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Preparation and evaluation of the clinical efficacy and safety of tomato lotion containing lycopene

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ABSTRACT

Introduction: Skin aging starts at around age of 30 with wrinkling and pigmentations as its main indicators. Delay and prevention of aging is a major concern for a great number of people. The purpose of this research was to develop and evaluate the efficacy of an anti-wrinkle tomato lotion, consisting of 5% w/w tomato powder in an oil-in-water (O/W) emulsion.
Methods: Different O/W emulsions were prepared and stability tests were done on them. The formulation that successfully passed the stability tests, was chosen and the *Solanum lycopersicum* powder was added to the oily phase of this O/W emulsion. The prepared lotion was evaluated for pharmaceutical tests. In vitro permeation studies were performed to measure permeation through cellulose acetate membrane by diffusion cell at sink condition. In vivo trial for examination of the anti-wrinkle efficacy of lotion was done on 10 healthy women as case group compared with 10 volunteers using the placebo lotion (lotion base without *Solanum lycopersicum* powder) as control group.
Results: According to the experimented results on the formulated lotion, the efficient time duration for lotion effectiveness was 42 days. Tomato powder formulated in base of lotion significantly decreased wrinkles. Our formulation was compatible with skin and caused no sensitivity reaction in human models
Conclusion: The Lycopene in *Solanum lycopersicum* in this formulation has anti-aging effect. This formulation might be a strong candidate for treatment of skin wrinkles.

Implication for health policy/practice/research/medical education:
Lycopene is a natural antioxidant that showed a good anti-aging effect when used with a suitable vehicle in lotion formulation.
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Introduction

Mechanism of skin aging includes intrinsic aging, which is the chronologic aging of skin, and extrinsic aging, which is influenced by physical and chemical factors (1).
There are multiple theories on the mechanisms of skin aging, which include oxidative stress, loss of telomeres, mutations in mitochondrial DNA (mtDNA), hormonal changes and diminish of dermal collagen and elastin.
In addition to the epidermis and dermis, changes to the subcutaneous fat also contribute to an aged appearance (2). When discussing extrinsic facial aging in specific, solar radiation, due to increasing exposure to ultraviolet rays (uvA and uvB) radiation over one's lifetime is the main reason. Intracellular and extracellular oxidative stress by reactive oxygen species (ROS) exacerbate skin aging, which is characterized by wrinkles and atypical pigmentation (3). Because ultraviolet (UV) enhances ROS generation in cells, skin aging is usually related to UV exposure. Nowadays, there are many cosmetic procedures and products which can effectively reduce the aging of skin and treat the wrinkles.
Topical administration of anti-aging agents comprises an important part of the therapy. The treatment of shallow-to-deep wrinkle usually begins with topical therapy (4). A variety of systemic and topical therapies are available for wrinkle treatment, and one of the most effective agents are

