

Evaluation of Hypolipidemic Activity of Simvastatin Nanosuspension in Triton WR-1339 induced Hyperlipidemic Rats

Athul P.V.*, Krishna Kumar

Rajivgandhi Institute Of Pharmacy, Trikaripur, Kasaragod, India.

*Corres.author: pv_athul@yahoo.com

Abstract: Simvastatin is a powerful lipid lowering agent that can decrease low density lipoprotein (LDL) level up to 50%. Simvastatin nanosuspension were prepared by high pressure homogenization method. The prepared formulation is evaluated for hypolipidemic activity in triton wr-1339 induced hyperlipidemic rats. The serum collected from different group of animals is evaluated by auto analyzer. The results shows that formulated nanosuspension have better efficacy compared to the unprocessed drug of simvastatin. Nanosuspension formulations has showed appropriate stability, formulation having drug content within the limits after 90 days.
Keywords: Simvastatin, nanosuspension, LDL, VLDL.

Introduction:

Nanosuspensions are defined as colloidal dispersion of nano-sized particles stabilized by surfactants. It is also defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of suspended particle is less than 1 μ m in size. The solubility of drugs that are poorly soluble in both aqueous and lipid media can be enhanced by using nanosuspension technique. The present study is aimed at overall improvement of therapeutic efficacy of hypolipidemic drug simvastatin through nanosuspension formulation. Simvastatin is a hypolipidemic agent whose bioavailability is reported as less than 5%. Nanosuspension of simvastatin is prepared by high pressure homogenization method using PVP K-30 as stabilizer. The prepared nanosuspension is evaluated for its hypolipidemic activity in triton induced hyperlipidemic rats.

Materials and Methods¹⁻³:

Simvastatin was obtained as a gift sample from microlabs, hosur, all other chemicals used are of analytical grade.

Preparing Nanosuspension:

Simvastatin nanosuspension is prepared by high pressure homogenization method. Simvastatin powder (1% w/v) was dispersed in aqueous surfactant solution using magnetic stirrer. After drug dispersion first size

reduction step is carried out using ultra turax T25 basic homogenizer at 9500 rpm for 10 min..Then obtained mixture is homogenized using micron lab 40 homogenizer (APV systems, Germany). The homogenization steps includes first two steps with 100 bar pressure and next two cycles with 500 bar pressure as initial step. Finally the suspension is homogenized for 15 cycles with 1500 bar pressure to obtain nanosuspension.

Production of Dry Nanoparticles:

The homogenized nanosuspension was freeze dried by using Virtis freeze dryer for increasing the shelf life of suspension. 1% mannitol is added to the suspension at the time of lyophilization as a cryoprotectant. At first samples are kept in deep freezer at -70°C overnight and kept in Virtis freeze dryer for 2 days in -50°C at 2 millitorr.

Particle Size Analysis:

The particle size analysis was carried out using Microtac blue wave particle size analyzer. Before measurement the samples has to be diluted with de-ionized water to obtain a suitable concentration for measurement. The results obtained for particle size distributions were used to confirm the formation of nano-sized particles.

Percentage Drug Content Determination:

The prepared nanosuspensions were analyzed for drug content by UV spectroscopic method. Nanosuspension equivalent to 20 mg of simvastatin weighed accurately and dissolve in 10 ml ethanol. The stock solutions were diluted with distilled water and analyzed by UV-Spectroscopy at 238 nm.

Animals:

Wistar albino adult male rats weighing 200-250g were used for the invivo hyperlipidemic study. The animals were grouped and housed in polyacrylic cages (38x 23 x 10 cm) with not more than five animals per cage and maintained under standard laboratory conditions (temperature $25 \pm 2^{\circ}\text{C}$) with dark and light cycle (14/10 hour).

