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Research Article
Novel Combination of Alprostadil-D-tocopheryl Polyethylene Glycol Succinate for Treatment of Erectile Dysfunction

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Abstract
Background and Objective: Erectile dysfunction (ED) is a common disease in diabetic patients. Alprostadil (Prostaglandin E1, PGE1) has shown higher success rates compared with phosphodiesterase type 5 inhibitors (PDE-5) in the treatment of ED. The combined formula of PGE1 with D-tocopheryl polyethylene glycol succinate (TPGS) was aimed to find a novel treatment for diabetic patients suffering from ED via protecting epithelial cells that lined penile vessels from the oxidative stress. **Materials and Methods:** PGE1-TPGS was prepared either with labrasol, polyethylene glycol 200 (PEG 200) or a mixture of labrasol and PEG 200, F1, F2 and F3 respectively. The prepared formulations were characterized for morphology and zeta potential and then were added to 1.5% of hydroxypropyl methyl cellulose solution to form topical hydrogel. Drug release study for all investigated formulations was conducted in comparison with raw-PGE1 and tested in endothelial cells (EA. hy926) under stress condition. **Results:** The obtained data revealed that PGE1-TPGS prepared with labrasol achieved the highest particle size while, PGE1-TPGS formula of PEG 200 and labrasol mixture showed the smallest particle size. Transmission electron microscope (TEM) images of the prepared formulations showed spherical shape micelles. Oxidative stress investigation showed a dramatic improvement in epithelial cell survival that attenuated the permeation enhancer's cytotoxicity. F1, F2 and F3 treatments showed improved cell viability when compared with blank formulations. At concentration 10 μg mL^{-1}, results showed that the investigated formulations improved cell viability from 9.13% (F1 blank), 7.55% (F2 blank) and 8.82% (F3 blank) to 90.52, 91.27 and 93.94% for F1, F2 and F3 formulations, respectively. **Conclusion:** The study revealed that combination of PGE1 with TPGS micelles formula loaded topical gel could be a successful strategy for the treatment of ED in diabetic patients.

Key words: Nitric oxide, free radicals, neuropathy, micelles, Impotence

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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 disease in diabetic patients (about 80%) as a result of lack of efficient blood flow, peripheral nerve damage and endothelial dysfunction[1]. High glucose level causes oxidative stress and release of high levels of reactive oxygen species (ROS) which affect endothelial function[1-4]. The reduction in the level of nitric oxide (NO) released affect penile vessels relaxation. This leads to insufficient blood flow and lack of the ability to maintain strong erection[5-7]. Although phosphodiesterase type 5 inhibitors are the drug of choice for ED treatment, its efficacy to cure diabetic patients is still limited[8,9]. Alprostadil (PGE1) has gained attention after successful clinical trials[10-12]. Food and drug administration (FDA) approved alprostadil (Caverject by Upjohn) in 1995, the first marketed intra urethral injection of PGE1 which considered an unfavorable route of administration.

Micelles have gained great attention nowadays due to its ability to reach the site of action and its nano-scale size[13]. Vitamin E, a potent antioxidant that showed good effect on the management of ED in older rats[14]. Furthermore, Vitamin E increased the blood flow and oxygen to the penis. Several studies suggested Vitamin E has several medical uses includes, lower blood glucose levels and antimicrobial activity. It can interact with free radicals that lead to reduction of the risk of diabetic complications, with symptoms like tingling, pain, burning, itching and numbness in arms and legs as a result of nerve damage that lead to peripheral neuropathy. D-tocopheryl polyethylene glycol succinate (TPGS) approved by FDA as a nutritional supplement, showed multiple applications in drug delivery systems[15-20]. The TPGS is characterized by biocompatibility, enhanced drug solubility, permeability and selective antitumor activity.

Topical gels were used previously in several studies for the management of ED; due to its applicability and ease of use with controlled manner and extra advantage of facilitation of intercourse for their use as lubricant[21,22]. Several drawbacks could affect life quality in diabetic patients in case of lack of sex as anxiety, depression, isolation, loneliness and loss of self-esteem. The available PGE1 injected intracavernous, could lead to penile pain, occurrence of hematoma, formation of corporal nodules and penile fibrosis. Accordingly, the aim of this study was the formulation of PGE1-TPGS micelles loaded in topical gel to enhance skin permeability as an alternative dosage form to the intra urethral injection.