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antioxidants (3,5).
The use of antioxidants is an effective approach to prevent symptoms of the skin aging. Antioxidants decrease ROS by direct scavenging, decreasing the amount of oxidants in and around our cells, prevention of ROS from attaining their biological targets, limiting the spread of oxidants such as the one that occurs during lipid peroxidation, and stopping oxidative stress thereby preventing the aging phenomenon (3).
Trichloroacetic acid (TCA), α-hydroxy acids (AHA) Or β-hydroxy acids, vitamin E, ascorbic acid, lycopene and phenolic compounds are some important antioxidants that are used in cosmetic products (6,7)
Tomato fruit is a source of natural antioxidant. Based on previous researches, someone that consumes a lot of tomato fruits regularly can reduce the risk of cancer diseases. In the daily life, the subjects who consume a lot of tomatoes can keep their physics healthy and stay young. Tomato contains carotenoids, lycopen and β-caroten, as well as other natural antioxidant compounds such as vitamin C and vitamin E. Since tomato has several good antioxidants, it is good if it is being developed as pharmaceutical product, especially as cosmetics (8).
Lycopene is a carotenoide that is present in *Solanum lycopersicum*, processed tomato products and some other fruits. Some of the fruits and vegetables that are known to be rich in lycopene are tomatoes, pink grapefruit, papaya, wolfberry, and goji. Lycopene is one of the most potent antioxidants among dietary carotenoids (9).
Lycopene's powerful antioxidant action and the ability to defend the skin against UV radiation are due, in large part, to its unique molecular design, which is responsible for lycopene's red appearance and its ability to block UV light. Lycopene's sun protection is only equivalent to approximately SPF-3 which is not adequate for sun protection by itself, but topically-applied lycopene has been shown to be able to defend against the harmful effects of UVB radiation (10).
It was found that topical application of lycopene suppressed the typical UVB-induced activity of an enzyme called ornithine decarboxylase, an important initiating and rate-controlling factor involved in stabilizing DNA structure in the nucleus of the skin cells as well as maintaining the DNA double-strand break repair pathway. What this means is that lycopene is able to offer significant protection to the cellular DNA and thus negated the need for the body to activate its internal DNA repair pathways (10,11).
UVB radiation also reduces an important substance in the skin known as PCNA (proliferating cell nuclear antigen), which is vital for DNA synthesis and cell repair. The topical application of lycopene was found to reverse the reduction of PCNA caused by UVB exposure to a significant degree.
In addition, it was shown that lycopene might also protect the skin through its ability to reduce inflammation, encourage cell renewal, and inhibit normal DNA damage following UVB injury which means it may help reduce the risk of wrinkles and protect skin against free radical damage (12).
The purpose of this research was to evaluate the physical stability and the anti-wrinkle activity in a lotion of tomato powder (consist of lycopene).
In this research, tomatoes (*Solanum lycopersicum* L.) were freeze dried and rubbed out to make a fine powder and formulated in the form of semisolid as a lotion. The form of semisolid as a lotion was chosen due to the form of a lotion that could easily be dispersed over the skin, not thickened and easily cleansed compared to the form of ointment. The test conducted in this research was physical stability test of the lotion based on stability parameters. Besides that the research measured anti-wrinkle activity with Visioface devices, and antioxidant activity with densyl chloride.
Lotions are emulsions of oleaginous substances and water, and spread more easily over skin than ointments. Oil-in-water (O/W) types of lotions are easily water-washable. Advantages of bringing the drugs in lotion bases are: lotion hydration prevents development of shallow wrinkles induced by dehydration of skin. Moreover moisture accumulates between the skin and the lotion layer that causes hydration of the stratum corneum. Hydration of stratum corneum allows 'opening up' of intra- and inter-cellular channels and pathways for easier passage of drug molecules. Additionally, the moisture layer provides a medium for dissolution of the drug that is otherwise dispersed as fine particles in the lotion base. Since only the dissolved drug presented to the skin as an individual molecular unit is able to enter the stratum corneum, skin occlusion generally results in enhanced percutaneous drug absorption (13).

Material and Methods

Isopropyl myristate, glycerin monostearate, cetostearyl alcohole, tween 20, tween 80, buthylen glycol, potassium sorbate, phenoxy ethanol and zinc sulfate all supplied from Merck Company (Germany). All ingredients used in this study were of analytical grade.

2.1. Identification of lycopene

Lycopene was authenticated according to USP and BP pharmacopeia by UV spectrum and paper chromatography.

2.2. Measurement of lycopene in tomato powder

For this experiment about 1.0 g of tomato material was accurately weighed into a 125 mL Erlenmeyer flask and 100 mL of mixed solvents (hexane:ethanol:acetone (2:1:1) were added with a graduated cylinder. The flask was sealed with a rubber stopper. After at least 10 minutes of extraction, 15 mL of water was added to separate the phases and absorbance of the upper phase determined. The lycopene concentration was estimated by:
Lycopene (mg/kg fresh wt.) = A503 × 171.7/W

*A503: tomato material absorbance in wavelength of 503 nm at spectrophotometer, where W is the exact weight of tomato added, in grams.

2.2.1. Determination of tomato powder solubility

In this study it was attempted to determine tomato powder solubility in different solvents such as isopropyl myristate, glycerin monostearate and canola oil. The solubility of tomato powder in glycerin was slight. For dissolving tomato powder we changed the solvent and further dissolved it in 3 different solvents. Eventually tomato powder was dissolved in the mixture of isopropyl mirystate and canola oil (5:2).

To develop a stable emulsion, several formulations as for the results from experimental design were prepared.

2.2.2. Explanation of software experimental design

In this experiment we changed the amount of 4 ingredients. The different volumes – minimum, mean and maximum – were defined for 4 substances according to their minimum and maximum in the formulation and they were introduced to the software. Later, the best 9 formulations were formed based on their priority. The compositions of prepared formulations are shown in Table 1 and physicochemical parameters of formulations are shown in Table 2.

