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Research Article
Tadalafil Transdermal Delivery with Alpha-lipoic Acid Self Nanoemulsion for Treatment of Erectile Dysfunction by Diabetes Mellitus

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Abstract

Background and Objective: Erectile dysfunction (ED) is a common condition, especially in diabetic males. Alpha-lipoic acid (ALA) can protect endothelial cells from oxidative stress damage. The purpose of this study was to find a new treatment for ED in diabetic patients via combined ALA with tadalafil (TFL) in a transdermal patch to enhance the skin permeability of TFL and protect epithelia cells lined penile vessels from the oxidative stress induced by hyperglycemia. **Materials and Methods:** A solubility study was carried out for TFL and ALA, followed by nine formulae with different ratios of oil, surfactant and co-surfactant. TFL released from the formulated patch was compared with a raw TFL patch and afterward, permeation of the formulated TFL was imaged. The difference between the treatments was estimated using one way-analysis of variance test. **Results:** Anise oil and Tween 20 showed highest solubility for both drugs, while polyethylene glycol (PEG 200) solubilized 0.49±0.1 and 7.8±1.2 mg mL^{-1} of TFL and ALA, respectively, Formula A4, achieved the lowest globular size (134.3±12.3 nm) according to ternary phase diagram. TFL released was enhanced significantly by about 2.83-fold in the transdermal patch in comparison with raw TFL after 12 h, images clearly confirm the efficacy of penetration of the formula within the skin layers. According to cell viability (EA. hy926) data to assess the role of ALA in improvement of resistance of endothelial cells to oxidative stress, ALA increased cell survival by about 1.3-fold. **Conclusion:** The combination of ALA and TFL in the form of a self-nano emulsion drug delivery system as a transdermal patch could be a successful strategy for treatment of ED in diabetic patients.

Key words: Oxidative stress, nanoparticles, protective effect, endothelial cells, transdermal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Erectile dysfunction (ED) is a common sequel in men with diabetes mellitus. Many studies have revealed that the ED prevalence rate is 30-50% in diabetic patients[1], which can be explained by associated neuropathy and peripheral vascular disease[2]. Erection depends on nitric oxide, which is released from penile arterial endothelial cells in response to sexual stimuli. Nitric oxide induces cyclic 3',5'-guanosine monophosphate formation, which results in relaxation of cavernosal smooth muscle and finally in erection[3]. Endothelial cells are highly involved in the maintenance of intravascular homeostasis: They play an important role in vascular tone regulation, hemostasis, fibrinolysis and in the production of several substances[4]. In diabetic patients, biochemical processes related to hyperglycemia damage the endothelial cells, causing their dysfunction[5].

Tadalafil (TFL) is a phosphodiesterase 5 inhibitor and is considered the most potent drug in this class. The main use of TFL is the treatment of ED6. According to the FDA biopharmaceutical classification system, TFL has low solubility and high permeability (class II), which leads to low bioavailability[1].

Alpha-lipoic acid (ALA) has a positive influence on the nitric oxide that is an essential mediator for vasodilatation. Many studies have revealed that ALA could be administered to prevent atherosclerosis and other cardiovascular diseases by improving endothelial functions[7,8] and moreover, to protect the diabetic patients from associated vascular and neuropathic diseases[9].

Transdermal patches are an efficient method of drug delivery and an alternative to oral delivery. These patches can overcome the limiting hepatic first-pass effect and are safe, painless and easy to use. Moreover, some drugs are able to reach therapeutic levels by penetrating the skin sufficiently[10]. The Self-Nano Emulsion Drug Delivery System (SNEDDS) has recently gained attention for drugs that are characterized by poor aqueous solubility, such as TFL. The improved aqueous solubility overcomes this main obstacle that limits its bioavailability[11,12].

The aim of this study was to enhance TFL skin permeability and convenience for patients and furthermore, to evaluate the role of ALA for diabetic patients suffering from ED.

MATERIALS AND METHODS

Chemicals: ALA and TFL powder, propylene glycol and polyethylene glycol 200 were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Clove oil, peppermint oil and orange oil were purchased from Fluka (Merkland, Germany). Oleic oil was purchased from Zhonghua Chemical Engineering Co., Ltd.(Main land, China). Anise oil and lemon oil were purchased from Abazeer (Jeddah, Saudi Arabia). Olive oil, decyl alcohol and Tween 20 were from ACROS Organics (New Jersey, USA). Caster oil was purchased from Asaggaf (Jeddah, Saudi Arabia). Almond oil, Span 80 and Tween 20 were from Fluka (Switzerland). Polyethylene glycol 400 was from Fluka (England) and 1,2-Propanediol propylene glycol was from Fluka (Germany).