Pharmacological Evaluation^{4,5}:

The animals were divided into four groups of five rats each. Group I, Normal control and receives standard pellet diet, water and orally administered with 5% CMC. Group II, Negative control and receives a single dose of triton administered at a dose of 400mg/kg, p.o. After 72 hours of triton injection, this group receives a daily dose of 5% CMC (p.o) for 7 days. The Group III receives a daily dose of nanosuspension of simvastatin, p.o., for 7 days, after inducing hyperlipidemia. Group IV receives standard drug simvastatin (10 mg/kg p.o).

On the 8th day blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes and serum samples were used for various biochemical experiments. Then animals were sacrificed by using 5ml Diethyl ether and collected the liver The liver was homogenized in cold 0.15M KCl and extracted with $\text{CHCl}_3:\text{CH}_3\text{OH}(2\% \text{v/v})$.The serum and liver were assayed for total cholesterol, triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL).

Stability Studies:

The formulated nanosuspension was divided in to 3 samples and stored at different temperatures for a period of 3 months.

- 4°C , refrigerated temperature
- $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ at 65% RH, room temperature
- $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ at 65% RH, accelerated temperature.

After 3 months formulation is evaluated for remaining drug content in by specific methods.

Table No-I: Result of particle size analysis

| Sample | Particle size (μm) |
|------------------|---------------------------------|
| Pure drug | 31.49 ± 0.07 |
| Homogenized drug | 0.240 ± 0.012 |

Table No-II: Effect of simvastatin nanosuspension on Total cholesterol, Triglycerides, LDL, VLDL in serum of control and experimental rats

| Groups and Treatment | Total Cholesterol | Triglyceride | LDL | VLDL |
|--------------------------------|------------------------|------------------------|------------------------|------------------------|
| Normal control | $87.63 \pm 2.26^{***}$ | $74.21 \pm 2.68^{***}$ | $21.86 \pm 0.94^{***}$ | $14.48 \pm 0.86^{***}$ |
| Negative control (Triton) | 268.30 ± 3.24 | 198.03 ± 3.16 | 115.34 ± 1.74 | 39.61 ± 0.98 |
| Positive control (simvastatin) | $173.24 \pm 1.98^*$ | $132.61 \pm 2.93^*$ | $51.86 \pm 0.78^{**}$ | $26.52 \pm 0.68^{**}$ |
| Test (Nanosuspension) | $107.04 \pm 2.39^{**}$ | $88.91 \pm 1.95^{**}$ | $27.08 \pm 0.96^{**}$ | $17.78 \pm 0.57^{**}$ |

Values are mean \pm SEM; n = 5 animals in each group; *P < 0.05, **P < 0.01, ***P < 0.001 when compared to triton treated group.

