



Microwave Generated Nanocomposites for Solubility Enhancement of Atorvastatin Calcium: *In Vitro- In Vivo* Characterization

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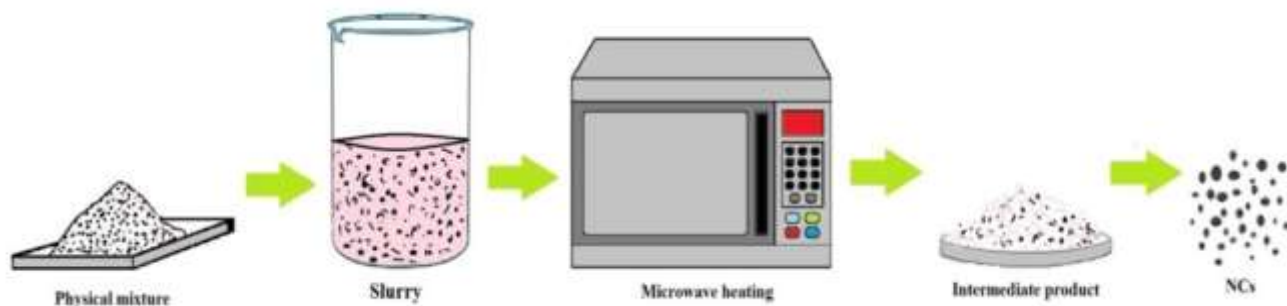
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Abstract : Atorvastatin calcium is anti-hyperlipidemic agent has low aqueous solubility resulting in low oral bioavailability thus presents a challenge in formulating a suitable dosage form to improve the aqueous solubility. Nanocomposites are novel technology for enhancing the solubility of Atorvastatin calcium. Nanocomposites formulation of Atorvastatin was prepared by microwave induced diffusion method (MIND). The natural polymers like gum acacia, modified gum karaya and synthetic polymer like PVP K-30 were used as carrier in the formulation. Six different formulations were prepared with varying ratios of drug and carriers and corresponding physical mixtures were also prepared. The selections of natural carriers were based on their surfactant and wetting properties. The optimum drug-to-carrier ratio was found to be 1:4 AA_N which enhanced solubility nearly 14 fold as compared to pure drug. *In vitro* drug release exhibited cumulative release of 84.72% as compared to 48.47% for the pure drug. The optimized nanocomposites were characterized by Fourier transform infrared spectroscopy, Differential scanning calorimetry, X-ray diffraction, Scanning electron microscopy, and Transmission electron microscopy. In a Triton-induced hyperlipidemia model, a 2-fold increase in the lipid lowering potential was obtained with the reformulated drug as compared to pure drug. These results suggest that nanocomposites using natural carrier is a promising approach for oral delivery of Atorvastatin calcium.

Keywords : Nanocomposites, Atorvastatin calcium, Microwave induced diffusion method , Natural and Synthetic carriers.

Graphical abstract



1. Introduction

The oral route is the major way of dosing both existing and new drugs. Most of the drugs which administrated by oral route are absorbed by passive diffusion through the gastro-intestinal (GI) cellular membranes. The solubility and permeability are the most important tools for determining the oral bioavailability of a specific drugs within the GI tract, which have aqueous environment. According to US pharmacopoeia more than 40% of the drugs are poorly soluble or insoluble in aqueous environments. The enhancement oral bioavailability of poorly water-soluble drugs represent an actual challenge for pharmaceutical research, with the aims of improving drug therapeutic effectiveness as well as creating new market opportunities. The BCS class-II drugs are water-insoluble (solubility equal or less than 100 μg of solute per 1 ml of solvent) but have high membrane permeability is only limited by dissolution. The energy-driven step is dissolution of crystalline solid in an process. In general, the kinetics of the process depends on solute, solvent chemical nature, microstructure and on the system conditions. It is possible to show that the dissolution rate of a crystalline drug in a given dissolution environment can be increased by forcing it to assume a microstructure by the theories of dissolution and non-electrolytes solubility characterized in nanoscale (short range) periodicity. The latest and most effective approaches to water-insoluble drugs solubilization are based on generating a drug dispersion (at molecular and/or nanoscale level) in a stabilizing media, preferably in solid-state form. Our main approach isthat the enhancing effects of Microwave (MW) heating on mass transport might provide a green, effective instrument for generating such dispersions [1].

Atorvastatin have an class of drugs which is statins and it used for lowering blood cholesterol level.It has very good intestinal permeability and short half-life(T_{max} , 1–2h).However, the factors likelow aqueous solubility(0.1mg/mL), crystalline nature and hepatic first pass metabolism responsible for low oral bioavailability (12%) of Atorvastatin[2]. Due to the poor performance, drug have to administrated in higher doses which can causes liver abnormalities, rhabdomyolysis,arthralgia and kidney failure [3].To avoid such side effects, salt formation [4] and inclusion complexes with b-cyclodextrin [5] has been tried. A nanocomposites is a combination of two or more different materials with different properties of each and that are fused, by an effort to blend the best properties of both. A composite consists of two materials of varying natures and combination of those shows improved in their properties greater than that of individual [6]. The melting or fusion technique is one of the simple and efficient technique in the preparation of nanocomposites or/and bionanocomposites for the solubility and dissolution enhancement. Particle size reduction provides more surface area for absorption and rapid dissolution[7].Microwave radiation consists of electromagnetic waves with frequencies between the infrared and radio waves, which is in the range of 0.3–300 GHz.It passes through materials and oscillate their molecules, which generating heat.The ability of microwave to penetrate any substance, which produced the heatin a sample at any point at a given time [8-9].The first and unique attempt was proposed by Kerk et al in the direction of bioavailability enhancement[10]. The pharmaceutical nanocomposites which prepared by MW processing which was silicon dioxide substrate with isolated molecular clusters which adsorbed on the surface. Nevertheless, it seems that re-crystallization is not definitively inhibited, as drug molecules have high mobility on inorganic surfaces. These approach consisting the replacement of inorganic surface with inert 3D-matrixes that havingthe suitable microstructural properties to prevent re-crystallization of the drug. The cross-linked polyvinylpyrrolidone (Crosopovidone) is first chosen matrix which constrains the drug into stable molecular clusters and/or nanocrystals by its 3D network [11-13].

The other matrix like cyclodextrin, is a torus-shaped molecule that forms molecular complexes with the drug [14].

In the present work, we developed a nanocomposites of atorvastatin calcium using natural carriers such as gum acacia, and modified gum karaya and synthetic polymer like pvp k-30. The atorvastatin NCs were evaluated for drug content, solubility and dissolution studies. Varying ratios of drug and carrier were formulated and evaluated. To deduce the possible effects of the carrier on the drug, their physical mixtures (PM) were also formulated and compared with NCs and plain drug for solubility analysis. Optimized nanocomposites were followed by DSC, XRD, SEM and TEM studies for confirmation of formation of NCs. Finally in-vivo studies were carried out to elucidate the anti-hyperlipidemic potential of optimized nano composites with comparison to pure drug.