All experiments were carried out at the Nanotechnology and Cell Culture Labs of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The experiments were started in April, 2017 and conducted to August, 2017.

At the nanotechnology lab of the Faculty of Pharmacy, King Abdulaziz University, an excess amount of ALA and TFL were added separately to 5 mL of various oils (clove oil, orange oil, peppermint oil, anise oil, olive oil, castor oil, lemon oil, almond oil and oleic acid), surfactants (decyl alcohol, Span 80, Tween 20 and ethyl alcohol) and co-surfactants (400 polyethylene glycol, propylene glycol, 1,2-propanediol propylene glycol, polyethylene glycol 200). The mixtures were placed in a thermostatically controlled shaking water bath at 25±0.5°C for 48 h. After reaching equilibrium, the mixtures were centrifuged at 10,000 rpm for 40 min. The supernatant was diluted with methanol. Concentrations were determined using high-performance liquid chromatography by using the Reddy *et al.*[13] and Poongothai *et al.*[14] methods for TFL and ALA, respectively.

Ternary phase diagram construction: Using different ratios of oil, surfactant and co-surfactant with the highest solubility for TFL and ALA, a ternary phase diagram was constructed to determine the area of globular size as smaller than 1 µm. As a result, nine formulae as in Table 1, were used, with anise oil, Tween 20 as a surfactant and PEG 200 as a co-surfactant. The total of the three components always summed to 100%. Ten milligrams of TFL and 100 mg of ALA were added to each formula.

TFL-ALA droplet morphology: The morphology of the selected formula droplets was evaluated using a transition electron microscope. To confirm the typical morphology of the SNEDDS, a transmission electron microscope(JEOL 4000EX transmission electron microscope, Tokyo, Japan) was used.

TFL-ALA droplet size: Two hundred microliters of each formula was diluted with 40 mL of distilled water and rotated for 5 min. Globular size was then determined directly using the Zetatrac instrument manufactured by Microtrac Inc. (PA, USA).

Table 1: Formula component ratios

Formula	Oil (Anise oil)	Surfactant (Tween 20)	Co-surfactant (PEG 200)	Size (nm)
A1	0.15	0.45	0.40	350.2±23.1
A2	0.30	0.20	0.50	432.9±87.2
A3	0.25	0.30	0.45	287.6±21.2
A4	0.10	0.40	0.50	134.3±12.3
A5	0.20	0.40	0.40	653.5±61.2
A6	0.30	0.50	0.20	215.3±45.3
A7	0.15	0.40	0.45	742.7±35.3
A8	0.25	0.45	0.30	379.3±42.3
A9	0.10	0.50	0.40	501.4±61.2

Transdermal patch formulation: Three polymers were tried as a matrix for the transdermal patches: Carboxyl methyl cellulose, hydroxyl propyl methyl cellulose (HPMC) and hydroxyl propyl cellulose solutions of each polymer with a concentration of 1%, formula No A4, was added to each solution. The solutions were stirred with a magnetic stirrer for 2 h and then poured into a petri dish and maintained at 40°C in an oven until the solvent was completely evaporated. Control films were prepared using raw TFL.

***In vitro* release study:** A Franz diffusion cell apparatus (Microette Plus, Hanson Research, Chatsworth, CA, USA) was used to assess the diffusion of prepared films and the same drug concentration was suspended in distilled water and used as a control (2 cm^2 used as a film). Each diffusion cell had a donor and receptor chamber, with a film placed between them. The receptor medium was phosphate-buffered saline (pH 7.2), the stirring rate was 400 rpm and the temperature was maintained at 32±0.5°C. The auto sampler collected aliquots at 0.5, 1, 2, 4, 8, 10 and 12 h and then, these were analyzed by spectrophotometer at 230 nm.

TFL-ALA SNEDDS skin permeation imaging by fluorescent microscope: To confirm the permeation of TFL-ALA SNEDDS into the skin layers, Rhodamine B dye (Rh B) used in a plain HPMC- film and solubilized in SNEDDS formula and poured on the HPMC solution to form the formulated film. The formulation was allowed to permeate the skin layers using the same conditions as in the previous study. After 1 and 3 h, skin samples were washed with distilled water and then preserved in 10% (v/v) formalin in phosphate-buffered saline (pH 7.4) for 24 h. Skin samples were cut and imaged using an inverted Zeiss LSM 510 META microscope (Carl Zeiss, Jena, Germany). The excitation wavelength used was 562 nm and the emission wavelength was 587 nm.

