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Rosuvastatin and Ezetimibe loaded PLGA: an investigation approach for the treatment of hyperlipidemia induced by Triton in male albino rats

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ABSTRACT

This study aimed to develop and optimize the anti-hyperlipidemic effect of Ezetimibe (EZE) and Rosuvastatin (RSV) combination using poly (lactic-co-glycolic acid) nanoparticles (NPs). To achieve this, in vivo Triton induced hyperlipidemia rats were used to evaluate the antihyperlipidemic activity of the marketed products in comparison with their NPs. Results revealed that after 24 hours, Triton treated animals showed altered lipid profiles including significantly ($P<0.05$) high cholesterol, triglycerides, LDL-C and Non-HDL-C and low HDL-C. They also exhibited an increase in the activities of ALT and AST enzymes, creatinine, urea, and blood urea nitrogen levels and a decline in the total proteins and albumin levels indicating liver and kidney injuries. Triton also altered the glycemic control as evidenced by the increase in glucose and insulin growth factor. Administration of orally EZE+RSV and their loaded NPs significantly ($P<0.05$) attenuated the lipids alters after 15 days of treatment. This attenuation was continued up to 30 days in many evaluated parameters. They also were effective in partial preventing liver and kidney injuries and the glycemic controls. The effects of NPs were more pronounced that the marketed forms. In conclusion, and based on our findings, the efficiency and convenience of anti-hyperlipidemic activity of EZE+RSV nanoparticles were well demonstrated.

Introduction

Lipid metabolic disturbances are one of the main factors, representing about 50% of the population-associated risk of cardiovascular disease (CVD) development (Hedayatnia *et al*., 2020). Dyslipidemia and/or hyperlipidemia can be characterized by an increased blood plasma level of cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and a reduced level of anti-atherogenic high-density lipoprotein cholesterol (HDL-C) (Dybiec *et al*., 2023). Both in developed and developing countries, dyslipidemia prevalence has increased over the last several years and become a serious public health problem, and it often represent the starting point of CVD, that is considered as one of the 5 death leading causes in the world (Shukr *et al*., 2019; Pirillo *et al*., 2021).

The synthetic 2-azetidinone (ezetimibe; EZE), by blocking the Niemann-Pick C1 like I protein, is a selectively cholesterol absorption inhibitor agent and thus act as a lipid lowering drug. For primary hypercholesterolemia treatment, it used as a mono therapy or in combination with other statins and characterized by low adverse effects incidence and a good tolerance (Shukr *et al*., 2019; Agrawal *et al*., 2021). In patients with hyperlipidemia, EZE reduces elevated cholesterol, LDL-C, Apo lipoprotein B (Apo B), and non-HDL-C (Agrawal *et al*., 2021). EZE variable bioavailability may be owing to its very low dissolution and solubility rate, in addition to extensive efflux by p-glycoprotein (Shukr *et al*., 2019).

Rosuvastatin (RSV) is one of the most effective hypolipidemic statins that is used to prevent CVD by reducing the LDL-C. Its mechanism of action, similar to other statins, is associated with competitive inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase enzyme (Ahmed, 2020). RSV possesses limited solubility in the gastrointestinal fluids owing to its low water solubility (Butt *et al*., 2019). After oral administration, RSV is subjected to extensive first pass metabolism and, thus, its oral bioavailability is approximately 20% (Ahmed, 2020). RSV maximum serum concentration is achieved in about 3-5 hours (Li *et al*., 2007).

To improve bioavailability of drugs with poor aqueous solubility, different strategies of pharmaceutical formulation have been used (Ahmed, 2020). Nanotechnology is functional systems engineering with an appropriate particle narrow size range, elevated dissolution rate which in turn can improve the bioavailability (Shukr *et al*., 2019). Nanoparticles (NPs) are of the most progressive ways for either hydrophobic or hydrophilic active compounds delivery (Begines *et al*., 2020). These particles have gained a considerable attention as promising and innovative carrier able to overcome several of drug undesirable features while improving or maintaining its therapeutic efficiency (Shukr *et al*., 2019). Besides, NPs could enhance drug features including targeting to infected tissue, sustaining drug release and prolonging drug effect in the target tissue (Shukr *et al*., 2019). Among biodegradable polymeric NPs, a FDA approved poly(lactic-co-glycolic acid) (PLGA) was evaluated using different statins (Dayar and Pechanova, 2022). Statin-loaded PLGA NPs showed a superior profile concerning minimizing adverse effects, dosing, drug release and the bioavailability (Li *et al*., 2017; Dayar and Pechanova, 2022). In hyperlipidemic animal models, administration of statin-loaded PLGA NPs reduced the drug dose, attenuated the increase of inflammatory markers activity, reduce the progression of hypertension and exhibit cardio protective properties without any adverse effects (Meena *et al*., 2008; Chen *et al*., 2011; Yokoyama *et al*., 2019).

