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RESEARCH ARTICLE

Novel Resveratrol-Loaded Nanocochleates and Effectiveness in the Treatment of Diabetes

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SUMMARY

Diabetes mellitus (DM) is a chronic metabolic disease in which insulin is not produced as competently or produced insulin has not been effective enough. There are basically two types, Type I and Type II. The basic function of beta cells in the pancreas is the release of sufficient bioactive insulin to maintain the physiological rate of plasma glucose. Insulin failure and diabetes are observed when these beta cells are damaged. Resveratrol (3,5,4'-trihydroxystilbene) (RSV) is a natural polyphenolic compound present in many plants. RSV, a potent antioxidant, is effective by increasing the activity of insulin, protecting beta cells that release pancreatic insulin, and lowering blood glucose levels. Nanocohleates are new drug delivery systems with a lipid-based, non-vesicular and cylindrical structure. A wide variety of drugs can be encapsulated, increasing the stabilization of biopharmaceutical molecules that improve low bioavailability. In our study, RSV-loaded nanocochleates have been developed, characterized and evaluated for their effects on diabetes on pancreatic beta cell groups that were rendered diabetic by glucose and streptozocin. Besides this, antioxidant activity of the developed formulation was examined.

Key Words: *Resveratrol, Diabetes Mellitus, antioxidant effect, nanocochleat, drug delivery system, cytotoxicity.*

Yeni Resveratrol Yüklü Nanokohleatlar ve Diyabet Tedavisindeki Etkinliği

ÖZET

Diyabet insülinin yetersiz miktarda üretilmesi veya üretilen insülinin yeterince etkisini gösterememesi sonucu ortaya çıkan kronik bir metabolizma hastalığıdır. Tip I ve Tip II şeklinde olmak üzere temelde iki tiptedir. Pankreasta bulunan beta hücrelerinin temel fonksiyonu, plazmadaki glukozun fizyolojik oranının devamlılığını sağlamak amacıyla yeterli biyoaktif insülinin salımıdır. Bu beta hücrelerinin hasarı sonucunda ise insülin yetmezliği ve diyabet gözlenir. Resveratrol (3,5,4´-trihidroksistilben) (RSV) pek çok bitkide var olan doğal polifenolik bir bileşiktir. Güçlü antioksidan olan RSV, diyabette insülinin etkinliğini artırarak, pankreastaki insülin salımını gerçekleştiren beta hücrelerini koruyarak ve kan glukoz seviyesini düşürerek etkili olmaktadır. Nanokohleatlar, lipit bazlı, veziküler olmayan, silindirik yapılı yeni ilaç taşıyıcı sistemlerdir. Çok çeşitli ilaçların enkapsüle edilebildiği, düşük olan biyoyararlanımı iyileştiren biyofarmasötik moleküllerin stabilizasyonunu artıran sistemlerdir. Çalışmamızda RSV yüklü nanokohleatlar geliştirilmiş, karakterize edilmiş ve glukoz ve streptozosin ile inkübe ederek diyabetik hale getirilen pankreatik beta hücre grupları üzerinde nanokohleatların diyabet üzerindeki etkinliği değerlendirilmiştir. Bunun yanısıra geliştirilen formülasyonun antioksidan aktivitesi de incelenmiştir.

Anahtar Kelimeler: *Resveratrol, diyabet, antioksidan etki, nanokohleat, ilaç taşıyıcı sistem, sitotoksiste.*

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INTRODUCTION

Diabetes mellitus is a disease characterized by a deficiency in carbohydrate, fat and protein metabolism, resulting in insufficient production of insulin or the inability of produced insulin to act in peripheral tissues (Surya et. al., 2014). There are two main types of diabetes, insulin-dependent Type I and insulin-independent Type II. Insulin insufficiency or absence is observed in Type II, as the beta cells responsible for insulin release in the islets of Langerhans are damaged. The main function of pancreatic beta cells is the release of sufficient bioactive insulin for regulation the physiological rate of plasma glucose. Insulin failure is observed when beta cells are destroyed and these cells are susceptible to damage caused by reactive oxygen species (ROS) (Yücel et. al., 2018; Lee et. al., 2013).