Protective effect of ALA on endothelial cells (EA. hy926) under high glucose stress: EA. hy926 cells were gifted from Dr. Ahmed Al-abd (Faculty of Pharmacy, King Abdulaziz University) and 33 mmol L^{-1} of D-glucose (Sigma) was added to Dulbecco's minimal essential medium, which contains 10% (v/v) fetal bovine serum. The cells were cultured to confluence for 18-24 h before use in an experiment. Serial dilutions of TFL (0.001-10 mg mL^{-1}), TFL-ALA formula (with concentrations of TFL 0.001-10 mg mL^{-1} and ALA 10-100 mg mL^{-1}) and SNEDDS free drugs were dissolved in 0.4% v/v of dimethylsulfoxide. After 72 h of incubation, cytotoxicity was determined by MTT assay according to the manufacturer's protocol, which has been prescribed previously in detail[15]. Analysis of variance was used to determine the significance of the treatments, with a $p<0.05$ considered to indicate a significant difference.

RESULTS

TFL and ALA solubility in different oils is shown in Fig. 1, surfactants and co-surfactants. Anise oil shows the highest solubility for both TFL and ALA (48.11±5.1 and 27.15±2.5 mg mL^{-1}, respectively). For surfactants, Tween 20 showed superior solubilizing potential for TFL (24.67±3.4 mg mL^{-1}), however, Tween 80 showed the highest solubilization for ALA (21.6±2.3 mg kg^{-1}) and TFL (21.56±3.2 mg mL^{-1}). Finally, PEG 200 solubilized 0.49±0.1 and 7.8±1.2 mg kg^{-1} of TFL and ALA, respectively. The detailed composition and characteristics of the ternary phase mixture are demonstrated in Fig. 2.

It was observed that the maximum concentration of anise oil that could be solubilized was 30% using 50% of Tween 20 and 50% of PEG 200. However, when the concentration of PEG 200 was increased with respect to Tween 20, the maximum concentration of oil that could be solubilized was decreased to 10%. This concentration was increased by increasing the amount of Tween 20 along with the amount of PEG 200.

