperatures (50°C,60°C,70°C) is followed every month for four months. 100mg sample was shaken with 10 ml distilled water at 100 r.p.m. for 15 minutes , filtered and 2ml of the filtrate was diluted with 5ml distilled water and analyzed spectrophotometrically at 365nm using calibration curve of the drug in the gel base and the same extraction procedure mentioned above was applied⁽²¹⁾.

RESULTS AND DISCUSSIONS:

Aloin was detected by TLC and it was the major content in the dried juice of the Iraqi plant Aloe vera. Quantitative estimation of aloin by U.V. spectrophotometer showed that its amount in the dried juice equal 15.4/w/w and this agreed with reported data for the plant Aloe vera grown in other countries⁽⁴⁾. It was reported before that Aloe vera having antimicrobial activity and aloin is the major compound responsible for its activity^(7,13), but no work has been on *Tinea* species.

Therefore, the dried juice of the plant (aloe) was used for the next experiments to prepare topical preparations using different types of ointment bases in order to select the most suitable topical preparation. Each ointment base was prepared to contain different concentrations (1,2,3, and 4%w/w). The diffusion of aloin from these bases is studied using excised mouse skin, and it was in the following order (as shown in figure 1) :

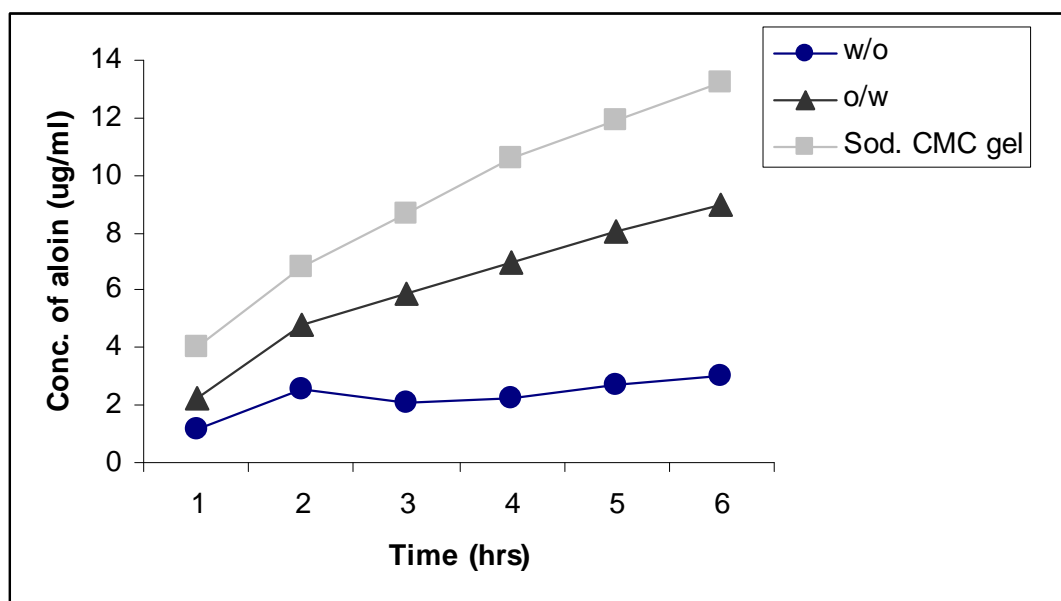


FIG 1 . THE DIFFUSION OF ALOIN FROM DIFFERENT OINTMENT BASES USING 1 % (W/W) CONCENTRATION

Gel base >o/w emulsion >w/o emulsion base. This can be explained on the basis of the limited solubility of dried juice (aloe) in water so partitioning of aloin is decreased according to the nature of the base. The results also showed that the diffusion increases as the concentration of aloe was increased.

The highest diffusion was obtained from 4% concentration using sodium carboxy methyl cellulose gel base (figure 2) so it was considered as the selected formula to which preservatives added (0.18% methyl paraben and 0.02% propyl paraben) and used for next experiments.