RSV is an antioxidant effective and polyphenolic compound that is found in grapes, wines, peanuts and blueberries. It exhibits antiinflammatory, cardioprotective and antitumor activities as well as blood sugar-lowering effect (Bonechi et. al., 2012; Isailović et. al., 2013; Matos et. al., 2014). RSV has come to the forefront in recent years with the effect on diabetes which reduces the blood glucose level, protects insulin-producing pancreatic beta cells and enhances insulin action (Ergin and Yaylalı, 2013). The activity of RSV is limited due to its poor solubility in water, its ability to be readily oxidized, its short half-life and its rapid elimination. Various formulations have been developed to protect RSV from light to enhance stability and aqueous solubility, to target desired site and / or prolonged release (Amri et. al., 2012, Matos et. al., 2014).

New micro and nano drug delivery systems can serve all these expectations and increase their effectiveness while encapsulation of the active substance. One of drug delivery systems, nanocochleates (NCs) are stabilized drug delivery systems, were discovered by D. Papahadjoupoulos et al. at 1975 that are cylindrical structures consisting of stacked layers with a negatively charged phospholipid precipitated together with a cation structure such as calcium and zinc. NCs in the form of spiral rolls are biodegradable and safe systems due to the use of phospholipids, a natural component of biological membranes in their preparation (Gould-Fogerite et. al., 1998, Ramasamy et. al., 2009).

Previous studies have included that using of many fields of RSV. These are related to increasing of solubility and bioavailability, evaluation of antioxidant and anticancer activity of RSV. Drug delivery systems including RSV are few in number and are generally one of them is based on comparison of several different methods for production of liposomes (LPs) incorporating RSV (Isailović et. al., 2013). There are no studies in the literature on diabetes with long-acting effects with lower doses of RSV loaded with drug delivery systems such as NCs.

This study was aimed to develop and characterize the new RSV loaded nanocochleates, to evaluate the antidiabetic effect on different pancreatic beta cells rendered with glucose and STZ of RSV-loaded NCs comparing with RSV solution and to determinate the antioxidant activity of released RSV from NCs with using 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS.+) radical scavenging activity assays.

MATERIALS AND METHODS

Materials

Resveratrol (RSV), Dioleyil phosphatidiylserine (DOPS), Streptozocin (STZ), Glucose, 3-(4,5-dimetilthiazol-2-il)-2,5-difenyl-tetrazolium bromide (MTT), Calcium chloride ($CaCI_2$), DPPH and ABTS were purchased from Sigma, USA. All other chemicals used were analytical grade. Pancreatic Beta TC (βTC) was provided from American Type Culture Collection (ATCC CRL 11506), Manassas, USA, Dulbecco's modified Eagle's medium (DMEM) was purchased from Biochrom, Germany. Cell culture flasks surface area 25 cm2 and 75 cm2 and cell culture plates 6 well were purchased from Corning®. Fetal Bovine Serum, Tripsin-EDTA solution, Dimethyl sulfoxide (DMSO) for cell culture and penicillin-streptomycin solution were purchased from Sigma (St. Louis, USA). Cedex Smart Slides and Trypan Blue solution were purchased from Roche (Switzerland). Glucose Liquicolor kit was purchased from Stanbio Lab, USA, Insulin mouse ELISA kit was obtained from Sunredbio, Shanghai.

Quantification of RSV

UV-spectrophotometer was used to measure drug content in NCs. The RSV spectrum's maximum was measured from 200 to 550 nm with spectrophotometer (Shimadzu 1800) and the maximum wavelength was found to be 306 nm. The calibration curve was linear from 30 to 0.3125 μg/mL with high correlation coefficient (r2=0.999). A stock solution of RSV was prepared in aqueous ethanol (1:9 v/v) at a concentration of 30 μg/mL and stored in the dark. Working solutions were prepared from stock solution with dilution down to a final concentration of 0.3125 μg/mL for calibration curves. The standard curve was calculated by linear regression, according to the following formula: y= ax + b, where x is RSV the concentration as μg/mL and y is absorbance at maximum spectrum of RSV.