2. Materials and method

2.1. Materials

Atorvastatin calcium was generous gift from Mylan lab, Nashik, Maharashtra (India). Gum acacia, Modified Gum Karaya and PVP K-30 were purchased from Modern science, Nashik, Maharashtra (India). All the materials were of analytical grade. The materials were used as received without any further purification.

2.2. Selection of natural gum [7, 15, 18]

Gum acacia and modified gum karaya were studied for swelling index, foaming index, viscosity method which described by Murali M. B GV et al [15, 23].

2.2.1. Swelling Index (SI)

Swelling index of gums was determined by modified method reported. 1 gm of acacia, modified gum karaya and gaur gum was accurately measured and transferred to 100 ml measuring cylinder. The initial volume which occupied by gum was noted. Made up the volume upto the 100 ml with distilled water. The open end of cylinder was sealed with aluminum foil and kept aside for 24 hrs. After 24 hrs volume of swelled gum was noted. The swelling index of gum was calculated by the following formula.

$$SI = \frac{H_f - H_i}{H_i} \times 100$$

Where, SI- Swelling index of gum,

H_i - Initial height of powder,

H_f - Final height of powder after 24 hr.

2.2.2. Foaming index

The foaming index of gum was calculated to check the surfactant properties of the gum. Accurately weighed 1 g of gum and transferred it in 250 ml measuring cylinder. 100 ml distilled water was added in measuring cylinder to make dispersion. Resultant dispersion was vigorously shaken for 2 minutes. The foaming index of gum calculated by the following equation,

$$\text{Foaming index} = H_f - H_i$$

Where, H_f = Height of solution of gum after shaking;

H_i = Height of solution of gum before shaking.

2.2.3. Viscosity

Viscosity of gums was calculated by dissolving one gram of each acacia gum and modified gum karaya and gaur gum in 100 ml of water (1% w/v solution). The viscosity of the carrier dispersions of acacia and modified gum karaya were measured by Brookfield viscometer using spindle 00 at 200 rpm.

2.3. Formulation of physical mixture [7, 15, 16, 18]

Physical mixture of drug with polymer acacia (AC), modified gum karaya, PVP K-30 were prepared by simple blending of drug with polymer in the ratio 1:1 to 1:6. The quantity of pure drug and respected polymers were mentioned in Table 1. The physical mixture prepared to check the solubility enhancing property of nanocomposites as compared with physical mixture.

Table 1: Formulation of physical mixture

Ratios (for physical mixture)	Quantity (mg)			
	Atorvastatin calcium (drug)	Gum acacia	Modified gum karaya	PVP k-30
1:1	500	500	500	500
1:2	500	1000	1000	1000
1:3	500	1500	1500	1500
1:4	500	2000	2000	2000
1:5	500	2500	2500	2500
1:6	500	3000	3000	3000

The resultant physical mixtures of atorvastatin calcium with carriers acacia gum, modified gum karaya and pvp k-30 are denoted by AA_p, AK_p and AP_p, respectively.

Data are means \pm SD, n = 3.

Table 2: Formulation of nanocomposites.

Ratio (for nanocomposite s)	Quantity (mg)			
	Atorvastatin calcium	Gum acacia	Modified gum Karaya	PVP k-30
1:1	500	500	500	500
1:2	500	1000	1000	1000
1:3	500	1500	1500	1500
1:4	500	2000	2000	2000
1:5	500	2500	2500	2500
1:6	500	3000	3000	3000

The developed nanocomposites of atorvastatin calcium with carriers acacia gum, modified gum karaya and pvp k-30 are denoted by AA_N, AK_N and AP_N, respectively.

Data are means \pm SD, n = 3.

2.4. Formulation of nanocomposites[15, 16, 17]

The nanocomposites were prepared by homogenous mixing of accurately weighed amount of individual drug with individual polymer. In this case the weight to weight (w/w) ratio of drug to polymer was taken from 1:1 to 1:6 keeping amount of mixture constant. The quantity of pure drug and polymer for different ratios were taken as mentioned in Table 2. Add 4 ml of water for each gram of polymer to this mixture for making homogenous slurry. The fixed amount of slurry was taken in glass beaker and irradiated with microwave radiation at power 560 W (CATA-2R, Catalyst System) with continuous stirring for 5 min. Nanocomposites were grounded in mortar and sieve to achieve the particle size of 80 to 250 μ m.

2.5. Evaluation of nanocomposites [7, 15, 16, 18]

2.5.1. *Invitro* solubility study:

The solubility of Atorvastatin calcium, AA_p, AK_p, and AP_p, AA_N, AK_N, and AP_N was determined in pH 6.8 phosphate buffer. The solubility of drug, physical mixtures and NCs was determined by taking an excess amount of drug (10 mg) and NCs (equivalent to 10 mg of drug) and adding them to 10 ml of solvent (pH 6.8 buffer), in Teflon-facing screw-capped vials. The samples were kept at equilibrium for a period of 24 hrs in an orbital shaker (Remi Instruments) at 37 \pm 0.5 $^{\circ}$ C and 50 rpm. The supernatant fraction collected from the vials

was filtered through a 0.45 micron membrane filter and analyzed by UV-visible spectrophotometer (Shimadzu) at a wavelength of 241 nm. Ratio optimization (drug: carrier) was done on the basis of the solubility results obtained.

2.5.2. *In vitro* dissolution profile:

In-vitro powder dissolution test was carried out on Atorvastatin calcium and nanocomposites which were performed by using USP XXIV apparatus II (Paddle) method by using 900ml pH 6.8 phosphate buffers as a dissolution media. Powder that contain accurate dose of drug (or equivalent to 10 mg of Atorvastatin calcium) was added in the dissolution media maintaining temperature at $37 \pm 0.5^\circ\text{C}$ and rotation speed of paddle at 75 rpm. 5 ml of sample were withdrawn at the interval of 0, 5, 10, 15, 20, 25, 30 minute by replacing 5 ml of pH 6.8 phosphate buffer solution in dissolution media. Samples were filtered by 0.45 μ membrane filter and analyzed spectrophotometrically at wavelength of 241 nm.

2.5.3. Drug content analysis

To calculate the amount of drug incorporated into nanocomposites drug content analysis was performed by dissolving nanocomposites mixture in 25ml of methanol. The resulting solution was filtered through 0.45 μ membrane filter and analyzed by UV-visible spectrophotometer at wavelength 246 nm for Atorvastatin calcium against methanol as a blank.

2.6. Solid state studies of optimized nanocomposites[7, 15, 16, 18]

From the results obtained by solubility and dissolution studies, the NCs that showed better results were selected for further characterization.