MATERIALS AND METHODS

All experiments were carried out at Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, in the period from February-October, 2018 at Pharmaceutical Technology and Tissue Culture labs. PGE1 was purchased from MOBELBIO (main land, China). TPGS, Labrasol, PEG200, D-glucose and hydroxypropyl methylcellulose (HPMC), acetonitrile HPLC grade, methanol HPLC grade were purchased from Sigma Aldrich, (USA). The EA. hy926 cells were provided as a kind gift from Dr. Serag Elbahairy (Faculty of Science, King Khalid University, Saudi Arabia).

Preparation of PGE1-TPGS micelles: The TPGS and PGE1 were dissolved in 10 mL ethanol as previously described with slight modification[17,19,23]. The ethanolic solution was added dropwise to a stirring permeability enhancer (labrasol, PEG and Labrasol-PEG mixture) aqueous solution and the solution was kept stirring to remove ethanol by evaporation. After that, the residual ethanol was removed by using rotary-evaporator (Bukhi, Switzerland). Formulations were prepared as indicated in Table 1.

Particle size analysis of PGE1-TPGS micelles: The prepared formulations were assayed for particle size using Malvern® ZS particle size analyzer. The formulations were diluted with a certain volume of water and the average of vesicle size was expressed using five replicate samples.

PGE1-TPGS micelles investigation by transmission electron microscope (TEM): The prepared PGE1-TPGS micelles were studied by using Transmission electron microscope (TEM).

Stability study of the formulation: The physical stability of micelle formulations was evaluated for morphology after 3 months of storage.

Preparation of PGE1-TPGS micelles loaded gel formula: PGE1-TPGS micelles (F 1-3) were utilized in the preparation of gel formulations. Hydroxypropyl methylcellulose (HPMC, 1.5 g) was dispersed in PGE1-TPGS micelles (100 mL) by using magnetic stirrer. The dispersion was sonicated for 2 min to

Table 1: PGE1-TPGS micelle formulation components

Formula	PGE1 (μg)	TPGS (mg)	Labrasol (mg)	PEG 200 (mg)
F1	30	5	10	-
F2	30	5	-	10
F3	30	5	5	5

diffuse any lumps. After that, the formed gel was stored at 4°C for 24 h. In addition, raw PGE1 gel was prepared using HPMC (1.5% w/v)[24].

Diffusion study of PGE1-TPGS micelles loaded gel formula: A Franz diffusion cell apparatus (Microette Plus, Hanson Research, Chatsworth, CA, USA) was used to assess the diffusion of the prepared PGE1-TPGS micelles loaded gel formulations. Raw PGE1 gel was used as a control. Each diffusion cell had a donor and receptor chamber, with rat skin, free from hair and subcutaneous fat, inserted between them. The receptor medium was phosphate-buffered saline (pH 7.2) with a stirring rate of 400 rpm and the temperature was maintained at 32±0.5°C. The auto sampler collected aliquots at 0.5, 1, 2, 3, 4 and 6 h. Collected PGE1samples were analyzed by HPLC at 230 nm according to Paul *et al.*[25] .

Oxidative stress study: Protective effect of TPGS on endothelial cells (EA. hy926) was investigated under high glucose stress. D-glucose (33 mmol) was added to Dulbecco's minimal essential medium, which contained 10% (v/v) fetal bovine serum. Cells were cultured for 18-24 h before use. Serial dilutions of each formula in terms of PGE1 concentration (0.001-10 mg mL^{-1}) and permeation enhancers (with concentrations of 0.001-10 µg mL^{-1}) were used. After 72 h of incubation, cytotoxicity was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's protocol, which has been prescribed previously in details[26].

Data analysis: One-way analysis of variance (ANOVA) with Dunnett's multiple comparison test used to compare data using GraphPad Prizm software (La Jolla, CA, USA). A $p<0.05$ indicated significant difference of the investigated data.