The aim of this study was to maximize EZE and RSV efficiency and their drug bioavailability and loading capacity by using PLGA NPs for hyperlipidemia treatment in Triton-induced hyperlipidemia rats.

Materials and methods

Chemicals

Tyloxapol (Triton WR-1339), chloroform solvent, pluronic acid and PLGA were purchased from Sigma-Aldrich (USA). EZE and RSV were purchased from Marcyrl-international pharmaceutical industries (Egypt). Diagnostic kits for measurements of cholesterol, triglycerides, HDL-C,

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creatinine, albumin, total protein, total lipids, blood glucose, urea, alanine (ALT) and aspartate transaminases (AST) were purchased from Bio diagnostic company (Egypt). Rat insulin like growth factor (IGF) was measured according to the manufacture instructions using commercial ELISA kit (Elabscience, USA).

Preparation of drugs loaded PLGA NPs

With slight modifications for proper NPs fabrication, EZE and RSV loaded PLGA NPs were prepared according to single oil-water emulsion solvent evaporation assay (Kızılbey, 2019). After four trials, the best one was as follow: At room temperature, PLGA (30 mg), EZE (6 mg) and RSV (15 mg) were dissolved in chloroform solvent (5 mL) to form the organic phase. At 75°C, 0.5% (w/v) of pluronic acid polymer was prepared in distilled water (50 mL) to give the aqueous phase. At 25°C under magnetic stirring, the organic phase was drop wise added into the aqueous phase. After that with an input energy of 40 W, the mixture was sonicated using probe homogenization (S-4000, Misonix, USA) for six hours at 60°C to generate the oil-water emulsion. On magnetic stirring, the generated emulsion was stayed overnight to allow organic solvents evaporation and ensure NPs solidification. At 4°C, NPs were then collected by centrifugation at 10000×g (Hettich centrifuge, Tuttlingen, Germany) for twenty minutes and washed 3 times with deionized water.

Animals

Adult male albino rats (215±20 g) were collected from the animal house of the faculty of Veterinary medicine, Zagazig University, Egypt. All of them were kept in stainless steel cages for two weeks acclimatization , given standard food and water, and maintained at 12/12 hours light / dark cycles and 22.0±3.0ºC. The study protocol was approved by Zagazig University Animal Ethics Committee.

Experimental groups and hyperlipidemia induction

A total of 24 male albino rats were divided into 4 groups (6 rats/each): Group (I): normal control rats were maintained on standard diet. The other three groups received Triton WR-1339 (100 mg/kg body weight, i.p.) every other day for hyperlipidemia induction. Group (II) was the positive control received Triton WR-1339 without any treatment. Group (III) received daily orally dose of marketed EZE and RSV in combination equivalent to 0.6 mg/kg every day for one month. Group (IV) received daily orally dose of the optimized NPs formula loaded with EZE+RSV equivalent to 0.6 mg /kg every day for one month. Under mild ether anesthesia, blood samples were collected through retro-orbital venous plexus puncture using heparinized microcapillaries tubes and serum was separated by centrifugation (4000×g for 20 minutes). The assessment of lipid profile, kidney, and liver functions and IGF were measured on days 15 and 30 (the end of the experiment).

Statistical analysis

Data were analyzed by SPSS version 21 software. Variables were expressed as mean± standard error of the mean (SEM) and assessed by one-way ANOVA followed by Bonferronitest as a post-hoc test.