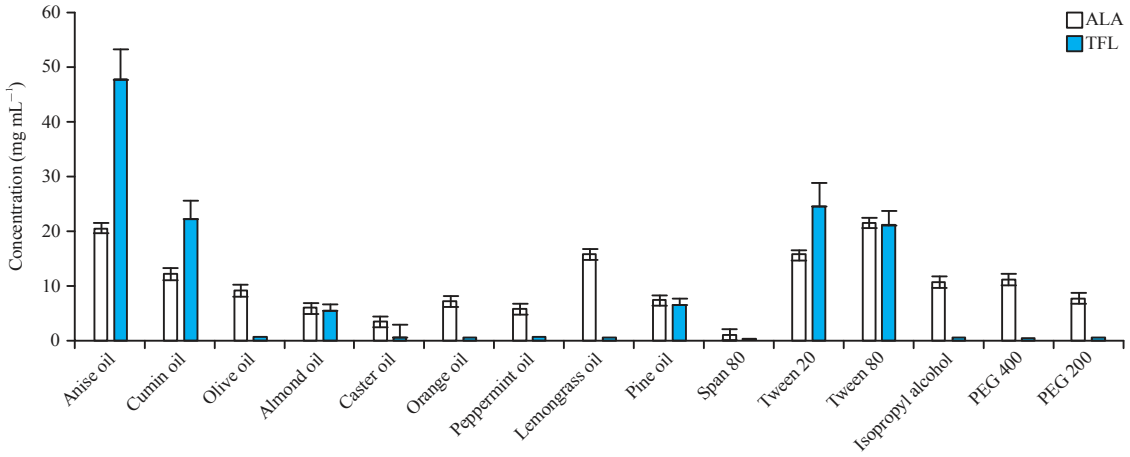

Fig. 1: TFL and ALA solubility in different oils, surfactant and co-surfactants

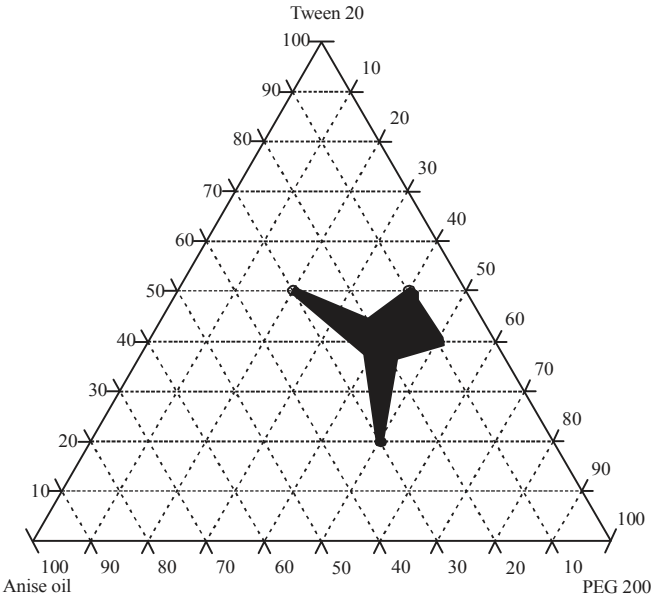

Fig. 2: Ternary phase diagrams of TFL-ALA indicating the oil-in-water (o/w) nanoemulsion region at different anise oil, Tween 20 and PEG 200 ratios

TFL-ALA nanoemulsion droplets of the selected formula in the range of 100-200 nm, which agreed with the dynamic light scattering results of the Zetatrac are shown in Fig. 3. With regard to TFL release, Fig. 4 shows a dramatic increase in the percentage of cumulative TFL released from the TFL-ALA patch (80%), whereas the plain film did not exceed 20%.

The fluorescence intensity within the rat skin layers is shown in Fig. 5. One hour after starting the experiment, very low intensity is observed in Fig. 5a in comparison with Fig. 5b, thus confirming the penetration of Rh B in the rat skin after 1 h of treatment with TFL-ALA SNEDDS patch. After 3 h, no significant change in the fluorescence was observed as in Fig. 5c, whereas Fig. 5d shows higher intensity with detailed skin layers as a result of penetration of Rh B dye into all skin layers. Data in Fig. 6 reveales a significant number of cells still viable after treatment with TFL-ALA SNEDDS, a higher percentage than cells treated with TFL alone. There are 88.54±3.43% still viable after treatment with 1 mg mL^{-1} TFL-ALA SNEDDS, while 68.6±6.54% of the cells are still viable after treatment with 1 mg mL^{-1} of raw TFL. Cell viability was expressed as the percent absorbance for cells exposed to TFL solution or each TFL-ALA SNEDDS relative to absorbance measured for cells without treatment.

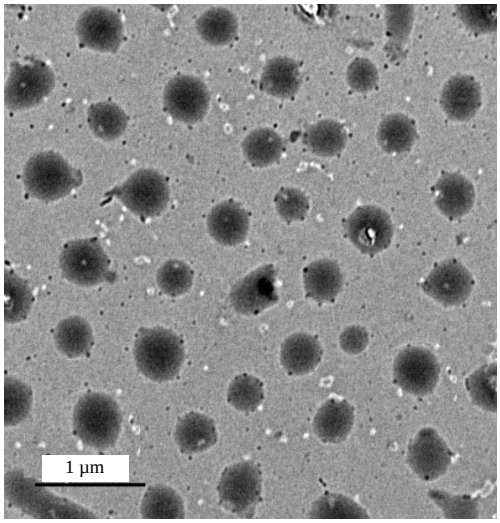

Fig. 3: TFL-ALA SNEDDS TEM image

DISCUSSION

ED is a complication of diabetes mellitus that affects about 80% of diabetic men, so it is necessary to find an efficient way to improve their life quality and convenience. TFL has very low solubility, so authors aimed to enhance its

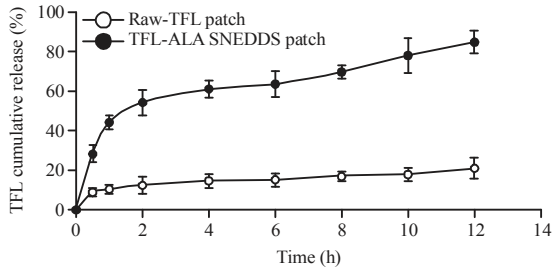

Fig. 4: Percentage of cumulative TFL release from raw drug patch and TFL-ALA SNEDDS patch