RESULTS

Formulation and characterization of PGE1-TPGS micelles: PGE1-TPGS micelles formulations (F1, F2 and F3) were prepared as a clear dispersion. The results of particle size and polydispersity index (PDI) for the prepared PGE1-TPGS micelle formulations are presented in Table 2. Size distribution of the prepared micelles formulations (F1, F2 and F3) were measured by particle size analyzer. Results revealed that F2 achieved the smallest PDI value that means PEG 200 produced homogenous micelle sizes in comparison with labrasol or mixture of labrasol and PEG 200 (Table 2).

Formula F1 achieved highest particle size (69.32±3.2 nm). On the other hand, the smallest particle size (10.91±1.1 nm) with PDI value of 0.62 was achieved by formula F3. Formula F2 prepared with PEG 200 showed the particle sizes relatively higher than F3. Figure 1 showed the TEM images of the micelle formulations (F1, F2 and F3). The prepared formulations showed a wide range of cluster sizes (micelle aggregates). The TEM images confirmed the formulation sizes measured by particle size analyzer and showed spherical particles with smooth edges. Stability study revealed no significant change in size after 3 months of the micelle preparation (F1, F2 and F3) when stored at room temperature for 90 days.

The results of *ex vivo* diffusion study via Franz cell diffusion apparatus revealed that the prepared formulations F2 and F3 completely released PGE1 within the first 30 min of the study when compared with F1 and raw-PGE1

Table 2: PGE1-TPGS micelle formulations size and PDI

Formula	Size (nm)	PDI
F1	69.32±3.2	0.51
F2	13.21±0.6	0.35
F3	10.91±1.1	0.62

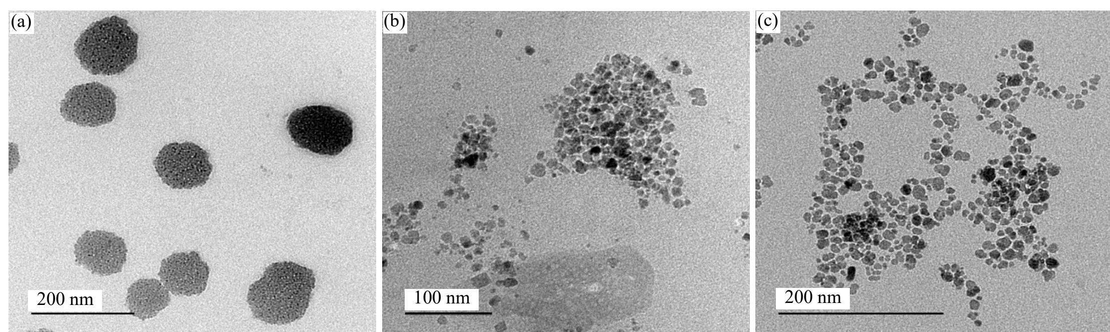

Fig. 1(a-c): TEM images of PGE1-TPGS formulations

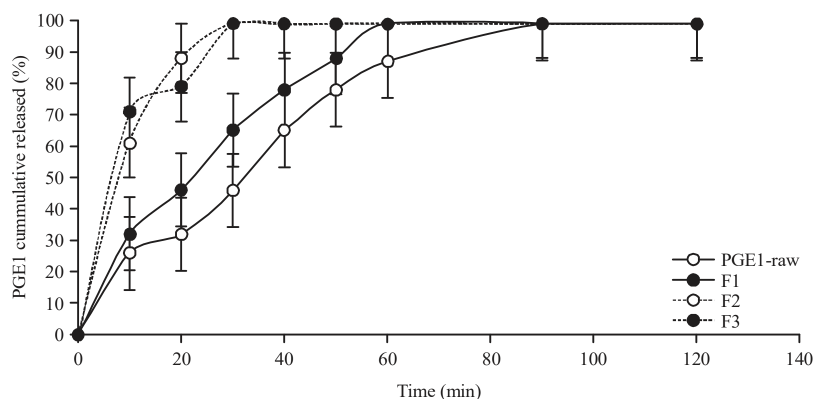

Fig. 2: Diffusion of PGE1 from different formulations and raw PGE1

Table 3: Endothelial cells (EA. hy926) survival (%) of raw PGE1 and different formulations and their blanks under stress condition