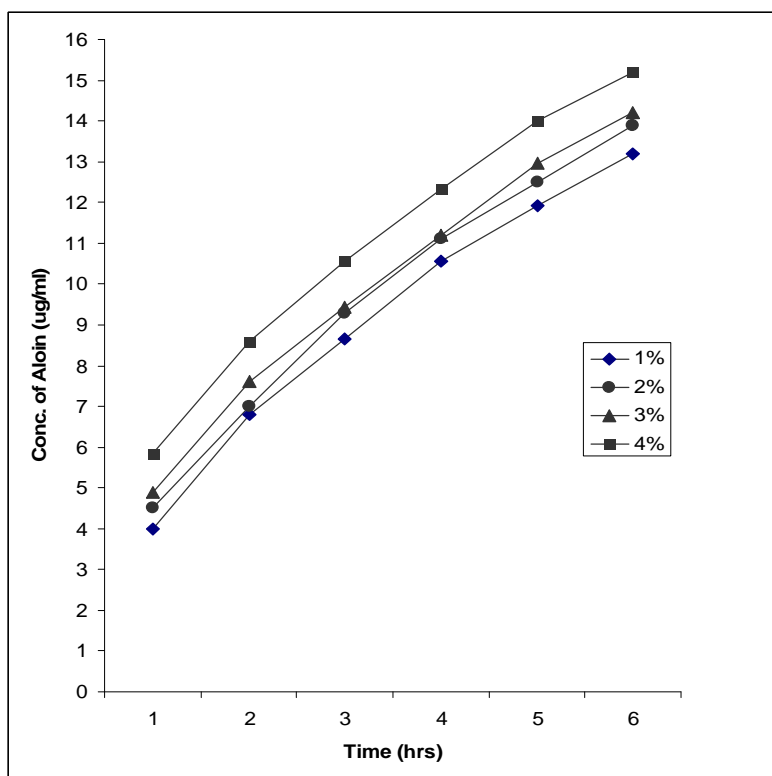


FIG 2 . EFFECT OF CONC. ON THE DIFFUSION OF ALOIN USING 1 %, 2 %, 3 % AND 4 % (W/W) ALOE GEL FORMULA.

Before applying on human skin it was very important to check whether the selected formula was irritant or not; therefore, irritancy test was done and it was found that the formula caused no irritation, therefore, it was decided to do clinical study on human skin lesions. It was found from the clinical study that patients taken 2% and 3% w/w aloe gel formula were not clinically cured while those taken 4% w/w showed significant improvement in the skin lesions caused by Tinea corporis and Tinea cruris after 4 weeks treatment with no adverse effect, as in (table 1 and 2).

Table 1 . Clinical follow up treatment with 4% w/w aloe gel formula.

| | Base line | 1st week | 2nd week | 3rd week | 4th week |
|-----------------|-----------|---------------------|--------------------|---------------------|---------------------|
| Itching | +ve | -ve | -ve | -ve | -ve |
| scale | +ve | moderately improved | -ve | -ve | -ve |
| Erythema | +ve | no change | no change | no change | no change |
| Border-activity | +ve | no change | moderately improve | moderately improved | moderately improved |
| Size | — | no change | no change | no change | no change |

Table 2 . Global clinical evaluation of improvement in skin lesion for patients treated with 4% w/w aloe gel formula

| | Base line | 1st week | 2nd week | 3rd week | 4th week |
|-----------------|-----------|----------|----------|----------|----------|
| Itching | +ve | 100% | 100 % | 100 % | 100 % |
| scale | +ve | 25 % | 100 % | 100 % | 100 % |
| Erythema | +ve | 0 % | 25 % | 48 % | 100 % |
| Border-activity | +ve | 25 % | 48 % | 72 % | 97 % |
| Size | — | — | — | — | — |

These two tables showed that itching has been cured since the first week of application and continued to the end of the treatment which might indicate the antipruritic activity of the selected formula. Also the amount of scale was decreased slightly in the first week and there was no scale after the second, third and the fourth week of treatment and this indicates the anti-inflammatory activity of the selected formula which may also have keratolytic activity. The absence of scaleness and the acceptable decrease in the border activity proves the effectiveness of the selected formula in stopping the disease activity and these results were supported by the fact that the size of the lesion remained stationary (no increase in the size of the lesion) which is a good marker for stopping disease activity.