Development and Preparation of NCs

NCs were prepared trapping method (Dévay et. al., 2007, Ramasamy et. al., 2009). Different amounts of phospholipid and vortexing times were used throughout formulation development studies (Table 1). Furthermore, the formulations were kept at 4°C for 12 hours and overnight after the addition of calcium ions to form the NCs. The optimal formulation was determined by examining the characterization parameters obtained with varying amounts of phospholipids, vortexing times and holding time at 4°C.

When we were preparing NCs, firstly, LPs were prepared using the dry-film hydration method. DOPS as a anionic phospholipid was dissolved (10 mg) in chloroform in a round-bottom flask and evaporated under a rotavapor at ~44°C. The dry film was hydrated with a RSV solution (30 µg/mL) and vortexed for 10 min. After preparation of LPs, dropwise calcium ions were added to LP suspension to form NCs. The resulting suspension was kept overnight at 4°C and then centrifuged at 3000 rpm for 30 min. Supernatants and NCs were separated.

Characterization of NCs

The particle size (PS), zeta potential (ZP) and polydispersity index (PDI) of NCs were measured by using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Physical appearances of the NCs were determined using a scanning electron microscope (SEM, Zeiss EVO LS 10). The encapsulation efficiency (EE) of RSV was determined after centrifugation of the NC dispersion and measured from the supernatant using following equation 1.

$$\text{Equation 1: } EE(\%) = \frac{\text{theoretical total amount of RSV-free RSV}}{\text{theoretical total amount of RSV}} \times 100$$

***In vitro* release study of RSV from NC formulation**

Release study was performed for 24 h at 37°C using Franz diffusion cells with a 12,000 Dalton pore size dialysis membrane. A 2.0 mL RSV-loaded NC suspension was placed in the donor compartment of the diffusion cells. The receiver compartment was filled with 2.0 mL phosphate buffer (pH 7.4). Sample of 2.0 mL was withdrawn at the end of 24 h, was analysed by UV spectrophotometer.

Cell culture studies

β TC cells were grown in a medium composed of DMEM containing 25 mM glucose, 5 mM glutamine supplemented with 10% fetal bovine serum, 1% gentamicin, and 7.5% sodium bicarbonate in an incubator at 37°C under 5% CO_2 atmosphere. The medium was changed with fresh DMEM every 48 h. The presence of a confluent monolayer was controlled with a microscope. β TC cell were seeded to Costar filter bottom cups at a density of $2.5x10^5$ cells/cm^2 (Suzuki et. al., 2002).

Cytotoxicity assay

Cell viability on β TC cells, was assayed using MTT test. In test, β TC cells were seeded (25.000 cells/well) (Lapidot et. al., 2002) in 96-well culture plates. Plates were kept at 37°C for 24 h for cell adhering. Then, the cells were treated with different RSV concentrations (2.5-100 µg/mL), blank and RSV loaded NCs. After 24 h of culture, medium was removed and 100 µL fresh medium and 13 µL MTT solution (5 mg/mL in phosphate-buffered saline (PBS) were added. After incubation for 4 h at 37°C, 100 µL of DMSO was added to each well to dissolve the formazan precipitate. The color density was measured at 570 nm with a multiwell ELISA reader (Biotech Synergy HT, USA) (Wan et. al., 1994). The wells containing only the medium were regarded as a control group with a cell viability of 100% and the results were presented as the percentage using the control group values.

Glucose and insulin amount determination

Glucose and insulin amounts were measured by assay kits. β TC cells ($2.5x10^5$ cells/cm^2) seeded in three different groups that including control and two diabetic groups with induced glucose and STZ. In control group, initial amount of insulin and glucose determined. In diabetic group, β TC cells incubated with Krebs-Ringer bicarbonate buffer (KRB; 119 mM NaCl, 4.7 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM MgSO4, 1.19 mM KH_2PO4, 25 mM $NaHCO_3$) containing glucose (25 mM) for 4 hr (Suzuki et. al., 2002). In third group, β TC cells inhibited with STZ for 4 hr. Glucose level was considered to be diabetic cell > 250 mg/dL (Ngoc et. al., 2011) in second and third groups. The glucose and insulin concentrations of the samples from each groups were measured using Glucose-LQ kit and Mouse Insulin ELISA kit respectively.