Concentration (µg mL^{-1})	F1	F2	F3	F1 (blank)	F2 (blank)	F3 (blank)
0	100.00±0.57	100.00±0.63	100.00±0.52	100.00±5.32	102.03±4.56	96.86±9.41
0.01	92.07±0.50	96.49±0.11	96.54±1.99	73.31±6.13	72.33±12.11	72.43±4.63
0.1	91.03±1.90	92.97±0.96	95.70±0.67	73.10±0.31	72.27±1.73	72.46±1.41
1	90.72±0.47	92.48±1.38	95.67±0.53	37.72±0.38	39.95±2.00	34.03±6.23
10	90.53±0.46	91.26±1.48	94.94±0.48	9.13±1.75	7.55±5.03	8.82±2.25
100	89.59±0.81	90.07±2.3	93.17±1.04	3.37±0.18	3.50±2.53	2.75±0.89

formulations that released PGE1 content within 60 and 90 min, respectively as shown in Fig. 2. The release of raw-PGE1 showed relatively slower release pattern when compared with the investigated formulations as shown in Fig. 2.

Oxidative stress study: Oxidative stress showed a dramatic improvement in epithelial cell survival. The improvement in cell survival was in the result of the effect of attenuated cytotoxicity of the permeation enhancers. According to Table 3, treatments F1, F2 and F3 showed improved cell viability when compared with blank formulations. At concentration 10 µg mL^{-1}, results showed that the investigated formulations improved cell viability from 9.13% (F1 blank), 7.55% (F2 blank) and 8.82% (F3 blank) to 90.52, 91.27 and 93.94% for F1, F2 and F3 formulations, respectively (Table 3). The results revealed no significant difference ($p<0.05$) in cell survival (%) at the investigated concentration range of F1, F2 and F3 formulations.

DISCUSSION

Results demonstrated that loading PGE1 with TPGS achieved small micelles less than 40 nm that facilitated formula permeation through skin layers according to previous results[23]. Nano-scale particle sizes facilitated PGE1 release completely within 20 min that showed rapid onset of action when compared with raw (unformulated) PGE1. Moreover, the study indicated that PGE1 protected endothelial cells from labrasol, PEG 200 in the oxidative condition. PGE1 increased the percentage of survived cells, which may be attributed to the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzymes. Moreover, PGE1 upregulated endothelial NO synthase (eNOS) and mRNA expression[27]. Oxidative stress played a crucial role in endothelial dysfunction and is a vital pathogenic element in the development of cardiovascular diseases, including atherosclerosis, hypercholesterolemia and disseminated intravascular coagulation. In a previous study, it was manifested that H_2O_2 as a precursor of ROS can markedly boom cellular permeability, negatively affect the antioxidant mechanisms and set off endothelial cell apoptosis[28] at concentration 200 µmol L^{-1}. It was reported that inhibition of ROS production can protect cells from the cellular damage effects of oxygen radicals[28]. The present study agreed with previously published articles that showed PGE1 not only eradicated the intracellular ROS caused by H_2O_2, but also efficiently improved the viability of H_2O_2-induced endothelial cells. PGE1 protected human retinal pigment epithelial cells from oxidative hazards and stopped the production of ROS in the intestinal mucosa of methotrexate-dealt with rats. These outcomes indicated that the protective impact is related to PGE1 scavenging intracellular ROS. Lipid peroxidation is one of the factor that induce radical-mediated cell damage[29]. Those effects confirmed that the scavenging of ROS is probably associated with the accelerated antioxidant enzymes. H_2O_2 is effective in reducing the viability of cells and also induces apoptosis[30-32].