2.6.1. Fourier –transform infrared spectroscopy (FTIR)

FTIR study of optimized ratio of nanocomposites (AAN 1:4) was carried out. Nanocomposites were mixed with potassium bromide (KBr) of IR grade in a ratio of 1:100 and compressed using a pellet pressed at 15 tones pressure. Then pellets were scanned using an FTIR spectrophotometer (Shimadzu, 8400S). The FTIR spectra of optimized nanocomposites were compared with that of the pure drug to assess any change in the principal peaks of spectra of optimized nanocomposites.

2.6.2. Differential Scanning calorimetry (DSC)

A DCS study of optimized nanocomposites ratio (AA_N 1:4) was employed to access what changes had actually made when nanocomposites were formulated and by what fact these enhances the solubility of drug. The DSC curves were obtained by Differential Scanning Calorimeter(Shimadzu, DSC 60, Japan) at the heating rate of 10 $^\circ\text{C}/\text{min}$ from 50 to 200 $^\circ$ in nitrogen atmosphere.

2.6.3. X-ray diffraction studies (XRD)

XRD studies of drug Atorvastatin calcium and optimized nanocomposites (AA_N 1:4) were carried out to investigate the change in crystallinity when drug was mixed with polymer. XRD pattern were recorded using with Cu- $k\alpha$ radiation (D500, Siemens Diffractometer). The scanning angle ranged from 10 $^\circ$ to 80 $^\circ$ of 2 θ . XRD Study was carried out to assess the changes in the crystallinity made when the drug was mixed with carriers.

2.6.4. Scanning electron microscopy (SEM)

Scanning electron microscopy was used to examine external surface morphology. The morphologies and detailed particle structural characterizations of pure drug and nanocomposites (AA_N 1:4) were observed by scanning electron microscope (JEOL JSM-630 J, Scanning Electron Microscope). NCs that showed the best results in the solubility and dissolution studies were subjected to scanning electron microscopy (SEM) studies to confirm the changes made during the formation of NCs. Samples were prepared by mounting powder onto a brass stub using graphite glue and coated with gold under vacuum before use. Images were recorded at the required magnification at an acceleration voltage of 10 KV using a scanning electron microscope.

2.6.5. Transmission electron microscopy (TEM)

The drug Atorvastatin calcium and optimized ratio of nanocomposites (AA_N 1:4) showing the best results in the solubility and dissolution studies was subjected to transmission electron microscopy (TEM) studies to confirm the formation of nanocrystals embedded in composites. The particle size and shape of pure drug crystal dispersed in polymer were analyzed with transmission electron microscopy. The morphology of the NCs was obtained by transmission electron microscope (PHILIPS CM200, Transmission Electron Microscope)

2.7. In-vivo pharmacological study [19, 20]

The anti-hyperlipidemic effect of the best optimized formulation were tested using a Triton-induced hyperlipidemia model. Male or female Wister rats weighing 200–250g were divided into four groups (n=5): Group I, normal control (0.9%, w/v sodium chloride solution); Group II, Triton treated; Group III, Plain atorvastatin; Group IV, optimized nanocomposites.

The study protocol was approved by animal ethics committee of MG V's pharmacy college, Nashik. All experiments comply with the Institutional Animal ethical Committee guideline under the purview of Committee for the purpose of control and supervision of Experimental Animals (CPCSEA). The animals were fasted for 18h and then injected intravenously with 200mg/kg Triton WR1339 (isooctyl – polyoxyethylenephenol, except group I). At 45 min after injection atorvastatin or the optimized NCs formulations were administered p.o. (25mg/kg) to groups III, and IV. Serum cholesterol, triglycerides and high density lipoprotein (HDL) levels were measured 24h after Triton injection. Animals were anesthetized with light anesthetic diethyl ether and 1ml blood was withdrawn from retro-orbital sinus. The blood was fractionated at 10,000 rpm for 10 min. at 40°C, and the serum was separated for analysis.

3. Results and Discussion

3.1. Preliminary Investigation of Atorvastatin calcium

Saturation solubility of pure Atorvastatin calcium in water, methanol and phosphate buffer pH 6.8 were determined. The results suggest that Atorvastatin calcium has very less solubility (0.028 mg/ml) in water. The melting point was observed 158-160°C.

3.2. Selection of Natural gum-

Swelling characteristics and viscosity of the gums are presented in Table 3. From this data, it can be concluded that the swelling characteristics and viscosity of acacia gum, modified gum karaya were low. The High viscosity and toughness of the carrier may affected its dissolution enhancement property [23]. According to past results it known that less swelling and low solution viscosity, they are more prone to dissolution enhancement. They are less prone to the formation of the tough matrix which will assist rapid liberation of the nanocrystals from the nanocomposites. The foaming index clearly indicates the greater foaming ability of acacia from other carriers. Hence, acacia enhances the solubility more efficiently than the other carriers.

Table 3: Physical characterization of polymer

Material	% Swelling	Viscosity (cp)	Foaming Index
Acacia	66.66 ± 1.20	2.22 ± 0.13	9 ± 0.40
Modified Gum Karaya	76.66 ± 1.02	2.21 ± 0.11	7 ± 0.70

Data are means ± SD, n = 3.

3.3. Evaluation of nanocomposites

3.3.1. In-vitro Solubility studies

Solubility studies were performed to find out the solubility enhancing properties of nanocomposites. Solubility studies provided the basis for selection of the best ratio that was to be forwarded for formulation.

Pure drug Atorvastatin and physical mixtures of Atorvastatin with individual carriers in varying ratios, as well as nanocomposites of Atorvastatin with individual carrier in varying ratios were analyzed for solubility determination. The solubility of pure drug was found to be 0.028 mg/ml in water and 0.112 mg/ml in phosphate buffer pH 6.8. The results of solubility studies of a various ratios of physical mixture and nanocomposites are presented in Table 4 and fig.1. Solubility studies reveals that physical mixtures improves the solubility of ATR significantly compared with pure drug .This may be due to the surfactant and wetting property of acacia gum, modified gum karaya. In case of nanocomposites solubility data indicates a tremendous rise in solubility compared with pure drug; this may be due to reduction of crystal size of the drug to a nanocrystalline form. This aspect is to be investigated by performing SEM and X-ray diffraction analyses.

