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Original article

Screening of topical gel containing lycopene and dexamethasone against UV radiation induced photoaging in mice

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ABSTRACT

Sun continuously emits UV radiation which produces series of pathological changes in the body, in long term, this ultimately results in photoaging and skin cancer on long term. Repeated UV exposure increases oxidative stress, nitrosative stress and inflammatory mediators which lead to activation of p38, MMPs and results in photoaging. Lycopene is an antioxidant and very low dose of dexamethasone provides anti-inflammatory effect. Animals were applied with lycopene gel, dexamethasone gel and combination of dexamethasone and lycopene gel after exposing to UV radiation. After experimentation period effectiveness, of treatment were evaluated by morphological, biochemical and histopathological parameters. The animals which had received lycopene and standard treatment showed less wrinkles in comparison to UV irradiated and dexamethasone treated group. Lycopene treatment decreased the TBARS level from $51.2 \pm 2.417\%$ to $23.593 \pm 3.945\%$, increased by chronic UV radiation. Similarly, collagen, catalase and GSH level was increased from $56.12 \pm 2.626\%$ damage to $20.56 \pm 2.029\%$, $64.59 \pm 1.743\%$ damage to $19.507 \pm 4.997\%$ and $48.780 \pm 1.682\%$ reduction to $21.927 \pm 7.248\%$. Histopathological study and epidermal thickness parameter also revealed that lycopene provide protection against UV radiation. In case of dexamethasone treatment, either alone or in combination did not provide significant ($P < 0.001$) protection, which may be due to its inherent property to generate oxidative stress in mice. It is noteworthy to report that lycopene provide protection against photoaging by virtue of antioxidant property.

1. Introduction

Aging is a multifactorial and complex process resulting in functional decline and the mortality of the cells and organism [1]. Aging of the skin by ultraviolet radiation is known as photoaging. Chronic UV exposure increases oxidative stress, nitrosative stress and inflammatory mediators. The generated reactive oxygen species (ROS) is responsible for sequential changes like generation of interleukin (IL) and tumor necrosing factor (TNF), activation of mitogen activated protein kinases (MAPK) and nuclear factor kappa B (Nf-kB) pathway, matrix mattalo proteinase (MMP) activation which are responsible for degradation of collagen. ROS also decrease the expression of tissue growth factor β (TGF-β) in the skin, which is responsible for collagen production. Since collagen imparts skin intactness [2], all these biochemical changes produces different symptoms like pigmentation on the skin, spider vein on the skin (because of dilation of narrow blood vessels), rough and leathery skin, wrinkles, a blotchy complexion, solar elastosis, erythema reaction, actinic keratosis, immunosupression and skin cancer on long term [3,4].

On the basis of pathological changes, the treatment should provide protection against elevated oxidative stress, nitrosative stress and inflammatory mediators. Lycopene (LYC) an aliphatic hydrocarbon, is one of the 600 caretenoids obtained from fruits, vegetables particularly tomatos. Lycopene has gained special attention as a highly potent antioxidant due to single oxygen and high free radical scavenging activity [5]. Baschant and Tuckermann in 2010, reported that low dose of dexamethasone (DEX) is effective against elevated inflammatory mediators [6]. Since photoaging mainly affects skin, topical treatment is preferred. Based on the previous findings and biochemical changes occurring due to chronic UV radiation exposure, authors decided to work on lycopene and dexamethasone. In this study, attempt had been made to evaluate

Abbreviations: UV, Ultraviolet; TNF, Tumour necrosing factor; MMP, Matrix metalloproteinase; ROS, Reactive oxygen species; DEX, Dexamethasone; LYC, Lycopene; SLS, Sodium lauryl sulphate.

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efficacy of topical gel containing LYC, DEX and two different concentration of lycopene and dexamethasone against UV radiation induced photoaging by determining morphological changes, *in vivo* antioxidant enzyme level and histopathological parameters.

2. Materials and Methods

2.1. Material

Lycopene was obtained as a gift sample from diligent neutraceuticals, Ahmedabad, Gujarat, India and dexamethasone was obtained as a gift sample from Zydus Cadila, Ahmedabad, Gujarat, India. Rest of the chemicals were obtained from drug store of Lovely Professional University which were of analytical grade.

2.2. Methods

2.2.1. Compatibility study between drugs and drug-excipient

Compatibility study was carried out according to ICH Q8 guidelines. Study was carried out by preparing samples in 1:1 proportion at two different conditions. They were analysed for compatibility by infrared spectroscopy (Shimadzu 8400S, Japan).

2.2.2. Gel formulation

Four different gel formulations containing LYC, DEX and different concentration of LYC and DEX was prepared by using carbopol 940. Accurately weighed carbopol was taken and dispersed in a beaker containing distilled water with constant stirring using a mechanical stirrer at 1000 rpm for 30 min. After dispersion of carbopol, dexamethasone and preservatives were dissolved separately in ethanol and added to carbopol dispersion followed by addition of SLS dissolved in water. The pH was adjusted with triethanolamine until clear consistent gel was obtained. Lycopene was dissolved initially in water (Table 1).