Previous studies have proven that PGE1 protects human liver sinusoidal endothelial cells and endothelial progenitor cells from apoptosis[32-34]. According to previous reports, H_2O_2 (200 µmol L^{-1}) induced cell apoptosis, while PGE1 (0.25-1.00 µmol L^{-1}) prevented H_2O_2 apoptosis in a concentration-dependent way[35,36]. As a result, PGE1 prevents apoptosis because of reduced intracellular ROS and extended antioxidant enzymatic activities[37,38]. The NO is a soluble material constantly synthesized via the endothelium and has a wide range of characters that keep vascular homeostasis, along with modulation of vascular dilator tone and protecting the vessel from injury, because of platelets and cells circulating in blood[39]. NO terminates chain reactions during lipid peroxidation, as observed in version lipid systems of low-density lipoprotein oxidation and in cells. These consequences proposed that the NO pathway plays an important function within the shielding activity of PGE1 towards H_2O_2-brought about oxidative ROS; however the mechanisms are nevertheless uncertain. PGE1 turns on adenylate cyclase and increases intracellular cAMP through binding to proteinoid receptor subtypes EP2, EP3, EP4 and IP[40,41]. The cAMP-responsive elements (CRE) inside the human eNOS promoter play a vital function in the inducible expression of the human eNOS gene. PGE1-responsive sequences are still uncertain[40]. PGE1 increases eNOS expression via protein kinase cAMP-dependent pathway activation and cAMP-responsive element binding protein (CREB) phosphorylation. Phosphorylated CREB pre-dominantly binds to CRE, that results in elevated promoter activity and upregulation of the eNOS protein and mRNA expression[30,39].

According to these results, diabetic patients who suffer from ED are recommended to use PGE1-TPGS micelles loaded gel formula. The investigated formula regenerated penile epithelial cells' function that could result in improved diabetic patient's sexual activity and enhancement of quality of life.

CONCLUSION

This study represented a novel combination of PGE1 and TPGS for treatment of ED in diabetic patients. This combination was included in a topical gel preparation for enhanced and efficient skin delivery. The damaged epithelial cells were regenerated by PGE1 that resulted in improved dilatation of penile blood vessels and erection. Formulated PGE1 released from micelles increased the onset of action by 4.5 folds when compared with raw PGE1 and dramatically improved epithelial cells survival under the stress condition. This novel mixture could improve patient's convenience and satisfactory of lifestyles, particularly in diabetic patients.

SIGNIFICANCE STATEMENT

This study discovered an exclusive combination of PGE1 and Vitamin E (TPGS) in the form of micelles loaded gel preparation for treatment of ED. This combination achieved efficient protection for the epithelial cells in diabetic patients. This study revealed the effective and applicable treatment for diabetic patients suffering from ED that opens the way for other researchers to evaluate the promising formula in clinical investigations. Finally, PGE1 loaded TPGS micellar form could provide a novel strategy for efficient transdermal delivery of PGE1 to replace the currently available invasive (injection) treatment for diabetic patients.

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REFERENCES

1. Jeremy, J.Y., R.A. Jones, A.J. Koupparis, M. Hotston, R. Persad, G.D. Angelini and N. Shukla, 2007. Reactive oxygen species and erectile dysfunction: Possible role of NADPH oxidase. Int. J. Impotence Res., 19: 265-280.
2. Maiorino, M.I., G. Bellastella and K. Esposito, 2014. Diabetes and sexual dysfunction: Current perspectives. Diabetes Metab. Syndr. Obes., 7: 95-105.
3. Volpe, C.M.O., P.H. Villar-Delfino, P.M.F. dos Anjos and J.A. Nogueira-Machado, 2018. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis., Vol. 9 10.1038/s41419-017-0135-z
4. Busik, J.V., S. Mohr and M.B. Grant, 2008. Hyperglycemia-induced reactive oxygen species toxicity to endothelial cells is dependent on paracrine mediators. Diabetes, 57: 1952-1965.
5. Ahmed, T.A., K.M. El-Say, B.M. Aljaeid, U.A. Fahmy and F.I. Abd-Allah, 2016. Transdermal glimepiride delivery system based on optimized ethosomal nano-vesicles: Preparation, characterization, *in vitro*, *ex vivo* and clinical evaluation. Int. J. Pharmaceut., 500: 245-254.
6. Fahmy, U.A., 2018. Augmentation of fluvastatin cytotoxicity against prostate carcinoma PC3 cell line utilizing alpha lipoic–ellagic acid nanostructured lipid carrier formula. AAPS Pharm. Sci. Tech., 19: 3454-3461.
7. Aldawsari, H.M., M.A. Elfaky, U.A. Fahmy, B.M. Aljaeid, O.A. Alshareef and K.M. El-Say, 2018. Development of a fluvastatin-loaded self-nanoemulsifying system to maximize therapeutic efficacy in human colorectal carcinoma cells. J. Drug Deliv. Sci. Technol., 46: 7-13.