Table 4: Solubility of Physical mixture and Nanocomposites of Atorvastatin calcium

Drug polymer Ratio	AA _P mg/ml	AK _P mg/ml	AP _P mg/ml	AA _N mg/ml	AK _N mg/ml	AP _N mg/ml
1:1	0.504± 0.01	0.347± 0.07	0.201± 0.04	1.055±0.02	0.829±0.03	0.246±0.05
1:2	0.650± 0.06	0.526± 0.09	0.280± 0.09	1.424±0.04	1.066±0.08	0.392±0.08
1:3	0.695± 0.07	0.617± 0.06	0.414±0.04	1.547±0.04	1.164±0.12	0.538±0.03
1:4	0.801± 0.04	0.695± 0.05	0.583± 0.08	1.627±0.08	1.200±0.02	0.639± 0.09
1:5	0.639± 0.06	0.549± 0.19	0.628± 0.10	1.402±0.05	0.661±0.10	0.740± 0.03
1:6	0.481± 0.14	0.448± 0.11	0.302± 0.01	0.897±0.06	0.436±0.06	0.515±0.06

Data are means ± SD, n = 3.

Solubility studies of physical mixtures and nanocomposites clearly indicated that as the ratio of drug to carrier increases solubility up to specified ratio. It was also found that the high solubility was shown by nanocomposites formulation and nanocomposites prepared by using acacia (AA_N) showing best solubility result at 1:4 ratio was considered optimal. The solubility of AA_N was found to be 1.627 mg/ml. AA_N (1:4), AK_N (1:4), AP_N (1:5) are the best ratios from different drug: polymers nanocomposites formulations, which were showing high solubility, hence those were subjected to *invitro* drug release study with pure drug and drug content analysis.

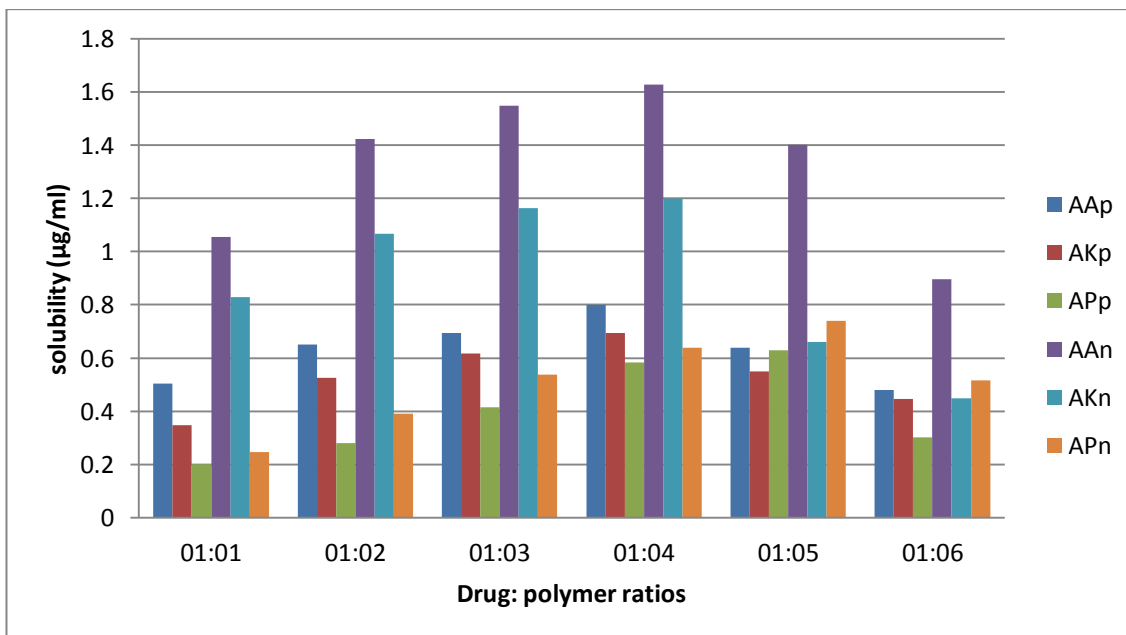


Fig. 1: Solubility graphs of AA_P, AK_P, and AP_P, AA_N, AK_N, and AP_N in phosphate buffer pH 6.8

3.3.2. *In vitro* Drug release

A powder dissolution test was performed because the solubility studies are not always a predictable parameter to determining the solubility enhancing properties of any material. The dissolution studies of Atorvastatin and Atorvastatin nanocomposites (NCs) give more specific information about the solubility and dissolution of drug. The dissolution profile of pure drug and NCs is presented in fig. 2. From the dissolution profiles of the NCs, there was evidently a remarkable improvement of the dissolution rates in all NCs compared with the pure Atorvastatin. All Of the NCs, the best result was shown by AA_N which show the drug released 84.72% in comparison to pure Atorvastatin which released 48.47%. From the solubility and dissolution studies the AA_N was selected for formulating the dosage form.

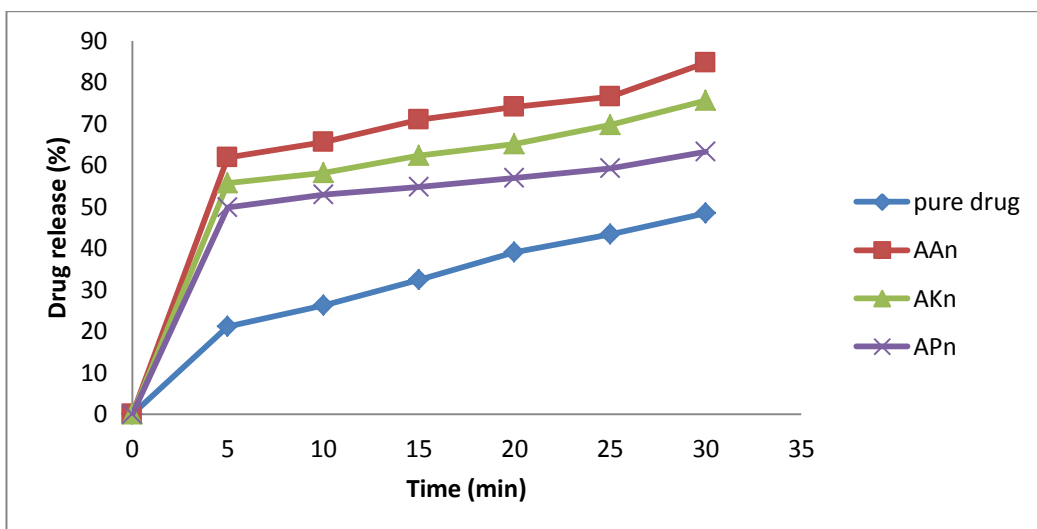


Fig 2: Dissolution profile of pure drug, AA_N, AK_N, and AP_N

3.3.3. Drug content analysis

Uniform dispersion of drug in the nanocomposites can be determined by drug content analysis. It was found that 85-92% drug was incorporated in the nanocomposites. AA_N (1:4) showing uniform dispersion of drug in the nanocomposites. Drug content analysis are presented in Table 5.

Table 5: Drug content analysis of nanocomposites

Nanocomposites	AA _N	AK _N	AP _N
Drug incorporated	91.42 ± 0.54%	89.82 ± 0.50 %	85.42 ± 0.66%

Data are means ± SD, n = 3.