Results

Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on lipid profile of adult male albino rats for different periods of treatment

Using a triton-induced hyperlipidemic model, anti-hyperlipidemic activity of each form of EZE and RSV combined drugs were evaluated by lipid lowering studies. As shown in Fig. 1, untreated rats (normal controls) showed no changes in lipid profiles. At 24 hours, triton-treated animals showed altered lipid profiles including significantly (P<0.05) high cholesterol, triglycerides, LDL-C and Non-HDL-C and low HDL-C. Administration of orally EZE+RSV and their loaded PLGA NPs significantly (P<0.05) attenuated the lipids alters after 15 days of treatment. Except total cholesterol, this attenuation continued up to 30 days (Fig. 1).

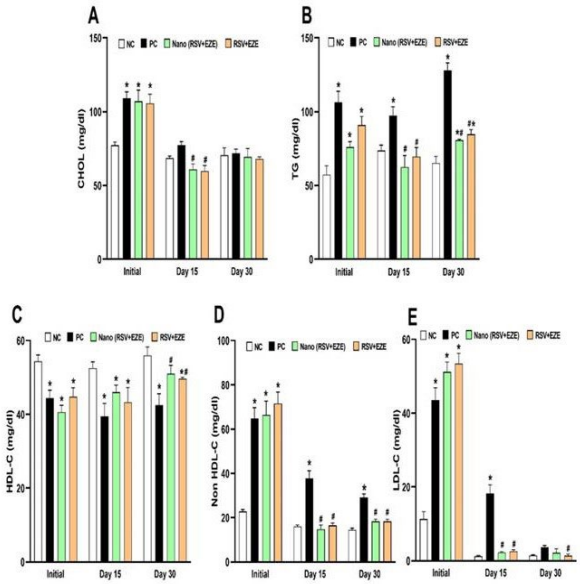

Figure 1. Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on lipid profile of adult male albino rats for different periods of treatment. NC: Normal controls, EZE+RSV: Ezetimibe+Rosuvastatin, PC: Positive control.

Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on liver related parameters of adult male albino rats for different periods of treatment

The data shown in Fig. 2 demonstrated that treatment with triton caused a significant (P<0.05) increase in the activities of ALT and AST enzymes and a decline in the total proteins and albumin levels. Administration of orally EZE+RSV and their loaded PLGA NPs was effective in partial preventing these liver injuries.

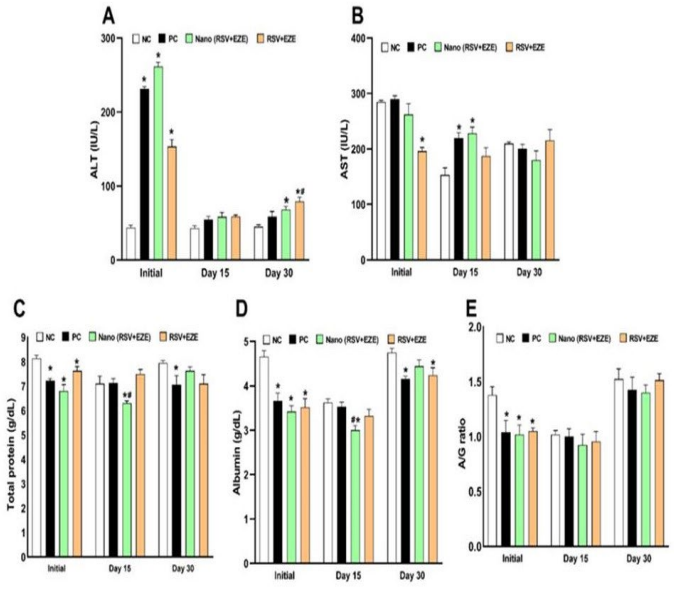

Figure 2. Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on liver related parameters of adult male albino rats for different periods of treatment. Triton positive control.NC: Normal controls, EZE+RSV: Ezetimibe+Rosuvastatin, PC: Positive control

Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on kidney related parameters of adult male albino rats for different periods of treatment

The data shown in Fig. 3 demonstrated that treatment with triton caused a significant (P<0.05) elevation in creatinine, urea, and blood urea nitrogen. Administration of orally EZE+RSV and their loaded PLGA NPs was effective in partial preventing these kidney injuries.