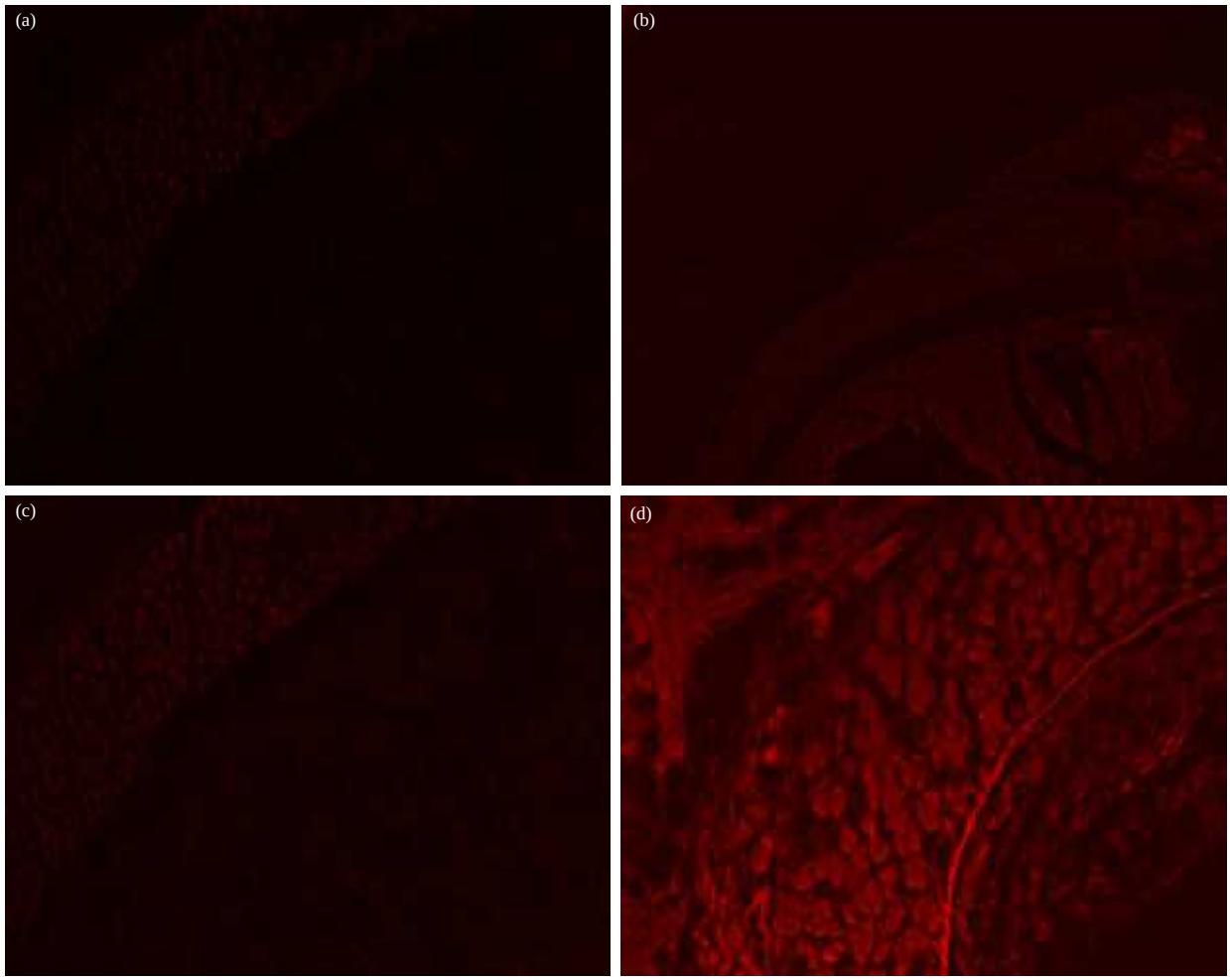

Fig. 5(a-d): Fluorescent microscope images (a, c) After treatment of skin layers with plain Rh B dye and (b, d) SNEDDS-loaded HPMC film after 1 and 3 h, respectively