3.4. Solid state studies of optimized nanocomposites

The optimized ratio AA_N1:4 further characterized by following parameters.

3.4.1. Fourier –transform infrared spectroscopy (FTIR)

FTIR studies are carried out for characterization of drug and to check the interaction between drug and polymers in the formulation. FTIR study of optimized nanocomposites and pure drug were carried out. FTIR spectra of optimized nanocomposites and pure drug are shown in fig. 3. The wave numbers of bands observed in the infrared spectrum of atorvastatin calcium listed as follows (intensity), 3668.12, 3363.67, 3055.35, 2920.32, 1651.12 and 744.55. These bands confirm the presence of characteristics groups like aromatic and aliphatic CH (3055.35 and 2920.32 respectively), while 3668.12 confirm the OH group, 3363.67 confirm amide NH group. The 1651.12 and 744.55 bands confirms the amide C=O and C-F respectively. The spectrum of optimized NCs was equivalent to Atorvastatin calcium. From this it can be concluded that principal peak values of the drug

remain unchanged in the microwave-treated NCs indicating no chemical interaction. Thus, it can be concluded that there is no chemical interaction between the drug and gum carrier.

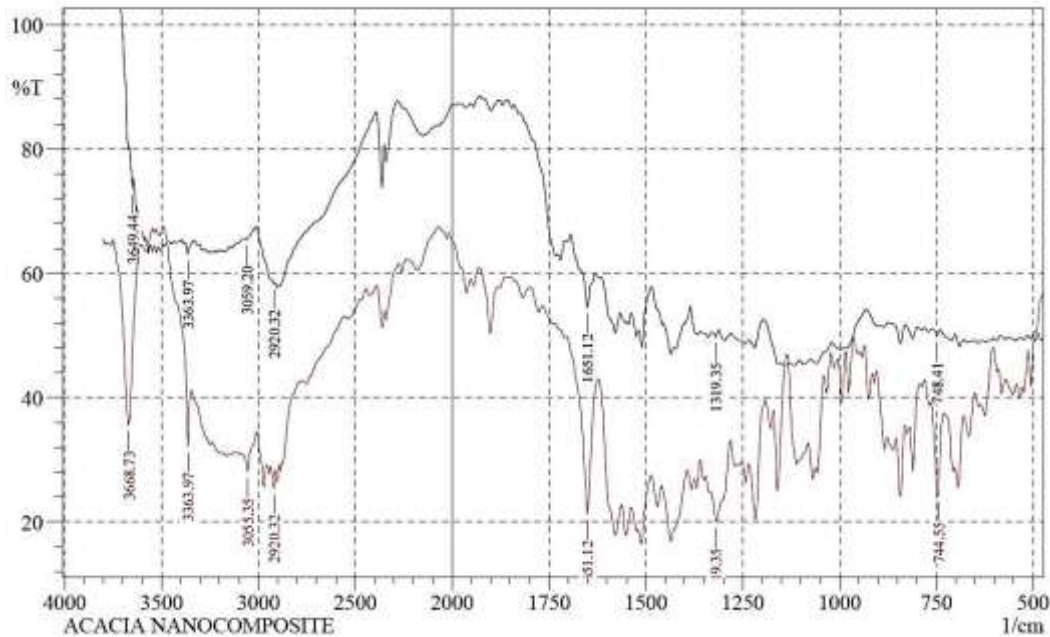
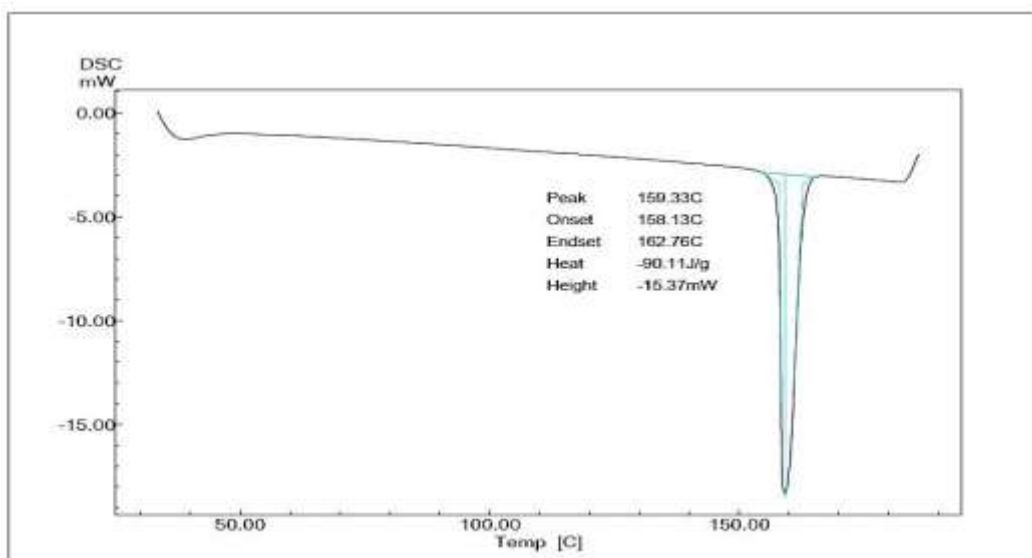


Fig 3 : FTIR of pure drug and optimized formulation.

3.4.2. Differential Scanning calorimetry (DSC)

DSC was performed to detect the interaction between Atorvastatin calcium and polymer. The DSC thermogram of pure drug shows a sharp endothermic peak corresponding to the melting point of crystalline drug was found to at 159.03°C. DSC of optimized nanocomposites AA_N 1:4 shows slight variation in endothermic peak as that of pure drug and intensity of peak is reduced this may be due to the decrease in the crystalline size of the drug. The DSC thermogram of AA_N at 143.05°C had shown a broad endothermic peak. The peak broadening indicated that most of the drug is embedded in NCs in the nanocrystalline form. Little shift in melting point was observed due to reduction of drug to the nanocrystalline form. This phenomenon is responsible for the solubility enhancement as the crystallinity has been reduced to the nanocrystalline form solubility get enhanced. DSC thermogram of pure drug and nanocomposites are shown in fig 4.

A.



B.

