Control and monitoring of lipoprotein levels in atherosclerosis induced rabbits using novel nanoparticulate medication of Lovastatin and Rosuvastatin

Guoyi Luo | Wenjie Chen | Jianjun Luo | Jie Liu

Pharmacy department, People’s Hospital of Chongqing Shuangqiao Economic and Technological Development Zone Chongqing, Chongqing, China

Correspondence
Jie Liu, Pharmacy department, People’s Hospital of Chongqing Shuangqiao Economic and Technological Development Zone Chongqing, 400900, China.
Email: liu107jie@163.com

Abstract
Different epidemiological studies have demonstrated higher level of uric acid and Lipoprotein levels as risk factors for atherosclerosis. The present investigation deals with formulation of novel combination of Rosuvastatin and Lovastatin in nanoparticulate form to assess atherosclerosis model. The ionic gelation method was found to be useful for the preparation of chitosan nanoparticles which are evaluated for different parameters like morphology, zeta potential, and polydispersity index found to be satisfactory for delivery of drug. Application of statistical model confirms the effect of Poloxamer 188 and chitosan on drug release and Encapsulation efficiency and drug release. The drug release of Rosuvastatin-Lovastatin nanoparticles (RST-LST-NP) was compared with individual Rosuvastatin nanoparticles (RST-NP) and Lovastatin nanoparticles (LST-NP). The results obtained from serum uric acid analysis as well as measurement of level of total cholesterol, high density cholesterol, low density cholesterol shows superiority of RST-LST-NP over RST-NP and LST-NP in lowering and maintaining serum lipoprotein levels.

1 | INTRODUCTION

Atherosclerosis (also called as arteriosclerosis) is one of the major causes of mortality and morbidity through world. When arteries of the blood vessel thickens, hardens due to deposition of plaque (fatty deposits) and clogs the arteries, the condition is called as atherosclerosis. This progressive disease is identified by the accumulation of lipidic substance and fibrous elements in large arteries [1]. The deposits are composed of cholesterol, fatty substances and also cellular waste products. Initially the disease shows no symptoms, but when worsens can lead to severe conditions. Depending upon the type of artery affected in particular organ [2], different conditions may precipitate like coronary heart disease, angina or peripheral heart disease, chronic kidney disease. The early lesions also called as ‘Foam Cells’. It is a disease of an arterial wall primarily infecting susceptible sites of major arteries. A Lipid retention, oxidation and modification can trigger the chronic inflammation causing thrombosis or stenosis or thrombotic occlusion [3]. One of the main underlying reasons behind atherosclerosis is considered to be hypercholesterolemia. Arterial endothelial permeability changes due to enhanced plasma cholesterol levels. This change in permeability leads to migration of lipids (LDL-C particles), into wall of arteries [4]. When monocytes circulating in blood adhere to endothelial cells leads to expression of adhesion molecules such as vascular adhesion molecule-1 (VCAM-1) and selectin which migrate to sub-endothelial space and get converted into foamy macrophages. Accumulation of intracellular cholesterol gets enhanced through LDL particles in sub-endothelial spaces. This binds to native and modified lipoprotein and anionic phospholipids. The end result of this cascade is vascular modification [5, 6]. The cascade can be expected to happen in a sequence like narrowing of vessels with some conditions of angina pectoris and acute coronary syndrome because of instability of plaque. Main reason underlying formation of thrombi is plaque rupture and/or erosion, sometimes calcified nodules [6]. Vulnerable plaque contains monocytes, macrophages and T-cells. The initial step for treatment mostly includes reduction in risk through some changes in lifestyle such as dietary, enhanced physical activity; avoid...
primary as well as secondary smoking. But when these changes are not sufficient to control the disease the Lipoprotein mediated therapy is utmost required [6]. Statins or HMG-CoA reductase inhibitors are the choice of drug for treatment of disease. Statin is a prevention and treatment therapy for CHD (coronary heart disease), hypertension, and cerebrovascular disease [7]. These statins causes increased expression of LDL receptors (LDL-R) on hepatocytes surface resulting in enhanced uptake of DL-C from circulation blood. This reduces the total plasma level of LDL-C along with apo-B containing lipoproteins, including TG-rich particles [6, 8]. In different trials statin therapy has shown its efficacy in lowering blood LDL levels. HMG CoA reductase inhibitors (or statins), a new class of lipid-lowering compounds, have raised expectations for more widespread use than that of the older lipid lowering drugs. Not only are they more effective in lowering LDL cholesterol, but they are better tolerated as well. No data exist concerning the effect of statins on early carotid atherosclerosis and clinical events in men and women who have moderately elevated LDL cholesterol levels but are free of symptomatic cardiovascular disease [8].