These results were supported by the laboratory findings using KOH examination test (for the lesion scraping) and follow up of % cure rate as shown in (table 3 and figure 3).

Table 3 . Laboratory follows up (6% positively of KOH test) and cure rate for patients treated with 4% w/w aloe formula.

| | Before | 1st week | | | 2nd week | | | 3 rd week | | | 4th week | | |
|-------------------------|--------|----------|-----|-----|----------|-----|-----|-----------|------|-----|----------|------|-----|
| | | Gel | Ny. | Hy. | Gel | Ny. | Hy. | Gel | Ny. | Hy. | Gel | Ny. | Hy. |
| %positivity of KOH test | 100 | 85.7 | 100 | 100 | 51.9 | 100 | 100 | 31.9 | 98.7 | 100 | 7.6 | 97.8 | 98 |
| %cure rate | 0 | 14.3 | 0 | 0 | 49.1 | 0 | 0 | 68.1 | 1.3 | 0 | 92.4 | 2.2 | 2 |

Gel: 4% w/w aloe gel formula. Ny: Nystatin ointment.

Hy: Hydrocortisone ointment.

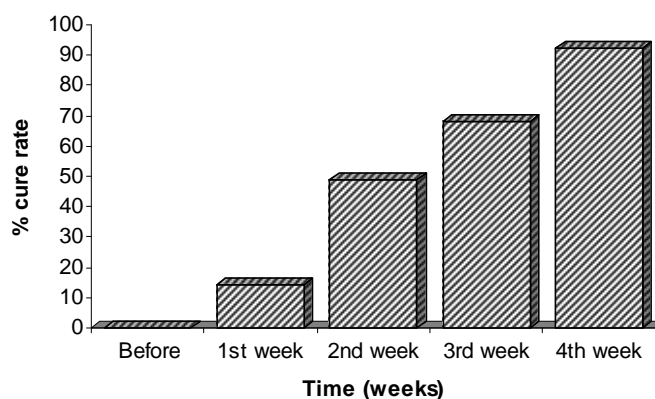


FIG 3 . % CURE RATE (ACCORDING TO LABORATORY FINDINGS) VERSUS TIME CURVE USING 4 % (W/W) ALOE GEL FORMULA

Where there is a significant decrease ($P\text{-value} < 0.005$) in the positivity of the test which means there is increase in the cure rate of the skin lesion using 4%w/w aloe gel formula in comparison with those treated with nystatin and hydrocortisone ointment where no improvement obtained. (Figure 4) shows a picture for the hand of a female with Tinea corporis lesion before and after 4 weeks treatment with 4%w/w aloe gel formula where a significant improvement is appeared.



FIG 4 . PICTURES OF A FEMALE HAND WITH TINEA CORPORIS SKIN INFECTION BEFORE AND AFTER 4 WEEKS TREATMENT WITH 4 % (W/W) ALOE GEL FORMULA.

The expiration date of aloin in the selected formula (4% w/w aloe gel formula) is determined by following its concentration at different temperatures (50°C , 60°C , 70°C) for four months and was found that the hydrolysis of aloin follows first order kinetic since it gave straight line upon plotting $\log\%$ remaining concentration versus time (figure 5) The observed degradation rate constants (K) were calculated from the slopes of the lines for the three different temperatures.