RSV-loaded NCs were incubated with this second and third group for 24 h after creating diabetic pancreatic beta TC cell. Treated cells were centrifugated (800 rpm, 5min) and insulin and glucose levels measured from supernatants as described above.

DPPH• and ABTS•+radicals scavenging activity

The ability of the formulations to scavenge DPPH• was determined by the method of Gyamfi et al. (Gyamfi et. al., 1999). A 50 µL aliquot of sample was mixed with 450 µL of Tris-HCl buffer (50 mM, pH 7.4) and 1.0 mL of 0.1 mM DPPH• in MeOH. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using equation 2.

$$\text{Equation 2: Inhibition \%} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

To further confirm the free radical scavenging activity of the formulations, an alternative synthetic radical $ABTS^{\bullet+}$ model was used, following the method of Re et. al. The $ABTS^{\bullet+}$ radical was generated by reacting an (7 mmol/L) $ABTS^{\bullet+}$ aqueous solution with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in the dark for 12-16 h, at ambient temperature, and adjusting the Abs 734 nm to 0.700 with ethanol. After 990 µL $ABTS^{\bullet+}$ solution was added to 10 µL sample, the absorbance at 734 nm was recorded 1 min after initial mixing and subsequently (for 30 min in total). The results are expressed as the Trolox equivalent antioxidant capacity (TEAC, mmol/L Trolox). Butylated hydroxyl anisole (BHA) was used as a positive control for both activities.

Statistical analysis

All data in this study were considered as means ±SD, and one-way ANOVA was used for statistical analysis. GraphPad InStat ver. 2 was used for the analysis program. Significant differences between means were determined by Tukey's pairwise comparison test.

RESULTS

Analytical method and calibration

For the detection of RSV, the optimal wavelength of 306 nm was used and the linearity plotting was (y=0.1197x+0.0207) (r^2=0.9997) for working solutions.

Development and characterization studies of RSV-loaded NCs

According to Table 1, formulation 2 was selected as optimal formulation.

Table 1. Characterization parameters obtained with different variables (Values are represented as mean±SD (n=3)

Formulations	PS±SD (µm)	ZP±SD (mV)	PDI±SD	EE±SD (%)
1	1.47±0.20	(-) 31.3±0.8	0.140±0.051	71±1
2	1.44±0.23	(-) 33.3±0.2	0.138±0.007	73±2
3	1.46±0.18	(-) 28.3±0.6	0.152±0.011	71±2
4	1.49±0.23	(-) 29.4±0.6	0.162±0.002	64±1
5	1.50±0.27	(-) 28.4±0.3	0.160±0.004	65±2
6	1.50±0.18	(-) 28.7±0.5	0.161±0.010	65±1
7	1.52±0.22	(-) 26.4±0.7	0.178±0.006	63±1
8	1.54±0.11	(-) 28.7±1.1	0.180±0.009	64±1
9	1.54±0.24	(-) 29.4±0.9	0.182±0.011	67±2

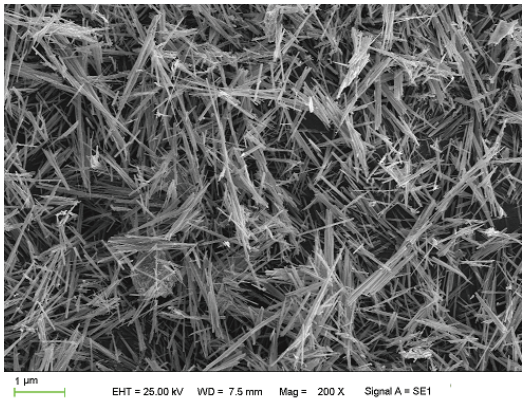

Figure 1. Scanning electron microscope image of RSV NCs

The morphology of NCs was determined using scanning electron microscopy (SEM) (Figure 1). Characterization of RSV NCs was given in Table 2.

***In vitro* release study**

Release study was performed at 37°C using Franz diffusion cells. At the end of 24 h, released RSV was found to be 68.5 % from NCs.

Cell viability

The effects of RSV solution, STZ and glucose with different concentrations and formulation components on β TC cell viability were investigated for 24 h using MTT method as described above (Figure 2).