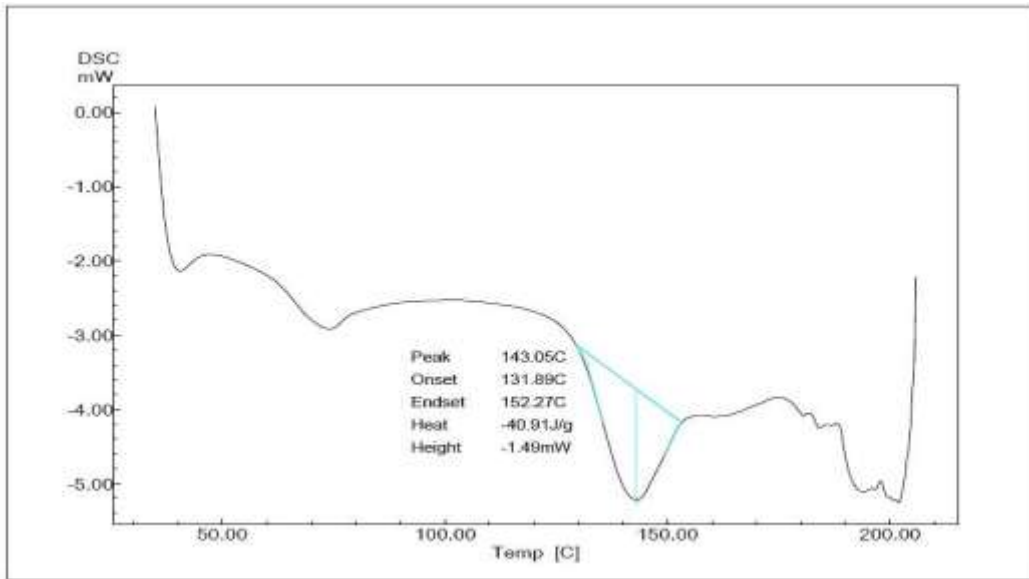
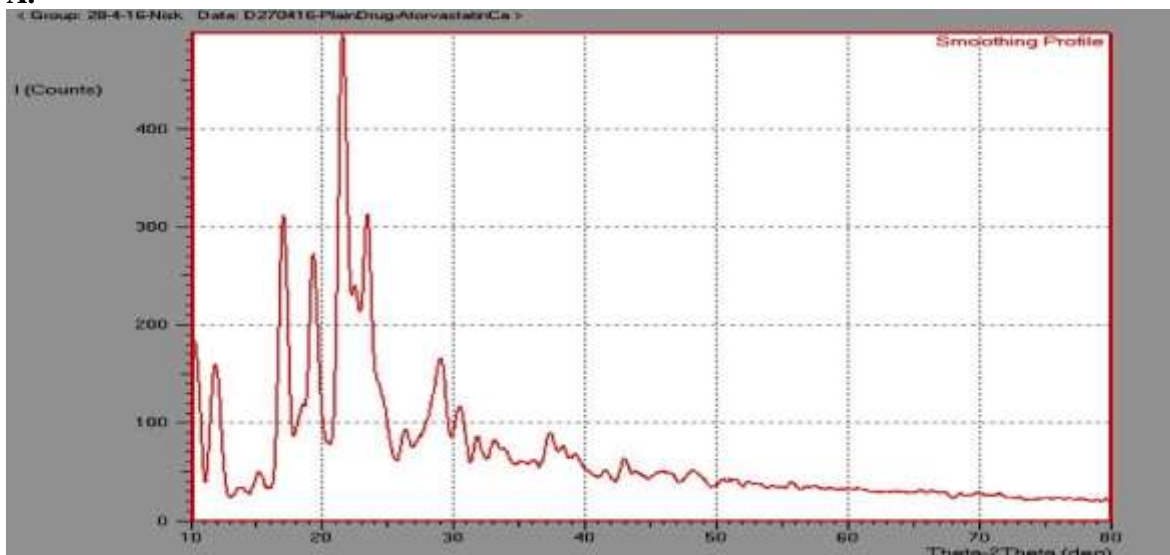


Fig. 4: DSC thermo gram of (A) pure drug and (B) optimized nanocomposites.

3.4.3. X-Ray Diffraction Studies (XRD)

The X-ray diffraction studies (XRD) of Atorvastatin calcium (ATR) and optimized nanocomposites AA_N 1:4 are shown in figure. The pure (ATR) exhibit intense crystalline peak between 10° and 50°, characteristic diffraction peaks at 10.44°, 11.90°, 17.04°, 19.33°, 23.40°, 24.82°, 28.83°, 30.43° and 37.33° were observed with intense peak at 21.58° indicating the crystalline nature of ATR. On the other hand AA_N it's observed that the peak intensity is reduced indicating reduction in crystallinity. This phenomenon is also responsible for enhancement of solubility. XRD pattern of pure drug and nanocomposites were showed in fig.5.

A.



B.

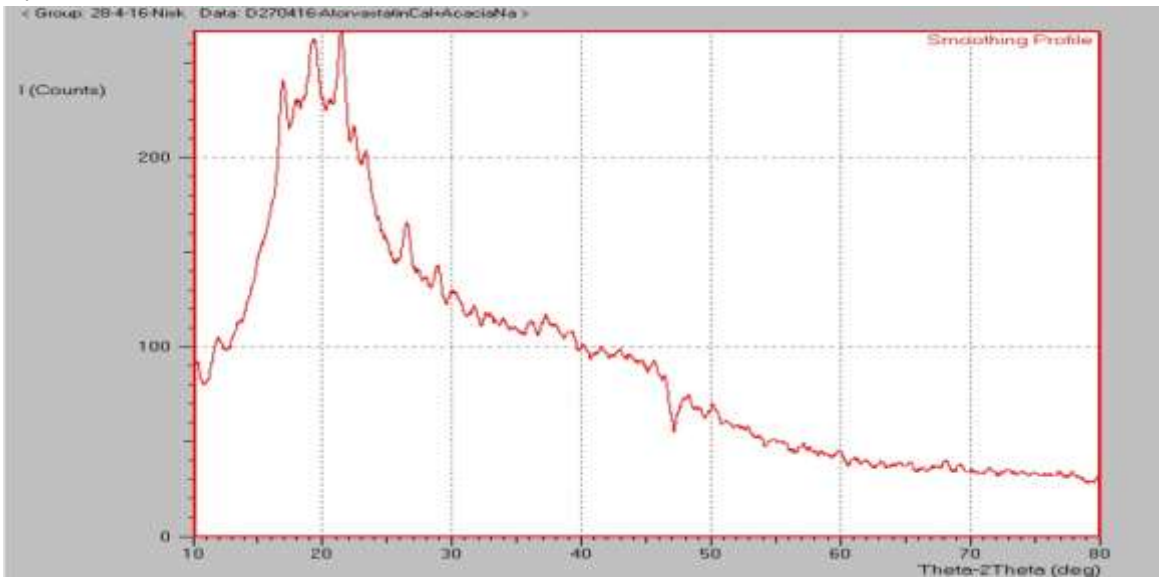
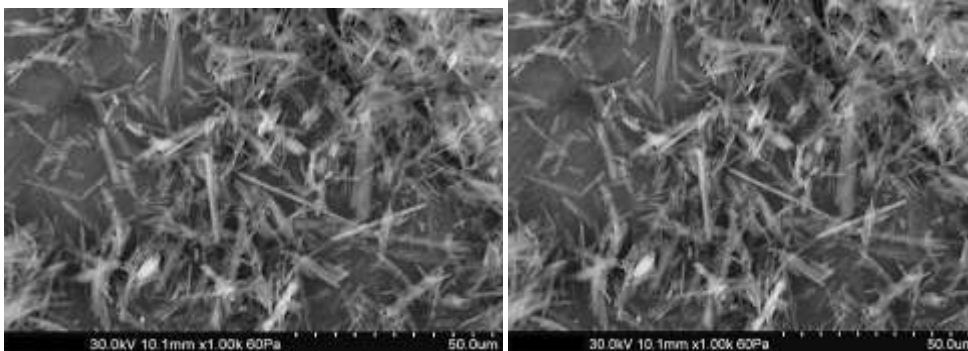


Fig. 5: XRD Pattern of (A) pure drug and (B) optimized nanocomposites.