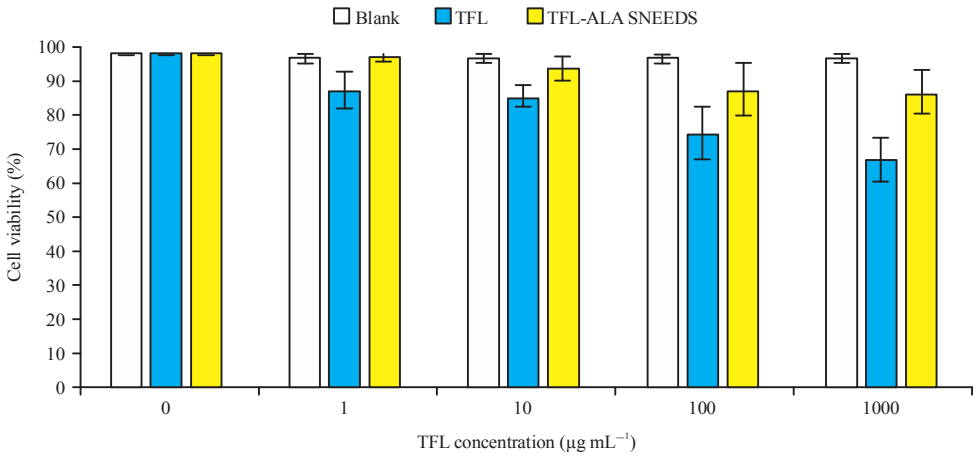

Fig. 6: *In vitro* cytotoxicity of TFL, TFL-ALA SNEDDS formula (A4) in EA. hy926 cells for 72 h
n = 3, Mean±SD, *p<0.05

solubility using a transdermal patch for topical use. Current study succeeded in formulating a nanoglobular size of TFL to further improve its release by about 2.8-fold in comparison with raw TFL. Cell viability data revealed significant improvement in epithelial cell survival under high oxidative stress, which was enhanced by 1.3-fold as a result of the presence of ALA. With regard to TFL release after 12 h, data revealed the role of oil, surfactant and co-surfactant in dispersing TFL in the buffered medium, while the sustained release was a result of the interaction of TFL with ALA, which increases the viscosity of the droplets, causing them to take more time to separate from the HPMC-film[16,17]. The greater intensity of Rh B dye of TFL-ALA SNEDDS could be attributed to the role of surfactant and co-surfactant, which are more lipophilic than plain dye and penetrate skin layers more easily. EA. hy926 cells were grown under high-glucose stress to evaluate the protective effect of ALA on endothelial cells. Dulbecco modified eagle medium[18] was supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units mL^{-1} penicillin and 100 µg mL^{-1} streptomycin in 5% CO_2 at 37°C. Diabetes is one of the major factors for precipitating atherosclerosis, which causes myocardial infarction, neuropathic injury and stroke. Excessive blood glucose leads to oxidative stress, resulting in overproduction of free reactive oxygen species (ROS)[19]. The ROS lead to mitochondrial damage that activates intracellular pathways (e.g., protein kinase C and hexosamine pathways) and causes endothelial cell damage[20]. Mitogen-Activated Protein Kinase (MAPK) cascades are activated in the endothelial cells and lead to degradation of insulin receptors due to excessive oxidative stress.

Many reports have revealed the role of ALA in the reduction of glucose uptake in both insulin-resistant and insulin-sensitive muscle tissues[21]. Because ALA enhances glycemic control, it has an important role in detoxification mechanisms. Previous studies have showed that daily ALA intake for 1 month activates a PI3K/Akt-dependent signaling pathway[22], thus preventing diabetes-mediated mitochondrial and endothelial dysfunction. Furthermore, ALA reduces ROS generation and increases Reduced Glutathione/Glutathione Disulphide (GSH/GSSG) redox status in penile tissue. A recent study confirmed that intravenous ALA enhances endothelium-dependent vasodilatation of the vasculature in patients with type-2 diabetes, furthermore, ALA supplementation was reported to improve neural blood flow and nerve conduction[23].

CONCLUSION

The study confirmed the protective effect of ALA on endothelial cells under high-glucose oxidative stress. Moreover, the prepared ALA-TFL transdermal patch based on SNEDDS achieved great enhancement of TFL release and increased the ability of the endothelial cells to survive high-glucose oxidative stress. Finally, loading ALA and TFL together may provide a novel combination to cure in diabetic patients.

SIGNIFICANCE STATEMENT

This study represents a novel combination of TFL and ALA for treatment erectile dysfunction in diabetic patients. This combination in form of a transdermal patch achieved an efficient TFL delivery with efficient protection to the epithelial cells that line the penile vessels. ALA will regenerate the damaged epithelial cells function which lead to reproduction of nitric oxide. That dilate penile blood vessels allow to fill with

blood after stimulation. TFL release enhanced by 2.83 folds and epithelial cells survival was increased by 130%. This novel combination will improve and patient's convenience and his life quality.

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