Table 2. Characterization parameters of RSV NCs (n = 3)

Formulation	PS±SD (μm)	ZP±SD (mV)	PDI±SD	EE±SD (%)
RSV NCs	1.44±0.23	(-) 33.3±0.2	0.138±0.007	73±2

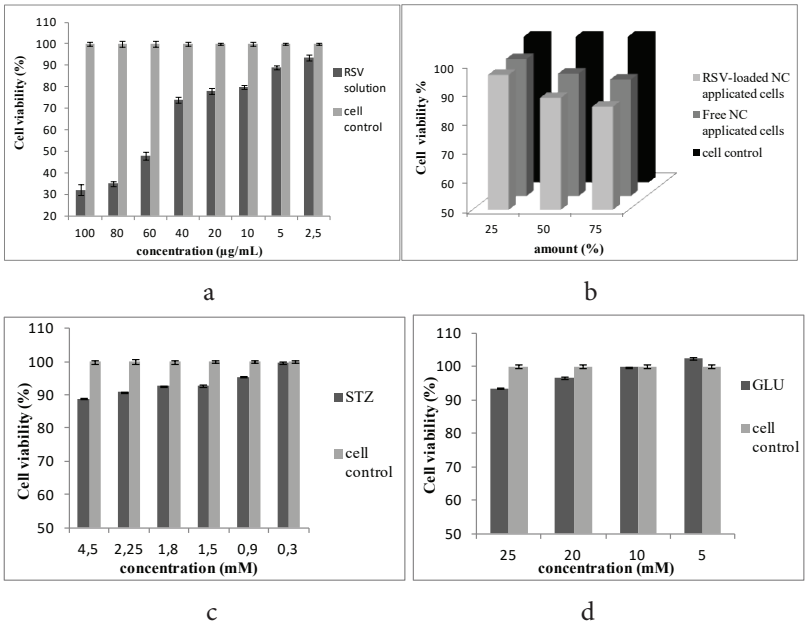

Figure 2. RSV solution with different concentrations (a), free and RSV-loaded NCs (b), STZ and glucose with different concentrations (c, d) on β TC cell viability.

Glucose and insulin determination

Glucose determinations after exposure of glucose and STZ and incubation of RSV solution and NCs on β TC cells were performed against control group were given in Figure 3. From normal, glucose-induced and STZ-induced cells, glucose concentrations were determined 201.6 (100%), 309.8 (153.6%) and 317 (157.2%) mg/dL respectively. After treatment with RSV solution and NCs for 24 h, glucose concentrations decreased to 265.8 (131.8%) mg/dL, 207.7 (103.0%) mg/dL, 282.2 (139.8%) mg/dL and 211 (104.6%) mg/dL at glucose-induced and STZ-induced groups respectively.

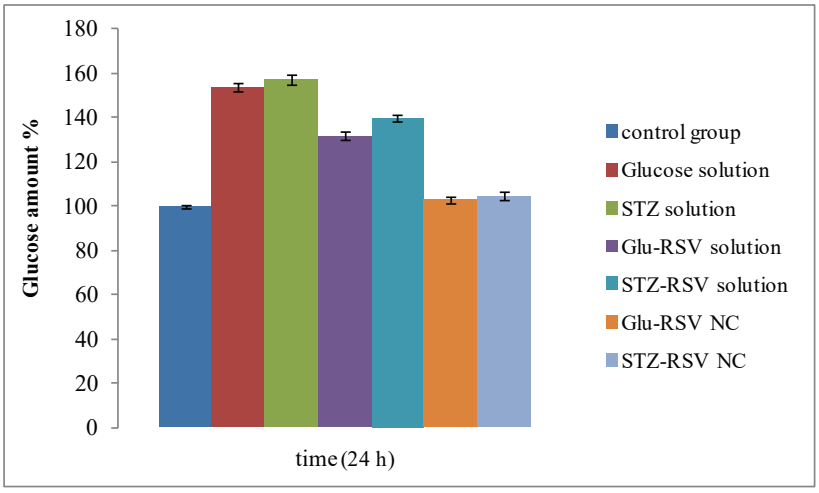

Figure 3. Increasing % glucose amount after incubation glucose and STZ solution against control group and glucose levels after incubation with RSV solution and NCs using glucose-induced and STZ-inhibited pancreatic β TC cells (error bars represent standard deviations, n=3).