3.4.4. Scanning electron microscopy (SEM)

SEM studies are usually done to study the surface morphology of drug particles. Atorvastatin calcium and optimized nanocomposites AA_N 1:4 were characterized by SEM. From the fig.6, it is concluded that pure Atorvastatin calcium drug showed needle and plate shaped with smooth surface while in case of AA_N it was observed that they were irregular shape and size. Figure clearly shown that crystal shaped of Atorvastatin calcium completely changes in nanocomposites which showing embedded Atorvastatin crystal in the matrix of polymer (acacia).

A.



B.

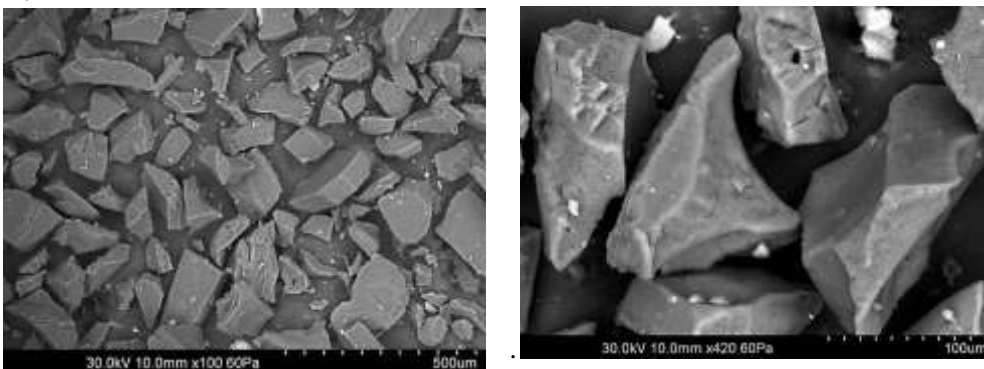
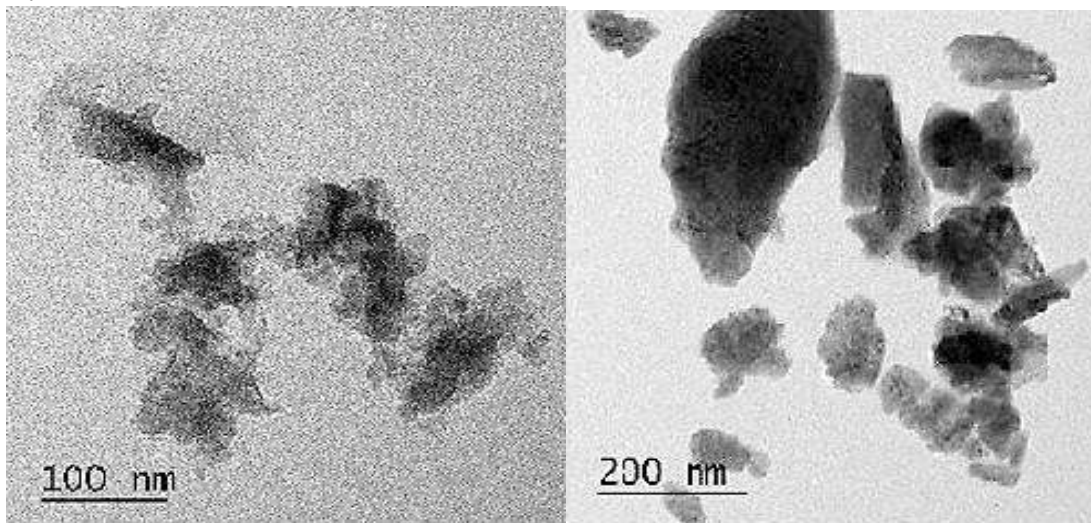


Fig 6: SEM images of (A) Atorvastatin calcium (B) optimized nanocomposites.

3.4.5. Transmission electron microscopy (TEM)

The TEM of pure drug and optimized nanocomposites AA_N 1:4 are shown in the following fig. 7. The TEM image of drug was observed first at 200 nm. In that image the drug is showing dark colour particles and pure drug image of TEM at 100 nm had shown the large free particles can be observed in somewhat cubic and rod shaped structure. After preparation of nanocomposites (AA_N) those were shown drug and polymer is mostly mixed looking flowery and most of the drug has transformed with the polymer. The polymer background which has more brightens and therefore drug is looking on the surface of polymer at 50 nm. When this preparation is observed at 20 nm the polymer is in background and the rod shaped structure of drug has converted into somewhat square shaped which shows that there is reduction of particle size of drug when it was treated with MIND method and all drug and polymer are finely embedded in each other.

A.



B.

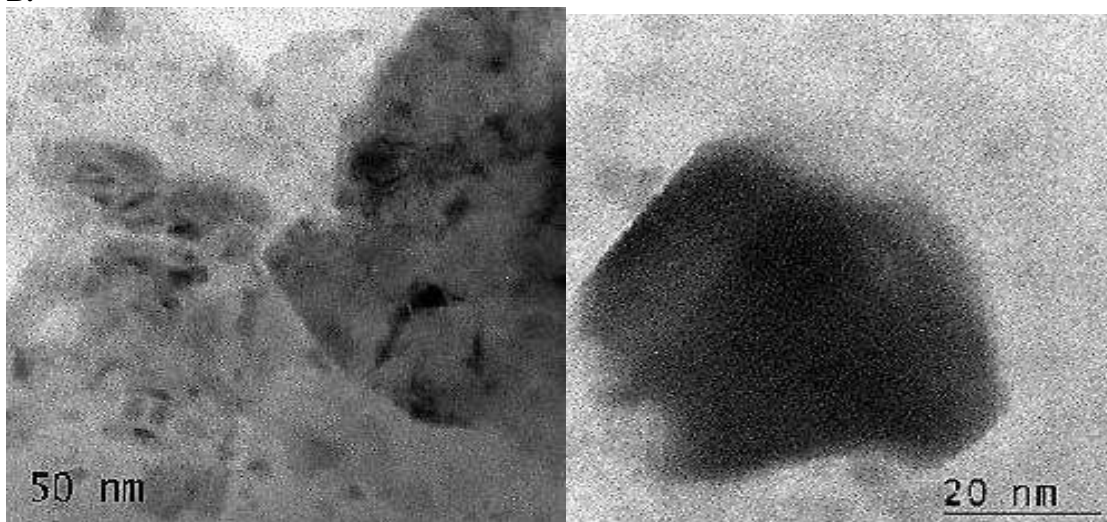


Fig.7: TEM images of (A) Atorvastatin calcium (B) optimized nanocomposites.