8. Gresser, U. and C.H. Gleiter, 2002. Erectile dysfunction: Comparison of efficacy and side effects of the PDE-5 inhibitors sildenafil, vardenafil and tadalafil-review of the literature. Eur. J. Med. Res., 7: 435-446.
9. Berner, M.M., L. Kriston and A. Harms, 2006. Efficacy of PDE-5-inhibitors for erectile dysfunction. A comparative meta-analysis of fixed-dose regimen randomized controlled trials administering the international index of erectile function in broad-spectrum populations. Int. J. Impotence Res., 18: 229-235.
10. Hanchanale, V. and I. Eardley, 2014. Alprostadil for the treatment of impotence. Expert Opin. Pharmacother., 15: 421-428.
11. Belew, D., Z. Klaassen and R.W. Lewis, 2015. Intracavernosal injection for the diagnosis, evaluation, and treatment of erectile dysfunction: A review. Sexual Med. Rev., 3: 11-23.
12. Swerdloff, R.S. and C. Wang, 2016. Sexual Dysfunction. In: Endocrinology: Adult and Pediatric, Jameson, J.L. and L.J. De Groot (Eds.). Elsevier Health Sciences, Netherlands, pp: 2154-2162.
13. USFDA., 1995. Caverject (alprostadil). In FDA Approved Drugs. Center Watchd.
14. Helmy, M.M. and A.M. Senbel, 2012. Evaluation of vitamin E in the treatment of erectile dysfunction in aged rats. Life Sci., 90: 489-494.
15. Guo, Y., J. Luo, S. Tan, B.O. Otieno and Z. Zhang, 2013. The applications of vitamin E TPGS in drug delivery. Eur. J. Pharm. Sci., 49: 175-186.
16. Mi, Y., J. Zhao and S.S. Feng, 2012. Vitamin E TPGS prodrug micelles for hydrophilic drug delivery with neuroprotective effects. Int. J. Pharm., 438: 98-106.
17. Zhang, Z., S. Tan and S.S. Feng, 2012. Vitamin E TPGS as a molecular biomaterial for drug delivery. Biomaterials, 33: 4889-4906.
18. Yang, C., T. Wu, Y. Qi and Z. Zhang, 2018. Recent advances in the application of vitamin E TPGS for drug delivery. Theranostics, 8: 464-485.
19. Ahmed, O.A.A. and S.M. Badr-Eldin, 2018. *In situ* misemgel as a multifunctional dual-absorption platform for nasal delivery of raloxifene hydrochloride: Formulation, characterization and *in vivo* performance. Int. J. Nanomed., 13: 6325-6335.
20. Saxena, V. and M.D. Hussain, 2013. Polymeric mixed micelles for delivery of curcumin to multidrug resistant ovarian cancer. J. Biomed. Nanotechnol., 9: 1146-1154.
21. Kim, E.D., R. El-Rashidy and K.T. McVary, 1995. Papaverine topical gel for treatment of erectile dysfunction. J. Urol., 153: 361-365.
22. Kim, E.D. and K.T. McVary, 1995. Topical prostaglandin-E1 for the treatment of erectile dysfunction. J. Urol., 153: 1828-1830.
23. Ahmed, O.A.A., K.M. El-Say, B.M. Aljaeid, S.M. Badr-Eldin and T.A. Ahmed, 2019. Optimized vinpocetine-loaded vitamin e D-α-tocopherol polyethylene glycol 1000 succinate-alpha lipoic acid micelles as a potential transdermal drug delivery system: *In vitro* and *ex vivo* studies. Int. J. Nanomed., 14: 33-43.
24. Fahmy, U.A., O.A.A. Ahmed and K.M. Hosny, 2015. Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. AAPS PharmSciTech, 16: 53-58.
25. Nicklin, P., A.C. Keates, T. Page and C.J. Bailey, 1996. Transfer of metformin across monolayers of human intestinal Caco-2 cells and across rat intestine. Int. J. Pharm., 128: 155-162.
26. Fahmy, U.A. and B.M. Aljaeid, 2016. Combined strategy for suppressing breast carcinoma MCF-7 cell lines by loading simvastatin on alpha lipoic acid nanoparticles. Expert Opin. Drug Deliv., 13: 1653-1660.
27. Morales, J.O., K.R. Fathe, A. Brunaugh, S. Ferrati and S. Li *et al*., 2017. Challenges and future prospects for the delivery of biologics: Oral mucosal, pulmonary and transdermal routes. AAPS J., 19: 652-668.
28. Gomes, M.B. and C.A. Negrato, 2014. Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. Diabetol. Metab. Syndrome, Vol. 6. 10.1186/1758-5996-6-80
29. El Saghier, E.O.A., S.E. Shebl, O.A. Fawzy, I.M. Eltayeb, L.M.A. Bekhet and A. Gharib, 2015. Androgen deficiency and erectile dysfunction in patients with type 2 diabetes. Clin. Med. Insights: Endocrinol. Diabetes., 8: 55-62.
30. Lv, R., L. Du, C. Lu, J. Wu and M. Ding *et al*., 2017. Allicin protects against H_2O_2 induced apoptosis of PC12 cells via the mitochondrial pathway. Exp. Ther. Med., 14: 2053-2059.
31. Wang, Z., D. Wang, Y. Li and X. Zhang, 2014. Protective effects of verapamil against H_2O_2-induced apoptosis in human lens epithelial cells. Biomol. Ther., 22: 553-557.
32. Gensch, C., Y. Clever, C. Werner, M. Hanhoun, M. Böhm and U. Laufs, 2007. Regulation of endothelial progenitor cells by prostaglandin E1 via inhibition of apoptosis. J. Mol. Cell. Cardiol., 42: 670-677.
33. Yang, H., P. Majno, P. Morel, C. Toso and F. Triponez *et al*., 2002. Prostaglandin E_1 protects human liver sinusoidal endothelial cell from apoptosis induced by hypoxia reoxygenation. Microvasc. Res., 64: 94-103.
34. Hammoutene, A. and P.E. Rautou, 2019. Role of liver sinusoidal endothelial cells in nonalcoholic fatty liver disease. J. Hepatol., 10.1016/j.jhep.2019.02.012.
35. Teramoto, S., T. Tomita, H. Matsui, E. Ohga, T. Matsuse and Y. Ouchi, 1999. Hydrogen peroxide-induced apoptosis and necrosis in human lung fibroblasts: Protective roles of glutathione. Jap. J. Pharmacol., 79: 33-40.