Rosuvastatin, a newer lipid lowering agent, is used largely in day to day clinical practice. Not only strong potential of lipid lowering but its great efficacy towards reducing inflammatory reaction, normalizing vascular endothelial function with plaque stabilization is some of the prime reasons for selection of drug [9]. It has been also stated that it can activate PPAR-γ via activation of extracellular signal regulated protein kinase with increased DNA binding activity. However its whole and sole effect on human monocytes is not clear [9, 10]. The drug was recently approved in US to delay progression of atherosclerosis to lower low density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) to target levels. It is reported to have more lipid lowering efficacy than any other drug of statin family. Some literature has reported that significant reduction in disease was seen with Rosuvastatin 40 mg/day in coronary heart disease patients which supports lipid lowering potential of Rosuvastatin. It is a well tolerated, lipid lowering agent in delaying atherosclerosis in primary prevention patients [11]. Lovastatin, another drug from class of statins, used to reduce risk of heart attack and stroke along with decreasing level of cholesterol (a fat like substance) from blood so as to reduce incident of atherosclerosis [12]. The drug acts through slowing the production of cholesterol and reduce total amount in body. Lowering blood cholesterol levels along with less fat deposits proves helpful in prevention of atherosclerosis [13].

Therapeutic efficacy of most drugs was greatly increased by employing nanoparticulate drug delivery system. The great platform has been provided for large number of drug. The inherent ultra small dimensions of nanoparticles makes it very easy get accumulate into host cell and exhibit its effect [14]. The distinct features of nanoparticles like biocompatibility, protection of nuclei degradation, in vivo delivery of drug to cell makes it a choice of drug for the most researchers [15]. The sustained release pattern of drug release, high loading capacity, and higher surface to volume ratio makes them suitable to carry large range of drug [15, 16]. Nanoparticles have uniquely appealing features that enable them to be programmed as cell and tissue-specific delivery systems, thus overcoming the low drug delivery and off-target effects that commonly impede developments in cardiovascular medicine [17]. The present investigation deals with formulation of novel nanocarrier system for most possible efficient delivery of Rosuvastatin and Lovastatin to control and maintain the level of Lipoproteins in Experimental animals.

2 MATERIALS AND METHODS

2.1 Materials

Rosuvastatin (RST) and Lovastatin (LST) were purchased from Baoji Guokang Bio-Technology Co., Ltd. (Baoji, Shaanxi, China). Chitosan, Poloxamer 188 were purchased from Sigma (USA), Tri ethylene polyphosphate (TPP), Dimethyl sulphoxide (DMSO), acetic acid (2 % v/v), Chloral Hydrate (10%), were purchased from Shanghai Chemical Co. (Shanghai, China).

2.2 Animal models

Adult male New Zealand Rabbits of weight ranging from 3 to 4.5 kg were selected for conduction of experiment. The animals were cage such that they are having free and easy access to food and water. The adaptation period of 8–10 days was given to every animal prior conduction of experiment. The guidelines of animal ethical committee were strictly followed during conduction of entire investigation.

2.3 Methods

2.3.1 Application of statistical design using 3-factor, 2-level full factorial

After performing preliminary demonstration of experimental trials it was found that concentration of chitosan, TPP and Poloxamer have visibly significant influence on drug release, encapsulation efficiency. Therefore these three parameters are considered to be independent parameters. A 3-factor, 2-level full factorial design applied optimises the nanoparticles and to find out the effect of independent variables on dependent variables percentage encapsulation efficiency and particle size. Independent variables and different levels are represented in Table 1.