Insulin concentrations of control, glucose-induced and STZ-induced cells were measured 50 µg/mL (100%) 33.6 µg/mL (67.2%) and 33 µg/mL (66%) respectively. After treatment with RSV solution and NCs for 24 h, these levels increased to above 85% (Figure 4).

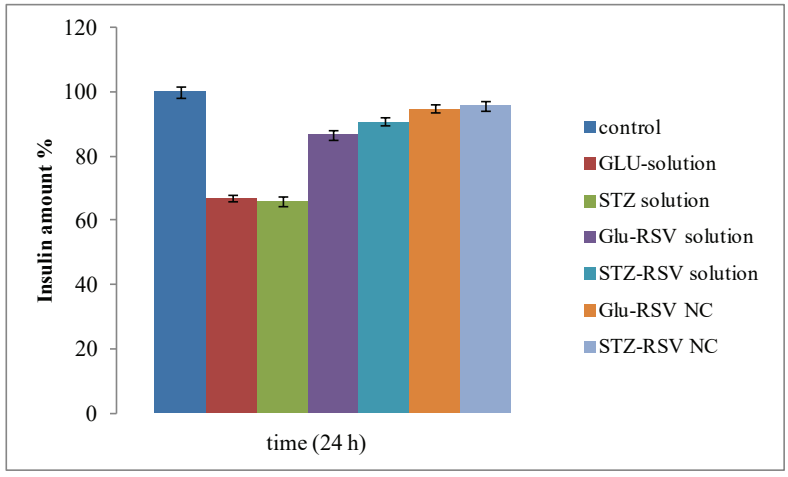

Figure 4. Decreasing % insulin amount after incubation Glucose and STZ solution against control group and insulin levels after incubation with RSV solution and NCs using glucose-induced and STZ-inhibited pancreatic β TC cells (error bars represent standard deviations, n=3).

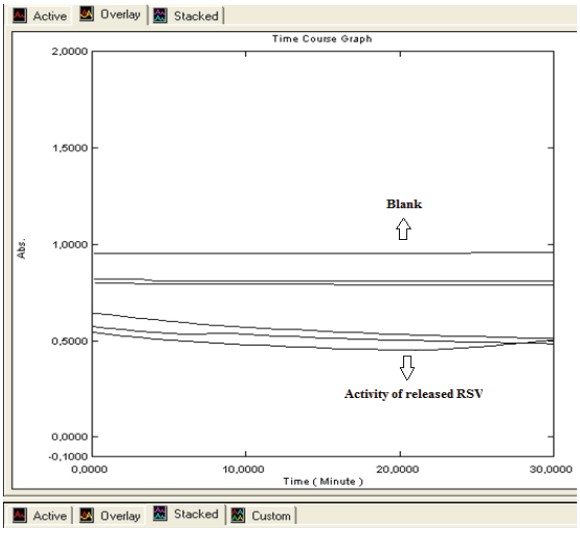

Figure 5. DPPH● scavenging activity of blank and released RSV

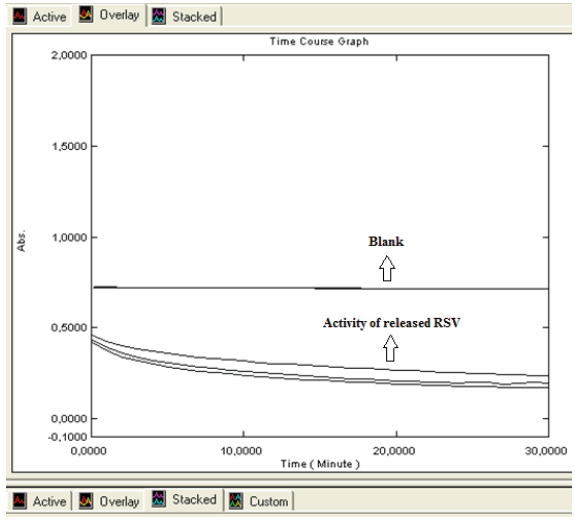

Figure 6. ABTS•+ scavenging activity of blank and released RSV

Radical scavenging activities

Released RSV succeeded in scavenging DPPH● radicals at physiological pH values. According to Figure 5, it was found that the ability of the released RSV to scavenge DPPH● was determined and the percentage inhibition was found 40.7±5 %.