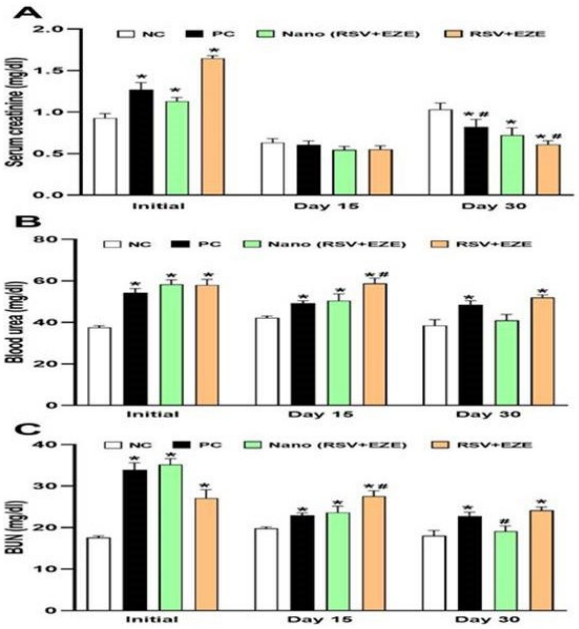

Figure 3. Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on kidney related parameters of adult male albino rats for different periods of treatment. Triton positive control.NC: Normal controls, EZE+RSV: Ezetimibe+Rosuvastatin, PC: Positive control.

Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on blood glucose and insulin like growth factor of adult male albino rats for different periods of treatment

The results presented in Fig. 4 show that both glucose and IGF of untreated triton-induced hyperlipidemic rats was significantly (P<0.05) higher than that of the normal/control. Rats treated with EZE+RSV and their NPs exhibited relatively lower glucose and IGF compared to the triton-induced hyperlipidemic group.

Discussion

The present study reported that all triton treated rats displayed hyperlipidemia as shown by their increased levels of serum cholesterol, triglyceride, Non-HDL, and LDL in addition to the decrease in HDL concentration. These results confirmed the triton method efficiency that was employed in the hyperlipidemia induction. Administration of orally EZE+RSV and the optimized loaded PLGA NPs significantly attenuated these lipids changes after 15 days of treatment. Except total cholesterol, this attenuation continued up to 30 days.

To induce cholesterol-induced hyperlipidemia, triton-induced hyperlipidemia is a well-known model and it was stated that triton inhibits triacylglycerol-rich lipoproteins catabolism by lipoprotein lipase and this, in turn, causing inhibition triacylglycerol extraction from plasma and inhibits lipoproteins uptake from the blood by the extra hepatic tissues (Shrivastava *et al*., 2013; Abdou *et al*., 2018; Parwin *et al*., 2019). Triton is a non-ionic surfactant that, by the emulsification process, enhances synthesis of liver cholesterol and elevates intestinal lipid absorption (Parwin *et al*., 2019).

The reported EZE anti-hyperlipidemic effect was confirmed by other studies reported in the literature (Morris and Tiller, 2003; Miura and Saku, 2008; Katsiki *et al*., 2013). Several mechanisms have been advanced for EZE anti-hyperlipidemic role, including EZE inhibitory effect on Niemann-Pick C1-Like 1 (NPC1L1), a cholesterol transporter located in epithelial cells of the small intestinal, so it reduces cholesterol intake by the intestine (Garcia-Calvo *et al*., 2005). It also could be due to reducing chylomicrons cholesterol content, which in turn reduces cholesterol amount delivered to the liver causing elevation in LDL-receptor expression and improving LDL clearance (Neal and Jones, 2003).