2.4 Development of Rosuvastatin–Lovastatin nanoparticles (RST-LST-NP)

The method of choice used to formulate RST-LST-NP was ionic gelation method using chitosan (CS) as coating material. The co-acetabers having nano size range were formed by interaction of positively charged amino group in CS with negatively charged Triplypolyphosphate (TPP). The pre-weighed quantities of RST (5 mg) and LST (10 mg) were dissolved separately in solu-
tion of dimethylsulphoxide (DMSO) (drug solution). CS was dissolved in 2% v/v solution of acetic acid (chitosan solution) and Poloxamer 188 was dissolved in this chitosan solution. The drug solution was added to chitosan solution using continuous stirring of magnetic stirrer up to 2 h at 1200 RPM. The aqueous solution of TPP (CS: TPP; 1:0.1) was also added in this step. This causes cross linking of CS and TPP to generate nanoparticles. Thus formed nanoparticles were recovered using centrifugation at 2500 rpm for 25 min. The diethyl ether was used as solvent for washing purpose. The formulation table for preparation of RST-LST-NP was shown in Table 2. By using similar method the nanoparticles of RST and LST were prepared individually for comparison in animals study.

2.5 | Particle size, zeta potential, polydispersity index and yield of nanoparticles

The suspension of RST-LST-NP was prepared by dispersing about 5 g of RST-LST-NP into 15 ml of distilled water and sonicated the solution 3–5 min. A 10 time’s dilution was performed using double distilled water. The drop of such suspension was mounted on foil paper, dried and examined under Scanning Electron microscopy (SEM) at operating distance of 8–8.5 mm. Zetasizer (Malvern instruments DTS Ver 4.10) was used to measure Particle size, Zeta potential, Polydispersity index (PDI) [18, 19].

2.6 | RST-LST entrapment by CS nanoparticles (entrapment efficiency)

The pre-weighed quantity of RST-LST loaded CS nanoparticle suspension was centrifuged at 10,000 rpm for 25 min at room temperature until clear supernatant is obtained. This clear liquid was separated by decanting the solvent. The concentration of non-entrapped or free drug for both RST and LST separately can be determined from this supernatant liquid by using spectrophotometric analysis [20]. The RST-LST entrapped in CS nanoparticles was calculated using following equation:

\[
\% \text{ E.E} = \frac{\text{Total Drug} - \text{free Drug}}{\text{Total drug amount}} \times 100
\]

2.7 | In vitro drug release of RST-LST from nanoparticles

The release pattern of both drugs was studied individually using same experimental technique. The dialysis bag was used as membrane replica with molecular cut-off of 5 kDa to observe release of RST-LST from RST-LST-NP’s in pH 7.4 phosphate buffer solutions (PBS). About 15 mg of RST-LST-NP’s were weighed and redispersed and placed into 150 mL of PBS solution in beaker with temperature of 370 °C. The whole system was assembled on magnetic stirrer. A 5 mL of sample aliquot was removed from buffer solution and again replenish with fresh PBS solution to maintain sink condition. The amount of RST in sample taken was analyzed using UV spectrophotometry at 308 nm. The same procedure was repeated for estimation of LST using maximum wavelength 248 nm [21].

2.8 | Development of atherosclerotic animal model

New Zealand Rabbits were procured from People’s Hospital of Chongqing Shuangjiao Economic and Technological
Development Zone, Chongqing, 400900, China. All experiments were performed according to guidelines of the Principles of Laboratory Animal Care and the Guide for Care and Use of Laboratory Animals. The animals were chosen randomly (3–4.5 kg) and divided into different groups as follow: the first group consists of 30 animals that were fed with regular diet (RD; n = 30), second group consist of animals kept on fat rich die (FRD; n = 40). Animals in FRD group were additionally supplied with intra-gastric (i.g.) Vitamin D3 (60 IU/kg) up to 3 days followed by a dose of 5 mL/kg of high fat emulsion containing pyrimidine twice daily by intra-gastric administration. This pyrimidine contains 200 g pork, 200 g cholesterol, 20 g bile salts, and 10 g propyl-thiouracils, dissolved in 1 L distilled water. Simultaneously animals were kept on equal volume of normal saline and fed with normal diet. The animals were housed individually suitable environmental conditions at temperature of 20–22 °C with relative humidity of 45–55% using 12 h of light dark cycle. After induction of atherosclerosis animals were subdivided as follow.