2.3. Preparation of lotion

Mixtures containing water phase (tween 20, tween 80, buthylen glycol, potassium sorbate, phenoxy ethanol, zinc sulfate and water up to 100%), tomato extract powder and oil phase (glycerin monostearate, cetoestearyl alcohole, vitamin E, isopropyl isopropyl myriststst, canola oil) were prepared in this way. We heated the oil and water phase until all components were melted (80°C). The emulsion was obtained adding the water phase to the oil phase gradually. The mixture was stirred by a set speed homogenizer at 3000 rpm for 10 minutes until the base was formed and the product was kept at room temperature.

All the excipients used in this study were of analytical grade (14).

Then different physicochemical properties like determination of pH, centrifuge test, determination of viscosity, in vitro release study were carried out, and formulation number 2 was selected for final evaluation.

2.4. Evaluation of selected formulations

The following physicochemical parameters were used for the evaluation of formulation.

2.4.1. Determination of particle size

The particle size of tomato powder was determined by zeta analyzer (Malvern Instruments Ltd).

2.4.2. Organoleptic evaluation

The prepared lotion was inspected visually for color and homogeneity (Table 3) (15).

2.4.3. Centrifuge test

The prepared formulations were centrifuged at 3000 rpm for 30 minutes (HETTIC D-7200, Germany) 24 hours after preparation and at one week intervals for 28 days (Table 3) (14,16).

Table 2. Physicochemical parameters of formulations (F)

Formulation	pH	Viscosity (rpm)	Centrifuge
F1	5.75	700	Not separated
F2	5.5	480	Not separated
F3	6.2	240	Separated
F4	5.6	400	Not separated
F5	6.1	1400	Separated
F6	6.3	1100	Separated
F7	5.4	200	After 24 h separated.
F8	5.5	420	Not separated
F9	5.5	180	Not separated

Table 3. Physicochemical evaluation of formulation

Parameters	Results
Physical appearance	Orange white emulsion, completely homogen
Centrifuge	+++
Freeze-Thaw	+++
pH	5.5 ±0.03
Drug content	25mg/100gr
Particle size	241±2.67

+: poor, ++: good, +++: excellent.

Table 1. Compositions of prepared formulations

Formulation ingredient	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Isopropyl myristate	10	10	10	10	10	10	10	10	10
Tween 80	2	1.5	2	1.3	1.5	1.5	1.3	2	1.3
Tween 20	1	1.5	2	2	1	2	1	1.5	1.5
Butylene glycol	2	2	2	2	2	2	2	2	2
Canola oil	1	1	1	1	1	1	1	1	1
Vitamin E	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Glycerol monostearate	2	2	4	4	4	1	2	1	4
Phenoxy ethanol	1	1	1	1	1	1	1	1	1
Potassium sorbate	1	1	1	1	1	1	1	1	1
Zinc sulfate	1	1	1	1	1	1	1	1	1
Cetostearyl alchole	4	1	1	2	2	4	1	2	1
Purified water up to (g)	100	100	100	100	100	100	100	100	100

2.4.4. Determination of pH

The pH value of prepared formulations were measured by a digital pH meter (Metrohm, Switzerland). The determinations were carried out in triplicate and the average of three readings was recorded (Table 3) (15).

2.4.5. Freeze and thaw cycle

Freeze and thaw treatment of the emulsion was performed immediately after preparation. Samples (20 ml) were stored at -20°C for 48 hours. The frozen samples were subsequently thawed in room temperature for 48 hours. This test was carried out in triplicate for each sample (Table 3) (17).

2.4.6. Rheological study

Viscosity of formulations was measured using Brookfield viscometer (DVIII ultra), at constant temperature of 25°C at 100 rpm. Measurements were carried out in triplicate (15).

2.5. Drug content

Five grams of lotion was dispersed in 50 ml ethanol 96%: ethyl acetate (1:1). After suitable dilution the drug content was measured by UV-spectrophotometer (Shimadzu, model UV mini-1240 CE) at 505 nm against corresponding emulsion formulation as blank (5 g lotion without tomato powder that prepared similar to samples (Table 3).

2.6. In vitro drug release study

Franz diffusion cells (with 30 ml volume) were used for the drug release studies. 30 ml of hexane was used as the receptor compartment; 1 g of lotion was applied on the surface of cellulose acetate membrane. The membrane was clamped between the donor and the receptor compartment. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37°C. The solution in the receptor compartment was stirred by magnetic stirrer. At predetermined time intervals 1 ml of solution from receptor compartment was pipette out and immediately replaced with 1 ml fresh hexane. After suitable dilution the drug concentration in the receptor fluid was determined spectrophotometrically against appropriate blank (1 g lotion without tomato powder). The experiment was carried out in triplicate. This test was studied under sink condition in the receiver compartment (Figure 1) (15).