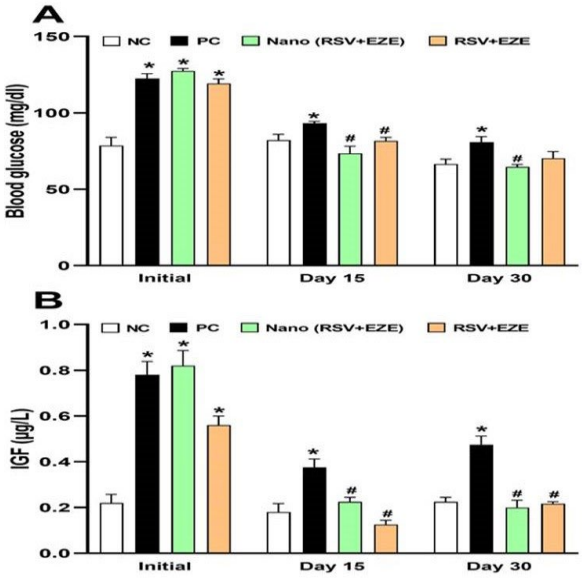

Figure 4. Effect of Triton, Ezetimibe-Rosuvastatin normal form combination and Ezetimibe-Rosuvastatin nanoparticles combination on blood glucose and insulin like growth factor of adult male albino rats for different periods of treatment. Triton positive control. NC: Normal controls, EZE+RSV: Ezetimibe+Rosuvastatin, PC: Positive control.

RSV is the most available powerful statin with superior findings in reducing LDL-C than with other statins (Boutari *et al*., 2021). RSV is a hepato-selective and hydrophilic potent HMGCoA reductase inhibitor which decrease triglycerides and LDL-C and increases HDL-C (Rizzo *et al*., 2009). In the therapeutic armamentarium, the position of the RSV/EZE drug combination is very high (Boutari *et al*., 2021).On the lowering of triglycerides and LDL-C, many studies have reported the additive effects of EZE/statin combination (Pearson *et al*., 2009; Hwang *et al*., 2018)

In this study, activities of serum AST and ALT, and creatinine, urea and blood urea nitrogen serum levels were increased, and total proteins and albumin levels were decreased after triton treatments indicating hepatic and renal injuries. Orally EZE+RSV and their loaded PLGA NPs were effective in partial preventing these liver and kidney injuries. Hepatic enzymes are located in the liver cells and, when liver cells are injured, they make their way into the blood (Mallo *et al*., 2013). Thus, the elevation in these enzymes activities in serum were directly proportional to cellular damage degree caused by hypercholesterolemia (Osman *et al*., 2010). Moreover, the reported decline in the total proteins, globulin and albumin, may be related to hepatic damage, kidney failure and nutritional deficiency(Abdou *et al*., 2018). The observed triton-induced alteration in these liver and related kidney parameters were in agreement with other reports (Sodipo *et al*., 2011; Abdou *et al*., 2018).

For treatment of some liver diseases, statins have been considered and some studies showed that statin therapy either attenuates steatosis and/or inflammation or trends toward reduce fibrosis (Schierwagen *et al*., 2017). For example, in patients with metabolic syndrome, RSV was reported to resolute non-alcoholic steatohepatitis (NASH) (Kargiotis *et al*., 2015). They found that patients with NASH and metabolic syndrome under treatment with RSV showed complete resolution of NASH, as reported by liver biopsy, and normalization of ALT and AST enzymes, blood glucose and lipid profile (Kargiotis *et al*., 2015). Also in patients with mild to moderate renal dysfunction, these drugs were reported to reduce renal tubular damage and degrading glomerular filtration function (Qiao *et al*., 2015).

Our results reported that triton injection induced alteration in glycemic control as indicated by elevation of glucose and IGF serum levels. Rats treated with EZE+RSV and their NPs exhibited relatively lower glucose and IGF compared to the triton-induced hyperlipidemic group. Treatment of rats with EZE+RSV and their NPs exhibited relatively lower glucose and IGF compared to the triton-induced hyperlipidemic rats. Similar results were obtained by Aboul-Enein *et al*. (1986) who found that blood glucose level was elevated after triton injection and also muscle and liver glycogen were highly elevated and the highest concentrations were obtained after32 hours of triton injection. Also, Hwang *et al*. (1985)

found that triton at concentration of >0.2% the IGF receptor binding was decreased by 50% and this may be associated with the elevated serum IGF (Hwang *et al.*, 1985).

Conclusion

The overall results highlighted that EZE/RSV combination appears to be potent, effective and safe lipid-lowering agents, able to significantly decrease cholesterol, triglycerides and LDL-C levels and increases HDL-C levels. Also, they have liver and kidney protective role. The oral administration of optimized PLGA NPs formula improves lipids and to some extent restoring it to the normal values. Evidently, Nano size of these drugs can improve cellular uptake and bio- distribution.

Conflict of interest

The authors declare that they have no conflict of interest.

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