About ten number experimental animals were chosen from group RD and were kept as negative control (RD-N; n = 10). The animals in FRD group (animals induced with atherosclerosis) were subdivided into four groups each group containing ten animals as follow:

The atherosclerotic animals receiving treatment of RST-LST-NP’s were put in T-RLN (n = 10) while another group is merely treated with RST-NP’s only (T-RST; n = 10). The animals in third group were treated with LST-NP’s only (T-LST; n = 10) and last group of animals received no treatment (NT; n = 10)

2.9 | Determination of serum uric acid level

Animals were fasted for the period of at least 8 h and injected with 10% choral hydrate to induce anaesthesia. Blood samples were collected after 4 h at room temperature and centrifuged at 3500 rpm for 15 min. Serum was separated (Sample 1). Uric acid levels were determined.

2.10 | Determination of serum lipid level

The serum lipid levels (total cholesterol, LDL, HDL) were determined from sample 1 using auto-analyser (Toshiba, Japan).

2.11 | Histological evaluation

After completion of the experiment the aorta was harvested, cut open in half portion and fixed in buffered formalin solution. The ring of aorta were cut at 4 μm and stained with haematoxylin and eosin (HE) staining.

3 | RESULT AND DISCUSSION

3.1 | Formulation of development of Rosuvastatin–Lovastatin nanoparticles (RST-LST-NP)

The nanoparticles of RST-LST-NP and RST-NP, LST-NP were prepared using ionic gelation method employing chitosan as coating material. The particles having nano size range were formed by interaction of positively charged amino group in CS with negatively charged tripolyphosphate (TPP). A CS is natural polymer and the nanoparticles prepared are highly acceptable due to its enhanced biocompatibility, non immunogenicity and non toxicity. The availability of drug concentration using nanoparticles in serum is also increases by use of CS as polymeric material. The surface characteristic of such chitosan prepared RST-LST-NP were examined using Scanning electron microscopy (SEM) as shown in Figure 1.

The spherical shape of nanoparticles with smooth surface showing no cracks and crevices has been observed. After drying of nanoparticles the free flowing nature of RST-LST-NP can be observed. The particle size is very crucial criteria for drug delivery. It has been observed that smaller the size of particle larger will be engulfment by cell and more will be therapeutical action. The zeta potential is one of the important parameter for kinetic stability of nanoparticle. The range of potential in between −25 and +32 mV is observed to show good repulsive force to attain better physical stability of dispersion or suspension. The zeta potential for RST-LST-NP was found to be this range and hence considered suitable administration. The size range of 97–210 nm was found to be best suited for intra-articulate administration to treat atherosclerosis. The average size range of nanoparticle is one of prime set of consideration making RST-LST-NP very effective in passive targeting of drug. PDI of RST-LST-NP was found to be reliable and within acceptable range that is less than 0.3 the values clearly
indicates homogeneity of nanoparticulate dispersion. The large value PDI might be result of longer chains of chitosan so as to prevent aggregation of nanoparticles. A quick glimpse at the results obtained showed that good stability with respect to PDI, zeta potential and particle size. Table 3 summarizes all physical parameters of RST-LST-NP. The percent encapsulation efficiency of RST-LST-NP’s was calculated using free (non entrapped) drug in supernatant solution. The percentage encapsulation efficiency of RST was found between 43.67% (RL 7) and 58.67% (RL 4) while the same value for LST was 41.98% (RL 7)–57.34% (RL 4). A direct proportion can be found between concentration of (polymers), that is, CS, Poloxamer 188. From results one can predict that higher the concentration of polymers presents larger will be encapsulation of drug. Sometimes the EE can be varied with change in concentration of independent variables. EE of both drug when used with CS showed good results at +1 level of both polymers, that is, CS and poloxamer and also with TPP. The highest encapsulation efficiency was found to be 57.34% for LST and 58.67% for RST. The polynomial equation obtained after application of statistical design for % EE (Y1) for RST can be given by:

\[
Y_1 = +52.19 + 19.28\,A + 3.46\,B + 9.18\,C \ldots \ldots \ldots \ldots \quad (1)
\]

The similar equation can be derived for % EE (Y1) for LST as per follow:

\[
Y_1 = +45.10 + 20.56\,A + 7.56\,B + 9.91\,C \ldots \ldots \ldots \ldots \quad (2)
\]

Here Y2 is % EE, A denotes concentration of Chitosan (%); B is TPP concentration (%) and C is concentration of Poloxamer 188 (%). The model F value of 489.12 for above data indicated significance of model. A synergistic effect is indicated if the value in the equation is positive while negative value represents antagonistic effect. The suggested model for Y2 is Linear as shown in Table 4.