2.7. Kinetic analysis of drug release

To study drug release kinetics, data obtained from in vitro release study were fitted in zero, first and Higuchi kinetic models equation. In order to evaluate mechanism of drug release, data of drug release were fitted in Korsmeyer-Peppas equation (18).

2.8. Stability test

The stability studies were carried out at 8°C (at refrigerator), 25°C (at room), and 40°C (in oven). At one week intervals for one month drug content and physical appearance (organoleptic characteristic) were evaluated (Table 4) (14).

2.9. Clinical tests

2.9.1. Skin irritation test

The formulation was applied over the arms of 10 women. The test sites were observed for erythema and edema for 48 hours after application (19).

2.9.2. Application of test formulations for anti-aging evaluation

In this research we chose 10 women to apply the lotion base with tomato powder and compared them with other 10 women as control that used placebo (lotion base without tomato powder).

These 2 groups were compared via charts get from Visioface software. Visioface is an apparatus for full face photography – comprehensive, economic analysis of the face. This apparatus consists of light facial booth in which the face is placed (frontally or sidewise) and has 200 white LEDs inside the booth diodes illuminating the face very homogenously and the operator could use it without requiring service (20).

To use the Visioface, at first we fixed the head of volunteers in front of the apparatus, on the marked place, and then we selected the capture from menu and then analyzed the photo. With this software we were able to measure the pixels of volume, area and depth of the wrinkles.

Technical data of this apparatus are written below:

Dimensions: 54 × 50 × 44 cm, Weight: approx. 11.4 kg (with accessories 13.8 kg) Illumination: 210 white light LEDs, Camera: Canon EOS 550D, 18 mega pixel, sensor CMOS, autofocus, images can be saved as jpg (recommended) or png, Objective: EF 20 mm/2.8, USM: focal

Table 4. Stability study parameters of selected formulation

Result Parameters	Condition	Initial	7 days	14 days	28 days
Physical appearance	8°C	√	√	√	√
	25°C	√	√	√	√
	40°C	√	√	√	√
	40°C/75% RH	√	√	√	√
Drug content (mean ± SD)	8°C	99% ± 0.14	96.8% ± 1	97.5% ± 1.12	97.5% ± 1.04
	25°C	99% ± 0.14	98.6% ± 1.02	97.3% ± 1.17	97.56% ± 1.18
	40°C	99% ± 0.14	98% ± 1	96.79% ± 1.013	96.5% ± 1.05
	40°C/75% RH	99% ± 0.14	98% ± 1.001	96.87% ± 1.1	96.74% ± 1.05

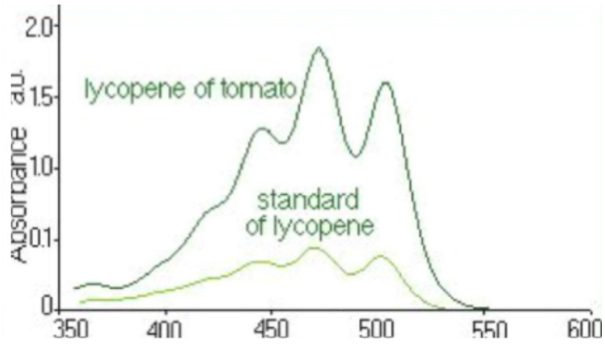

Figure 1. Spectrophotometer absorbance curve of tomato paste.

length 20 mm, filter diameter 72 mm, focus by ultrasound, Power Supply: external 100-250 V, 47-63 Hz, DC 12V/4A, Port: USB.

Results

3.1. Identification of lycopene

3.1.1. UV spectrum lycopene

As Figure 1 shows the maximum absorption of lycopene in hexane solution was in 445, 472 and 503 nm. The UV spectrum obtained from tomato powder in hexane solution was similar to standard lycopene. The maximum absorption of tomato powder with concentration of 10 µg/ml in hexane in range of 450 to 550 nm was in 503 nm.

3.1.2. Retention (Rf) of lycopene

On the thin-layer chromatography (TLC) plate, the tomato powder in hexane solution gave red (Rf = 0.14), orange (Rf = 0.6), and yellow (Rf = 0.72) spots. The Rf value of the red spot was the same of that of authentic lycopene. The Rf obtained from tomato powder in hexane solution was similar to standard Rf of lycopene, obtained from references.