3.6. *In-vivo* pharmacological Study:

Triton is a hyperlipidemic agent that induces hyperlipidemia by inhibiting lipoproteinlipase enzymes peripherally which responsible for the elimination of lipid particles from the body[21]. The administration of Triton causes transient elevation of lipid levels. Antihyperlipidemic activity of Atorvastatin causes reduction in elevated total Cholesterol, LDL and TG levels in blood. On the other hand, it increases the plasma HDL level, which promotes the removal of cholesterol from peripheral cells and delivered it back to the liver[20]. The various formulations of atorvastatin was administrated to affect the serum lipid levels which altered by Triton. Better results were observed on treatment with optimized nanocomposites (AA_N 1:4). It successfully reduced

the levels of triglycerides and cholesterol by 45.46 % and 54.65 % respectively. However, treatment with pure drug show unsatisfactory results (fig.8). On the contrary, the levels of HDL rise from 21 mg/dL to 41 mg/dL by optimized formulation. Again, the change in HDL level by pure drug treatment was not significant as it increased the level from 21 to 30 mg/dL. From above results, it can be concluded that the effect of optimized formulation on the serum lipid level was statistically significant ($P < 0.05$) when compared with plain atorvastatin treatment. Atorvastatin reduces the levels of total cholesterol, low density lipoprotein and triglycerides and elevated the level of HDL in blood, according to previous results which reported as per Ankush Choudhary [22]. From various formulations, optimized nanocomposites were effectively reduced the levels of bad cholesterol and increase the level of HDL (Good cholesterol) in blood. The results shown by optimized nanocomposites were 2 times better than pure drug treatment. The higher lipid-lowering activity of the optimized nanocomposites formulation can be explained by the fact that the NCs formulation resulted in complete solubilization and dissolution of atorvastatin, which increased bioavailability of the drug by means of increasing in its absorption. Hence, the activity of atorvastatin was significantly increased by using optimized NCs indicating better performance of atorvastatin calcium in comparison to pure drug.

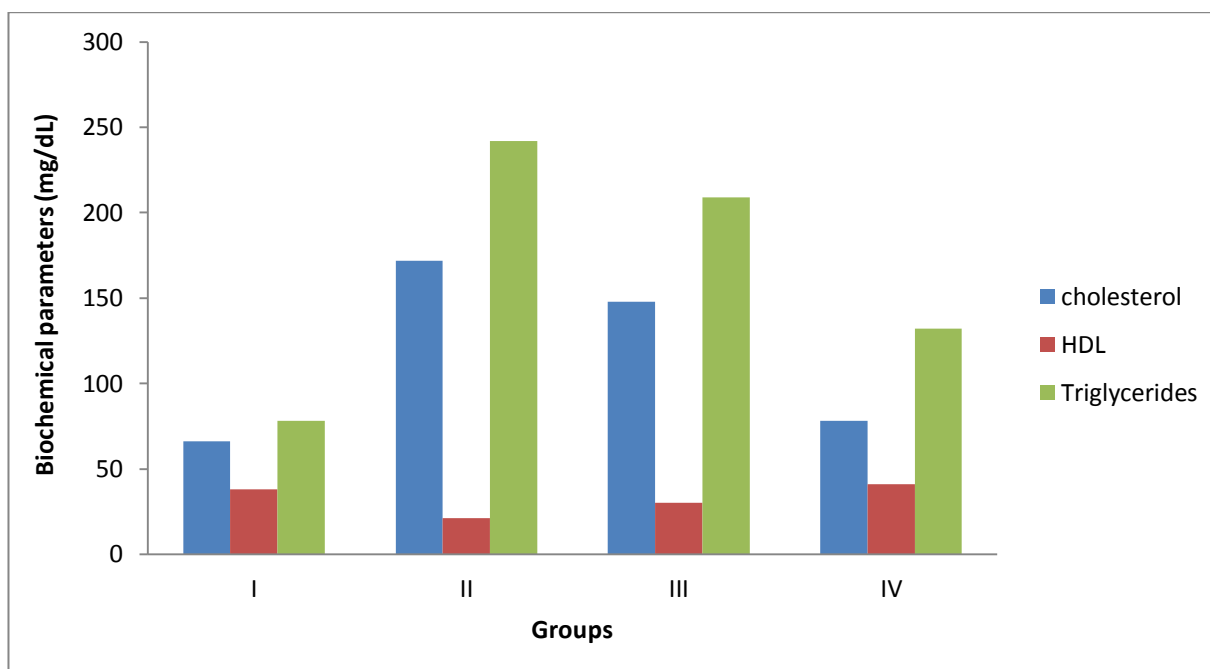


Fig. 8: Effect of Atorvastatin formulations on cholesterol, HDL, triglycerides in serum of control and experimental rats. Normal control (Group I), Triton induced (Group II), Plain atorvastatin treated (Group III), Optimized nanocomposites treated (Group IV).

4. Conclusion

In the present study we investigated the possibility of preparing an Nanocomposites of atorvastatin calcium by a MIND technique. This study successfully demonstrated the use of Acacia gum, Modified gum Karaya, PVP K-30 as carriers for the formation of microwave generated NCs in the solubility and dissolution enhancement of Atorvastatin calcium. *In-vitro* assessment of optimized formulations further confirmed the use of NCs for enhancing solubility and dissolution by use of natural carriers. FTIR, DSC, XRD, SEM and TEM characterization it can be concluded that Atorvastatin calcium had been converted to nanocrystals in the composites and this was responsible for the solubility enhancement. The pharmacological evaluation of the formulation revealed significant increase in its hypolipidemic effect as compared to pure drug. Therefore, the present methodology can be regarded as a novel and commercially feasible technique for improving the *in-vitro* and *in-vivo* performance of atorvastatin calcium.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgement

The authors are grateful to Mylan Lab Ltd (Nashik, Maharashtra, India) for providing gift samples of Atorvastatin calcium. They are also grateful to the MGV's Pharmacy college, Panchavati, Nashik for providing all the necessary facilities and infrastructure to carry out this study. The authors also acknowledge Diya lab and Indian institute of technology, Mumbai for providing the necessary facilities to carry out SEM, XRD and TEM.

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