The correlation coefficient ($R^2$) for Y2 was found to be 0.8612 indicating model as good fit. The p value was found to be < 0.001 indicating statistical significance of model.

Also among the three independent variables selected all factors $A$ ($p<0.0001$), $B$ ($p = 0.0022$), $C$ ($p = 0.0001$) as shown in Table 5. Higher concentration of chitosan imparts higher ionic gelation resulting in enhanced entrapment efficiency. The low EE was attributed to lesser amount of CS and TPP used during the nanoparticles formulation. It was general concept that, increased EE may be due to the higher concentration of polymer with respect to the amount of the drug used during the nanoparticles formulation. Higher concentration of the polymer forms the denser mass of the drug polymer dispersion which helps to entrap the drug molecule in nanoparticles. Nanoparticles prepared with high cross linking agent showed increase in the EE.

### Table 3: RST-LST-NP’s physical evaluation parameters

| Batch code | RL1 | RL2 | RL3 | RL4 |
|------------|-----|-----|-----|-----|
| Morphology | Smooth, Spherical | Smooth, Spherical | Smooth, spherical | Smooth, Spherical |
| Particle size | 109.44 nm | 99.50 nm | 110.59 nm | 106.05 nm |
| PDI | 0.132 | 0.140 | 0.283 | 0.301 |
| Zeta potential | 20.12 ± 5.5 | 24.18 ± 2.5 | 24.20 ± 2.5 | 21.19 ± 3.0 |

### Table 4: Summary of results of regression analysis for responses Y1 and Y2

| Models | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Std. dev | Press | Remarks |
|--------|-------|----------------|-----------------|---------|-------|---------|
| Response Y1 | | | | | | |
| Linear | 0.8294 | 0.7983 | 0.8923 | 3.98 | 27.98 | suggested |
| 2FI | 0.9912 | 0.9813 | 0.9823 | 2.46 | 48.24 | ……… |
| Quadratic | 0.6918 | 0.8723 | 0.6845 | 2.44 | 24.78 | ……… |
| Cubic | 0.7913 | 0.8912 | 0.9812 | 3.19 | 41.23 | ……… |
| Response Y2 | | | | | | |
| Linear | 0.8798 | 0.8429 | 0.8612 | 2.46 | 9.95 | suggested |
| 2FI | 0.9642 | 0.9912 | 0.9637 | 1.45 | 19.78 | ……… |
| Quadratic | 0.9032 | 0.8624 | 0.8372 | 2.57 | 10.00 | ……… |
| Cubic | 0.8423 | 0.8362 | 0.6714 | 3.01 | 13.78 | ……… |

Regression equations of the fitted models

$Y_1 = +52.19 + 19.28\,A + 3.46\,B + 9.18\,C$

$Y_1 = +45.10 + 20.56\,A + 7.56\,B + 9.91\,C$
**TABLE 5** ANOVA of models for Y1 and Y2

| Source          | DF | Sum of squares | Mean square | F value | P value |
|-----------------|----|----------------|-------------|---------|---------|
| Model for Y1    | 3  | 3812.32        | 1298.12     | 587.12  | <0.0001 |
| A               | 1  | 2134.12        | 2215.09     | 851.21  | <0.0001 |
| B               | 1  | 261.12         | 161.21      | 47.01   | 0.0022  |
| C               | 1  | 498.12         | 578.98      | 26.21   | 0.0001  |
| Model for Y2    | 3  | 502.32         | 242.12      | 231.12  | <0.0001 |
| A               | 1  | 304.12         | 365.21      | 320.73  | <0.0001 |
| B               | 1  | 58.12          | 28.98       | 28.20   | 0.0057  |
| C               | 1  | 99.02          | 89.12       | 41.23   | 0.0012  |

**FIGURE 2** In-vitro release pattern of RST-LST-NP's. Showing comparison of release pattern with conventional formulation and pure drugs

### 3.2 RST-LST release from RST-LST-NP's

The in vitro drug release study was carried out in phosphate buffer saline (pH 7.4) with an initial burst release of 15.24% (RST) and 13.98% (LST) from RST-LST-NP's followed by a sustained release of 98.95% (RST) and 99.67% (LST) respectively at the end of 14 h of the experimental period. The results can be observed as graphical form in Figure 2. The sustained pattern of drug release can be clearly observed from the graph which would eventually help for the prolonged therapy.