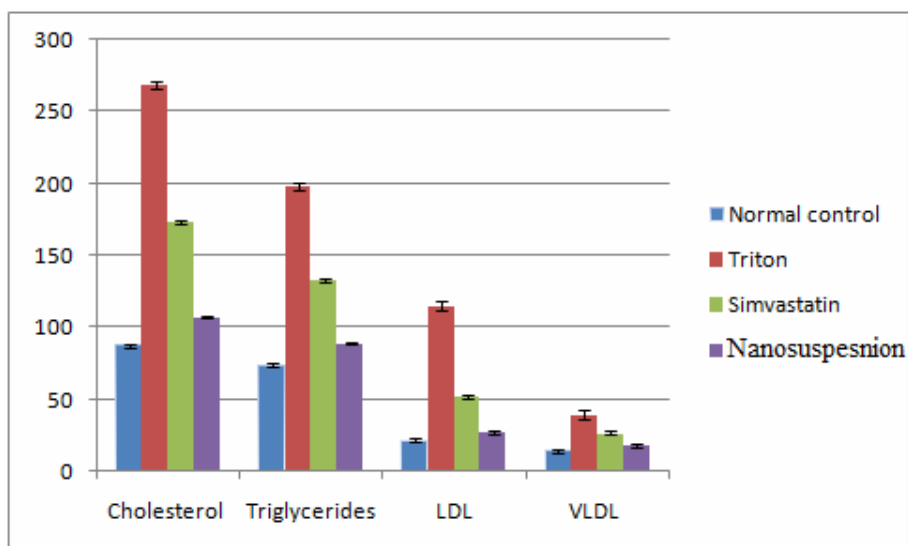
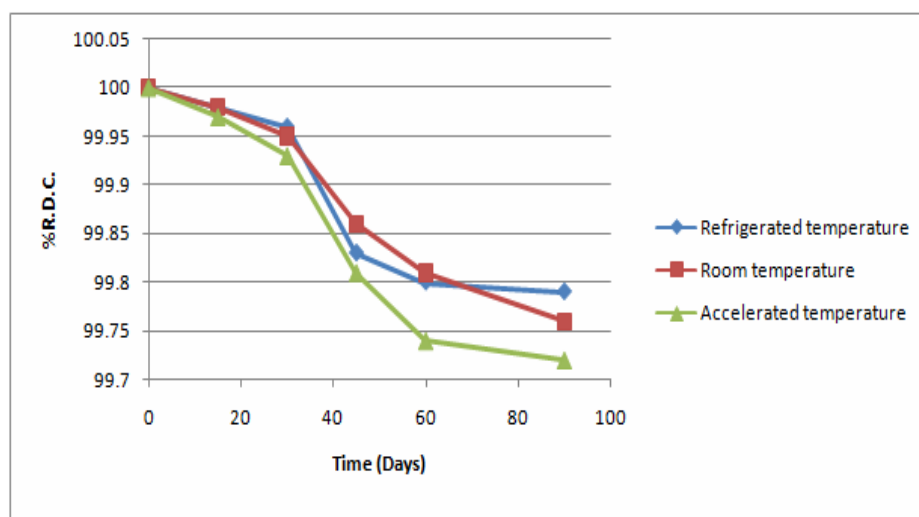
Table No. III: Stability studies of nanosuspension

| Sl.No. | Days | % R.D.C. 4°C | % R.D.C. 27°C | % R.D.C. 40°C |
|--------|------|--------------------|--------------------|--------------------|
| 1 | 0 | 100.00 ± 0.000 | 100.00 ± 0.000 | 100.00 ± 0.000 |
| 2 | 15 | 99.98 ± 0.032 | 99.98 ± 0.021 | 99.97 ± 0.018 |
| 3 | 30 | 99.96 ± 0.010 | 99.95 ± 0.011 | 99.93 ± 0.021 |
| 4 | 45 | 99.83 ± 0.013 | 99.86 ± 0.023 | 99.81 ± 0.045 |
| 5 | 60 | 99.80 ± 0.034 | 99.81 ± 0.043 | 99.74 ± 0.029 |
| 6 | 90 | 99.79 ± 0.041 | 99.76 ± 0.030 | 99.72 ± 0.023 |

R.D.C. = Remaining drug content

Results and Discussion

The particle size analysis studies show that homogenized drug was found to be 240 nm and the unprocessed drug 31.49 μm in size. All particles are found to be in nanometer size range and showing ideal surface morphology. All the formulations were found to be easily redispersible on moderate shaking. The formulation shows a percentage drug content of 99.51% which is acceptable according to United state pharmacopoeia. The pharmacodynamic study for the formulations and unprocessed drug was carried out in triton induced experimental animals and results are recorded. Treatment with nanosuspension of simvastatin produced a significant decrease in the serum level of lipids in the triton induced hyperlipidemia rats compared to unprocessed drug. The serum analysis of control and test animals shows that total cholesterol, triglyceride levels, LDL, VLDL levels are decreased more in triton induced test animals (simvastatin nanosuspension) compared to the positive control group (unprocessed drug). The results indicates that the formulated dosage form of simvastatin having better therapeutic efficacy compared to unprocessed drug. The stability studies were carried out for formulation as per ICH guidelines at refrigerator temperature (4°C), room temperature (27°C), and accelerated temperature (40°C) for a period of 90 days. The result shows that formulation having drug content within the limits after 90 days, indicating better stability.

Fig No-1: In-vivo Hypolipidemic activity**Fig No-II : Stability studies of formulated nanosuspension**

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