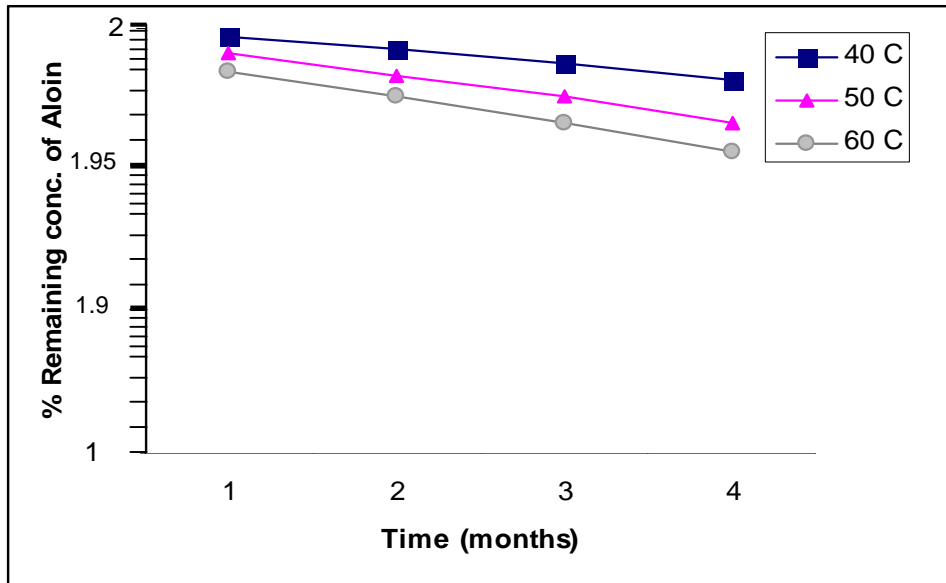


FIG 5 . % REMAINING CONCENTRATION VERSUS TIME CURVE FOR ALOIN IN 4 % (W/W) ALOE GEL FORMULA AT DIFFERENT TEMPERATURES.

The Arrhenius plot (21) was applied and the degradation rate constants at 25°C (K_{25}) was obtained from the plot as shown in (figure 6) and the expiration date was calculated and was found to be ≈ 2 years. The overall results indicate that the dried juice of the Iraqi plant Aloe vera can be used to prepare stable, effective and non-irritant topical preparation to treat some skin infections without adverse effect.

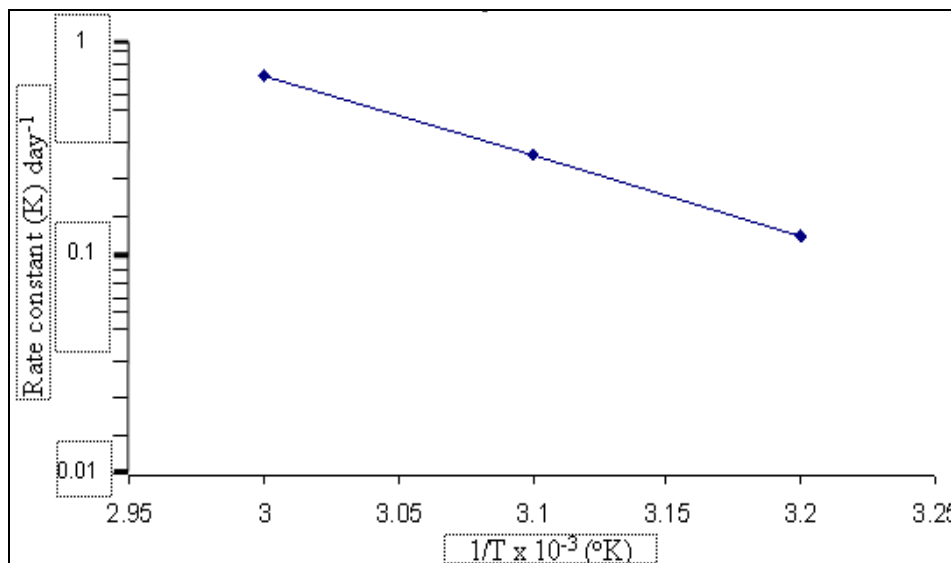


FIG 6 . ARRHENIUS PLOT FOR EXPIRATION DATE DETERMINATION OF ALOIN IN 4 % (W/W) ALOE GEL FORMULA

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