When applied mathematical model like zero order, first order, Higuchi and Koremeyer-Peppas model, the Higuchi model was found to be fit for this diffusion release pattern. The concentration of polymer plays important role drug release [28]. Larger quantity of polymers quit ensures steady, sustained release. The polynomial equation obtained for EE (Y1) is given by

\[
Y2 = +75.10 + 3.90A + 4.32B + 4.87C
\]

Here Y2 is drug release, A denotes the concentration of Chitosan (%); B is the TPP concentration (%) and C the is concentration of poloxamer 188 (%).

### 3.3 Determination of change in uric acid (UA) level after administration of RST-LST-NP’s

The serum uric acid (UA) levels were analysed using auto-analysers. In animal group of RD (regular diet) the uric acid level was found to be normal, that is, 160 μmol/L while in experimental group received no treatment (group NT) the peak level of UA was found (210 μmol/L). The experimental group who receive treatment with Rosuvastatin nanoparticles (T-RST) and Lovastatin (T-LST) only showed slightly lower levels of UA (180 and 175 μmol/L respectively) than NT group. The significant lowering of UA levels was observed in treatment group received treatment with RST-LST-NP’s, that is, 165 μmol/L, the values obtained above were put in graphical format (Figure 3) showed that RST-LST-NP's have significantly more potent in lowering serum uric acid levels [29].

### 3.4 Determination of change in lipid profile after administration of RST-LST-NP’s

In elaborating lipid profile of from serum sample, the concentration of total cholesterol (TC), Low density lipoprotein (LDL), high density Lipoprotein (HDL) were determined. The study population in group RD showed no significant rise in level of any lipid. The concentrations were found to be in the normal range (i.e TC = 1.6 μmol/L, LDL = 0.37 μmol/L and HDL = 1.46 μmol/L). A very sharp and significant change in level of lipid profile was observed in experimental group NT. All lipid levels were remarkably increased. The TC was found to be 3.2 μmol/L, LDL was 0.99 μmol/L and HDL level was 2.1μmol/L. These values are high enough to cause mortality due to atherosclerosis. The animals in treatment group receiving RST-LST-NP’s, the levels of different lipids were as follow: TC was 1.72 μmol/L, HDL was 1.51 μmol/L and LDL was 0.43 μmol/L. The values obtained are very close to the normal lipid profile of animals. The values of TC, HDL, and LDL in group treated with RST nanoparticles were found to be 1.97, 1.8, 0.59 μmol/L respectively while for treatment group of LST nanoparticles the values are 2.1, 1.75, 0.63 μmol/L respectively. Although the both nanoparticles (RST- NP and LST-NP) were found to be effective in slight lowering of lipidic profile they were not as efficient as combination nanoparticles (RST-LST-NP’s). Therefore the results obtained proved the efficiency of RST-LST-NP’s in lowering the lipid content in blood serum sample.

### 3.5 Histological Evaluation

From Histological evaluation it can be observed that no significant changes in aortas of RD group were observed. Large no of foam cells following cell infiltration and nuclear condensation was observed in case of NT group (see Figure 4(A)). However no nuclear condensation or calcium deposits, no cholesterol or fat deposits were observed for treatment group T-RLN group
FIGURE 3  Blood Serum analysis showing: (A) Serum uric acid; (B) serum total cholesterol; (C) serum high density lipoprotein; (D) serum low density lipoprotein levels

FIGURE 4  Histological observations of aorta: (A) Deposition of fats, cholesterol in aorta of hypercholesterolemia animals; (B) aorta with no fat deposition or cholesterol in T-RLN group animals
(see Figure 4(B)). Slight amount of foam cells can be observed but are not to significant extent to generate atherosclerosis.

4 CONCLUSION

In present experiment a successful establishment of animal model to evaluate the effect of RST-LST-NP’s on atherosclerosis is done successfully. Thus formulated RST-LST-NP’s nanoparticles were found to be very effective in lowering the uric acid levels and maintaining the level of different Lipoproteins (TC, HDL, and LDL) when compared to non treatment group. The synergistic action of RST and LST can also be confirmed by combining them in RST-LST-NP’s. This action was confirmed by comparing their effect with RST-NP and LST-NP individually. The results obtained can assure the efficiency of RST-LST-NP’s in lowering and maintaining lipoprotein levels in atherosclerotic model.