There were 520 mg lycopene in 100 g tomato powder. The best solvent system for tomato powder was isopropyl myristate and canola oil.

3.2. Emulsion preparation

As for the results from experimental design, the best formulation was F2 containing isopropyl myristate: 10%, glycerin monostearate: 2%, cetostearyl alchole: 1%, tween 20: 1.5%, vitamin E: 1%, canola oil: 1%, tween 80: 1.5%, buthylen glycol: 2%, potassium sorbate: 1%, phenoxy ethanol: 1%, zinc sulfate: 1% and water up to 100%. Both the oil and aqueous phases were separately heated to 70°C-80°C; then the aqueous phase was added to the oily phase with continuous stirring. When the emulsion was cooled to room temperature, control tests were done (14).

3.3. Quality control tests of selected formulation

The prepared formulation was all uniform in appearance, having orange white color. Observation of prepared formulation under the microscope revealed homogeneity of globules and internal phases.

pH values of 5% (w/w) formulation was in range of skin and appropriate for application of this lotion on the skin surface.

3.4. Drug content

There was 25 mg lycopene in 100 g lotion.

3.5. Drug release through cellulose acetate membrane from formulation

3.5.1. The in vitro drug release study of the formulation release for a period of 140 minutes

According to release exponent ($n < 0.5$), diffusion mechanism is fickian, and base on the correlation coefficient it has first degree kinetic.

3.5.2. Kinetic analysis of drug release

The in vitro drug release study of the formulation exhibited release for a period of 2 hours. According to release exponent, diffusion mechanism was fickian which refers to combination of both higuchi and first degree release rate, and base on the correlation coefficient, first degree kinetic was dominant.

3.6. Stability studies

According to Table 4 the formulation was found to be stable with no sign of change in physical appearance, organoleptic characteristic and drug content.

3.7. Skin irritation test

Skin irritation test was conducted to evaluate the irritation by the prepared formulation on intact skin. The prepared formulation did not show any erythema or edema; this indicates that the prepared formulation containing 5% tomato powder was non-irritant on skin.

3.8. Rheological study

viscosity of formulation F_2 was 480 rpm.

3.9. Antiaging activity evaluation

The wrinkles measurements via charts were obtained from Visioface in days of 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70. The digits produced by Visioface in week 1 and week 6 are

Table 5. The digits produced by Visioface in week 1 and week 6

Case 1	**Week 1**	**Week 6**
Volume	$Px^3 = 150.451$	$Px^3 = 100.145$
Area	$Px^2 = 10.199$	$Px^2 = 8.45$
Depth	$Px = 18$	$Px = 15$
Case 2	**Week 1**	**Week 6**
Volume	140.541	120.654
Area	9.205	7.456
Depth	15	12.02
Case 3	**Week 1**	**Week 6**
Volume	178.45	90.81
Area	5.65	3.58
Depth	16.52	15.36

presented in Table 5.

The most efficacy about wrinkle decrease was in day 42 (week 6) and after that there was no more advance in wrinkle reduction.

Wrinkle chart in day 0 and day 42:

Px^3 = volume of wrinkles at pixel

Px^2 = area of wrinkles at pixel

Px =depth of wrinkles at pixel.

Discussion

An optimized formulation of tomato lotion was prepared in this study using the "heating and mixing" method. Formulation and optimization procedures were facilitated by Taguchi designs.

The driving force for passive transport through a membrane is the chemical potential gradient or flux expressed across the membrane. To create the gradient necessary to deliver a drug across the skin, one normally dissolves a drug in a solvent or vehicle to establish a certain concentration, and determines the activity of the drug at the outer surface of the skin. Since different vehicles have different capacities to dissolve the drug, at any fixed level of activity one can have different concentrations of the drug at the interface of application depending on the solvency of the vehicle.

In this research we studied anti-wrinkle effect of tomato lotion in 10 cases with 10 controls receiving placebo. The effects of applied lotion were significant after 6 weeks and were measured by Visioface devices. Based on the experimental results, the average decrease in depth, area and volume of wrinkles were 26.4%, 24.2% and 38.1%, respectively, which were improved compared to control group. These effects were observable in all of the cases receiving treatment with the tomato lotion formulation.

Conclusion

Tomato powder formulated in lotion base significantly decreased wrinkles in test group. Our formulation was compatible with skin and caused no hypersensitivity reaction in tested human model.

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Authors' contributions

All contributed to the design of the study. SK carried out the study. MAS, AHS and SK prepared and confirmed the final manuscript.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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