36. Quintero, A., C.A. Pedraza, E. Siendones, A.M.K. El-Said and A. Colell *et al*., 2002. PGE1 protection against apoptosis induced by D-galactosamine is not related to the modulation of intracellular free radical production in primary culture of rat hepatocytes. Free Radical Res., 36: 345-355.
37. El-Say, K.M., A.R.M. El-Helw, O.A.A. Ahmed, K.M. Hosny and T.A. Ahmed *et al*., 2015. Statistical optimization of controlled release microspheres containing cetirizine hydrochloride as a model for water soluble drugs. Pharm. Dev. Technol., 20: 738-746.
38. Fahmy, U.A., A.E. El-Sisi, H.A. El-Ghamry and A.S. Zidan, 2013. Effect of concomitant administration of amoxicillin on the pharmacokinetics and bioavailability of metformin. Latin Am. J. Pharm., 32: 1074-1081.
39. Jones, W., X. Li, Z.C. Qu, L. Perriott, R.R. Whitesell and J.M. May, 2002. Uptake, recycling and antioxidant actions of α-lipoic acid in endothelial cells. Free Radical Biol. Med., 33: 83-93.
40. Yu, J., G.N. Prado, B. Schreiber, P. Polgar, P. Polgar and L. Taylor, 2002. Role of prostaglandin E_2 EP receptors and cAMP in the expression of connective tissue growth factor. Arch. Biochem. Biophys., 404: 302-308.
41. Hu, Y.P., Y.B. Peng, Y.F. Zhang, Y. Wang, W.R. Yu, M. Yao and X.J. Fu, 2017. Reactive oxygen species mediated prostaglandin E_2 contributes to acute response of epithelial injury. Oxid. Med. Cell. Longevity., Vol. 2017 10.1155/2017/4123854.