REFERENCES

1. Insull, W., Jr: The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. Am. J. Med. 122(1), S3–S14 (2009)
2. Stary, H.C.: The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur. Heart J. 11(suppl_E), 3–19 (1990)
3. Shattock, S.G.: A report upon the pathological condition of the aorta of King Menephtah, traditionally regarded as the Pharaoh of the Exodus. Proc R Soc Med. 2(Pathol Sect):122-7(1909)
4. Sakakura, K., et al.: Pathophysiology of atherosclerosis plaque progression. Heart Lang Circ. 22(6), 399–411 (2013)
5. Ylä-Herttuala, S., et al.: Stabilization of atheroslerotic plaques: an update. Eur. Heart J. 34(42), 3251–3258 (2013)
6. Catapano, A.L., et al.: ESC/EAS guidelines for the management of dyslipidaemias: The task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis 217, 1–44 (2011)
7. Fukuda, K., et al.: Statins mediate anti-atherosclerotic action in smooth muscle cells by peroxsome proliferator-activated receptor-γ activation. Biochem. Biophys. Res. Commun. 457(1), 23–30 (2015)
8. Tamminen, M., et al.: Utrastructure of early lipid accumulation in ApoE-deficient mice. Arterioscler. Thromb. Vasc. Biol. 19(4), 847–853 (1999)
9. Keating, G.M., Robinson, D.M.: Rosuvastatin. Am. J. Cardiovasc. Drugs 8(2), 127–146 (2008)
10. De Lorenzo, F., et al.: Prevention of atherosclerosis in patients living with HIV. Vasc. Health Risk Manage. 5, 287 (2009)
11. Crouse, J.R. III: An evaluation of rosuvastatin: pharmacokinetics, clinical efficacy and tolerability. Expert Opin. Drug Metab. Toxicol. 4(3), 287–304 (2008)
12. Frisinghelli, A., et al.: Regression or reduction in progression of atherosclerosis, and avoidance of coronary events, with lovastatin in patients with or at high risk of cardiovascular disease: a review. Clin Drug Investig. 27(9), 591–604 (2007)
13. La Ville, A.E., et al.: Primary prevention of atherosclerosis by lovastatin in a genetically hyperlipidaemic rabbit strain. Atherosclerosis 78(2–3), 205–210 (1989)
14. Huang, M., et al.: Uptake of FITC-Chitosan nanoparticles by A549 cells. Pharm Res 19, 1488–1494 (2002)
15. Pohlmann, A.R., et al.: Spray-dried indomethacin loaded polyester nanocapsules and nanospheres: development, stability evaluation and nanostructure models. Eur. J. Pharm. Sci. 16, 305–312 (2002)
16. Wu, X.S., Wang, N.: Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers. Part IV: biodegradation. J. Biomater. Sci. Polym. Ed. 12, 21–34 (2001)
17. Flores, A.M., et al.: Nanoparticle therapy for vascular diseases. Arterioscler. Thromb. Vasc. Biol. 39(4):635–646 (2019)
18. Bathool, A., et al.: Development and characterization of atorvastatin calcium loaded chitosan nanoparticles for sustain drug delivery. Adv. Mater. Lett. 3(6), 466–470 (2012)
19. Nesalin, J.A., Smith, A.A.: Preparation and evaluation of chitosan nanoparticles containing zidovudine. Asian J. Pharm. Sci. 7(1), 80–84 (2012)
20. Kalimutu, S., Yadav, A.V.: Formulation and evaluation of carvedilol loaded Eudragit E 100 nanoparticles. Int. J. PharmTech Res. 1(2), 179–183 (2009)
21. Sanlıer, S.H, et al.: Development of gemcitabine-adsorbed magnetic gelatin nanoparticles for targeted drug delivery in lung cancer. Artif. Cells Nanomed. Biotechnol. 44(3), 943–949 (2016)

How to cite this article: Luo, G., et al.: Control and monitoring of Lipoprotein levels in atherosclerosis induced rabbits using novel nanoparticulate medication of Lovastatin and Rosuvastatin. Micro Nano Lett. 2021;1–8. https://doi.org/10.1049/mna2.12081.