2.2.3. Evaluation parameters

2.2.3.1. Homogeneity. Gel formulation was tested for homogeneity by visual inspection after the gel has been set in container. The observation was made on the basis of appearance and presence of aggregates.

2.2.3.2. Grittiness. The ideal gel formulation should not contain any lumps or gritty particles hence, measurement of grittiness is an essential parameter. The thin smear of gel was prepared and observed microscopically for any gritty particles.

2.2.3.3. Spreadibility. Spreadibility was measured by putting excess sample between 2 glass slides and compressed to uniform thickness by placing weight of 1000 g for 5 min. The time in sec required to separate two slides was taken as a measure of spreadibility. It was calculated by formula $S = (m \times l)/t$, where S is spreadibility, m is weight tied to upper slide, l is length moved by upper slide and t is time taken to slide [7].

2.2.3.4. In vitro permeation study. In vitro release was carried out using Franz diffusion cell by using phosphate buffer saline (pH 6.5) at 37 ± 0.5 °C temperature and 600 rpm. Cellulose membrane was mounted in between receptor and donor compartment. The donor compartment was filled with 1 g of gel formulation. At appropriate time, interval of 15, 30, 45, 60, 120, 180, 240, 300, 360 min 5 mL of sample was withdrawn and immediately replaced by equal amount of receptor solution. The samples were analysed at respective absorbance maxima of DM and LYC [8].

Amount of drug release at particular time was determined by formula given below:

$$\text{Amount released}\,(t_1) = (A_u/A_s)(C_s)(1000)(V_c/A_o)$$

$$\text{Amount release}\,(t_2) = [(A_u/A_s)(C_s)(1000)(V_c/A_o)] + (Ar_{t1}.V_s/V_c)$$

$$\text{Amount release}\,(t_3) = [(A_u/A_s)(C_s)(1000)(V_c/A_o)] + \sum_{t-1}^{n-1} ARt - 1.Vs/Vc$$

where, A_u is Abs of current sample, A_s is Abs of standard, C_s is concentration of standard (mg/mL), V_c is volume of diffusion cell (mL), AR is amount released (mcg/cm^2), A_o is area of the orifice (cm^2), V_s is volume of sample aliquot (mL).

2.2.3.5. DPPH assay. The free radical scavenging activity of the compound was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH as described by Blois [9]. In an ethanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (final concentration 1.0×10^{-4}mol), LYC at different concentrations (10, 20, 30, 40, 50 mcg/mL) were added followed by incubation for one hour. The absorbance of the resulting solutions was measured at 517 nm, against blank. Methanol was used as a blank. Vitamin E was used as a standard. Antioxidant capacity of LYC is expressed as IC_{50}. The data was analysed by unpaired two tailed *t* test.

2.2.4. Animal treatment

Forty-two Swiss albino female mice were procured from National Institute of Pharmaceutical Education and Research, Mohali, India. Whole experiment was performed as per the procedure approved by animal ethics committee of Lovely Professional University, India. The mice were quarantined for 7 days and evaluated for weight change and any sign of injury before the study begins. The animals were housed in plastic cages with saw dust and each cage contained 3 animals. They were kept in 12/12 hour light dark cycle at temperature of 25 ± 2 °C. They were given standard diet and water *ad libetum*. The animals were divided in seven different groups, each containing six animals. Initially, dorsal region of mice were depilated using depilatory cream. Osram Ultra Vitalux 230V-E27 (300 W) was used as UV radiation source. The bulb was fixed in UV chamber and mice were kept in a restrainer to restrict their mobility while exposure to UV radiation. The positive control (group I) was not exposed to UV radiation. The negative control (group II) was exposed to UV radiation but it did not receive any treatment. The animals in group III, group IV, group V, group VI and group VII were exposed to UV radiation and then they received DEX, LYC, DEX (0.005%) – LYC (5%), DEX (0.01%) – LYC (10%) and tretinoin as a treatment. Initially for 6 weeks, animals were exposed to UV radiation for 5 min, then next three weeks for 10 min, and then in remaining 3 weeks they were exposed for 12.5 min.

2.2.5. Evaluation of protective effect of prepared gels against photoaging

2.2.5.1. Severity of wrinkles. Severity of wrinkles was determined by taking photographs and comparing them.

2.2.5.2. In vivo parameters.

2.2.5.2.1. Lipid peroxidation. Levels of thiobarbituric acid reactive substance (TBARS) were estimated by the following method, which was given by Ohkawa et al. [10]. Also, 0.2 mL of tissue homogenate, 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8% TBA were mixed. The mixture was made up to 4 mL with distilled water and then heated at 90 °C for 60 min followed by addition of 1 mL of water and 5 mL n-butanol/pyridine mixture (15:1) and centrifuged at 600 g for 10 min, absorbance of organic layer was measured at 532 nm. 1,1,3,3-tetramethoxypropane was used as the standard. The TBARS were expressed in mmol/mg