As can be seen in Figure 6, released RSV manage to inhibit ABTS•+ radical in a time depended fashion. For released RSV and BHA, Trolox Equivalent Antioxidant Capacity (TEAC) values were found to be 1.769±0.27 mmol/L/Trolox and 2.26±0.086 mmol/L/Trolox respectively.

DISSCUSION

The aim of the present study was to develop and characterize RSV encapsulated NCs and assess their sustained efficiency on diabetic pancreatic β TC cell line. RSV was chosen as a model drug in this study which is chemically unstable and has poor water solubility and bioavailability. For improving bioavailability and stability of phenolic compounds, encapsulation is an alternative way and offers a potential approach for stabilizing and improving their bioavailability (Isailović et. al., 2013). In this study, the efficacy of RSV on diabetes has been explored and interpreted using

the advantages provided by new drug delivery system such as NC to overcome problems such as solubility and bioavailability problems, application frequency of RSV solution form, side and toxic effects caused by high drug concentration. NC seems to be a safe delivery system that is composed of naturally occurring products, phosphatidylserine and calcium. Phosphatidylserine is a natural component of all biological membranes (Zarif et. al., 2000).

Preparation, characterization and antidiabetic and antioxidant activity of NC containing RSV as an active compound has not been studied. In literature, there is a limited number of studies with drug delivery systems prepared with RSV that are generally comparing the preparation methods, increasing the resolution and bioavailability of RSV and determining antioxidant and anticancer activity.

For characterization of NCs PS, ZP, PDI and EE% were measured and given Table 1. We found that mean PS was 1.44±0.23 μm, ZP was -33.3±0.2 mV PDI was 0.138±0.007, EE% was 73±2 % respectively. ZP is effective in assessing the stability of colloidal systems which is defined as the stability of colloidal systems greater than +30 mV and less than -30 mV (Isailović et. al., 2013). In addition, polydispersity value was determined to be 0.150 or less, and the resulting NC formulation showed a monodisperse distribution. Systems with a polydispersity index up to 0.5 are regarded as monodisperse. In colloidal systems, monodisperse distribution is desirable and it means that particle size distribution is narrow (Bennett and Kim, 2013; Grau et. al., 2000). There is no literature data to compare our characterization study results, so this is the first study of NCs containing RSV as an active molecule.

Before starting cell culture studies, the cytotoxic effect of the active ingredient and formulation components used in the experiments on the cells should be examined. Cell viability % should be above 50 % at the end of the cytotoxicity test (Ingels et. al., 2002). For cytotoxicity study, we used MTT test that is the most commonly used (Karamustafa et. al., 2009). We tested impact of RSV solution with different concentrations (2.5-100 μg/mL) and free and RSV-loaded NCs on β TC cells. RSV did not cause any cellular toxicity with the used dose as a 30 μg/mL was chosen as the starting concentration in preparing NCs. Additionally, free and RSV-loaded NCs were also not found to be toxic to cells even at the highest concentrations. These high viability rates have shown that NCs can be used safely. In literature, to our knowledge, there are not any cytotoxicity studies with RSV-loaded NCs using pancreatic β-TC cell. According to Bonechi et. al., free and RSV-loaded LPs were tested on two types of cell lines as mouse tumoral fibroblasts NIH3T3 and human astrocytes U3763-MG and it was found that RSV liposomal formulation did not affect cell viability (Bonechi et. al., 2012). In previous study, authors evaluated free and encapsulated RSV on keratinocytes and they has been reported that cytotoxicity of RSV decreased considerably by encapsulation into LPs (Isailović et. al., 2013).

At the concentrations of STZ and glucose (4.5 μg/mL and 25 mM) used to make the cells diabetic, 89% and 93% viability rates were determined respectively. At these concentrations, STZ and glucose have also been shown to turn the cells into diabetics by increasing the amount of glucose above 250 mg/dL (Bildirici et. al., 2005; Palsamy and Subramanian, 2008).

In vitro RSV release experiment from NCs was performed with a dialysis membrane using Franz-type diffusion cells and using pH 7.4 phosphate buffer at 37°C. RSV release from NCs was found 68.5% at the end of 24 h. The medium for release experiment was selected as pH 7.4 because the pH of DMEM (cell culture medium) was measured as 7.38 pH. Released RSV was then studied for antioxidant activity.

In this paper, we investigated that the hypoglycemic effect of RSV NCs, was investigated by decreasing glucose, increasing insulin levels and inhibiting oxidative stress in glucose and STZ-induced diabetic groups. We divided β TC cells to three groups including normal, glucose-induced and STZ-induced cells. From normal, glucose and STZ-induced cells, glucose concentrations increased significantly from 201.6 to 309.8 and 317 mg/dL respectively ($p<0.01$). The glucose concentrations of these groups incubated with RSV solution and NCs through 24 h decreased to 265.8 and 207.7 mg/dL at glucose-induced group, 282.2 and 211 mg/dL at STZ-induced group. The glucose levels of treated diabetic β TC cell groups returned back to the normal range with RSV NCs ($p>0.05$) (Figure 3).

Besides that, we measured insulin concentrations in diabetic cells simultaneously and investigated relationship between glucose and insulin concentrations. Initially, insulin levels of control group and diabetic groups were found 50 μg/mL (100%), 33.6 μg/mL (66%) and 33 μg/mL (67.2%). These differences are significant ($p<0.01$). After incubation of RSV solution and NCs with diabetic cells for 24 h, insulin secretion was increased to above 85% (Figure 4) in all groups. When compared to insulin levels of RSV solution and NCs significant difference was observed ($p<0.01$). Although the treated groups with NCs were more effective than RSV solution, this treatment is not enough due to the significant difference with the control group ($p<0.01$)

To our knowledge, no previous report evaluating the antidiabetic and antioxidant effects of RSV-loaded NC formulations against the DM-related oxidative stress. Oxidative stress is considered to be involved in many diseases as diabetes (Pinholt et. al., 2011), the state of imbalance between the level of antioxidant defence system and production of ROS that has a primary role at diabetes-associated pathological damages (Deliorman Orhan and Orhan, 2016; Baynes and Thorpe, 1999; Sayın et. al., 2011). ROS produced by the induction of STZ, reduces the activities of antioxidant enzymes in pancreatic tissues. Also glucose rapidly stimulates generation of intercellular ROS (Lee et. al., 2003; Susztak et. al, 2006). RSV is a known polyphenolic antioxidant compound and this has been proven in many literatures (Husseln 2011; Khanduja and Bhardwaj 2003; Murakami et. al., 2015). The important thing for us in our study is to measure the radical scavenging capability of released RSV from NC after 24 hours and two different DPPH● and ABTS•+ radical scavenging activity experiments were run for this purpose. The DPPH radical is frequently used to determine the free radical scavenging activity of natural products (Siddhuraju and Becker, 2003). Occasionally, however, it is inappropriate to use this particular free radical due to sample solubility or spectral interference (Koleva et al., 2002). An alternative synthetic radical is $ABTS^{\bullet+}$ a moderately stable nitrogen-centred radical species. Although the principle underpinning the use of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radicals is essentially identical, $ABTS^{\bullet+}$ based models are more versatile as both non polar and polar samples can be assessed and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Pukalskas et al., 2005). In the radical scavenging assays, absorbances were taken in every minute during 30 min, at 517 and 734 nm. Released RSV and BHA, a synthetic antioxidant, studied in both assays. In $DPPH^{\bullet}$ scavenging assay released RSV showed 40.7% inhibition at 25 µg/mL concentration and it was found to be as active as BHA, while its IC_{50} value was found 77.7±0.001 µg/mL. In ABTS•+ scavenging assay released RSV and BHA studied at the same concentration and also no statistically significant difference ($p>0.05$) were found between the TEAC values of the released RSV and BHA.

CONCLUSION

These results demonstrate that, NCs were developed as suitable for our purpose and antidiabetic and antiradical effect of RSV-loaded NCs was found considerably. They improved the decreased insulin levels rendered with glucose and STZ and reduced the increased glucose levels significantly. They also showed prolonged antiradical activity for 24 hours. These NCs may be useful and effective to treatment of DM and free radicals that occur in type II DM.

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