Chapter 10

In Vivo Studies

Chapter 10

10.1. Acute Toxicity Study

In vivo acute toxicity studies on animals are an essential part of drug development process. Such acute toxicity studies are carried out for various objectives i.e..

1. To determine the Median Lethal Dose (LD_{50}) after a single dose administered through one or more routes, one of which is the intended route of administration in humans..
2. To determine Maximum Tolerated Dose (MTD) and No Observable Effect Level (NOEL)..
3. To identify potential target organs for toxicity, determine reversibility of toxicity, and identify parameters for clinical monitoring..
4. To help select doses for repeated-dose toxicity tests..

A number of methods are available to have an insight about the acute toxicity of any chemical or drug product. These include classical Litchfield and Wilcoxon method (Dosing of animals of both sex with increasing amounts of chemical and plotting dose-response curve to determine LD_{50}/MTD). This type of study has a disadvantage that it uses a large number of animals. So two methods are available now as alternatives which reduces the use of animals i.e. Fixed Dose Procedure (FDP) (1) and Up-Down Procedure (UDP) (2). Both methods produce data consistent with classical LD_{50} methods (3, 4). Among these methods Up-Down procedure requires the least number of animals (6-10) of single sex and provides results in terms of LD_{50} along with data for the hazard classification system, unlike FDP that does not estimate results in terms of LD_{50} value (5). Instead FDP gives better evaluation of the maximum tolerated dose of drug/drug product..

MTD of a drug can be defined as the highest dose of a drug or treatment that does not cause unacceptable side effects. The maximum tolerated dose is determined in clinical trials by testing increasing doses on different groups of people until the highest dose with acceptable side effects is found. Toxicity parameters to be considered include..

1. Mortality
2. Clinical pathology
3. Gross necropsy
4. Weight change
5. Signs of toxicity – convulsions, rashes, akinesia, licking, tremors

Drug doses at or below this level should not induce (6)

- Overt toxicity, for example appreciable death of cells or organ dysfunction,
- Toxic manifestations that are predicted materially to reduce the life span of the animals except as the result of neoplastic development or
- 10% or greater retardation of body weight gain as compared with control animals.

In some studies, toxicity that could interfere with a carcinogenic effect is specifically excluded from consideration..

For determination of MTD of APOE lipoplexes, fixed dose procedure of OECD-Organization for Economic Cooperation and Development was used. Typical protocol includes administration of a drug/drug product in escalating doses through intravenous route and observing animals for any signs of toxicity..

10.1.1 Method

All experiments and protocol described in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of Pharmacy Department, The M. S. University of Baroda and with permission from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India..

10.1.1.1 Selection of animals species

Male C57BL/6 mice were used for the study as females are generally slightly more sensitive to such studies (4). Healthy young adult animals (with 8-12 weeks age) which were nulliparous and non-pregnant were used for study.

10.1.1.2. Housing and feeding conditions

The temperature in the animal room was 20-25°C. Artificial lighting with the sequence of 12 hr light and 12 hr dark was kept in animal housing. The animals were housed individually. For feeding, conventional rodent laboratory diets was used with an unlimited supply of drinking water..

10.1.1.3. Preparation of animals

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing for acclimatization to the laboratory conditions..

10.1.1.4. Preparation of doses

Test substances (lipoplexes) were administered in a constant dose volume of 4 mL/kg by varying the concentration of the dosing preparation. (The dosing volume was chosen such that the volume did not exceed 0.4 mL/100g bodyweight). All doses were prepared prior to administration. Above certain dose, only liposomal carrier was tested to ascertain the safety profile of developed liposomal carrier systems.

Lipoplexes were lyophilized and lyophilized lipoplexes were reconstituted with sufficient quantities of normal saline to produce pDNA concentrations desired for administration. All the test substances were sterilized by filtering through 0.2 µ membrane filter prior to administration.

10.1.1.5. Procedures

a) Administration of doses

Prior to dosing, all the animals were fasted by withholding food but not water for 3-4 hr. The fasted body weight of each animal was determined and the dose was calculated according to the body weight.

The test substances were administered via tail vein of animals using sterile single use disposable polystyrene syringes (BD syringes). In the circumstance that a single dose was not possible, the dose was given in smaller fractions over a period not exceeding 24 hr at 1hr time gap between two doses.

b) Main test

The test substance was administered in a single dose by intravenous injection using a polystyrene single-use disposable injection. In the circumstance that a single dose was not possible, the dose was given in smaller fractions over a period not exceeding 24 hr.

Animals should be fasted prior to dosing (e.g. with the mice, food but not water was withheld for 3-4 hr). Following the period of fasting, the animals weighed and the test substance was administered. After the substance was administered, animals were provided with food and water *ad libitum*.

c] Sighting Study

The purpose of the sighting study was to allow selection of the appropriate starting dose for the main study. The test substance was administered to single animals in a sequential manner starting from DOSEfirst to DOSElast. The sighting study was completed when a decision on the starting dose for the main study was made (or if a death is seen at the lowest fixed dose)..

.The starting dose for the sighting study was selected from the fixed dose levels as described in **Table 10.1.** Starting dose selection was obtained from the available literature showing toxicological data for specific chemicals..

d] Main study - MTD Determination

Single animals were dosed in sequence usually at 48 hr interval. The first animal was dosed at a level selected from the sighting study (

Table 10.3). A period of at least 24 hr was allowed between the dosing of each animal. All animals were observed for at least 14 days for any signs of toxicity. If the animal survived, the second animal received a higher dose. If the first animal died or appeared moribund (Moribund status: being in a state of dying or inability to survive, even if treated), the second animal was administered a lower dose. Animals were to be euthanized by intraperitoneal injection of pentobarbital (50 mg/ml) after study or if moribund status (inability to ambulate, inflammation, anorexia, dehydration, or more than 20% weight loss) was observed. The weight of each animal was recorded immediately before intravenous injection, 1 day after injection, and at the end of study..

e] Numbers of Animals and Dose Levels

1. The action to be taken following testing at the starting dose level is indicated based on the observations. One of three actions will be required; either stop testing and assign the appropriate hazard classification class, test at a higher fixed dose or test at a lower fixed dose. However, to protect animals, a dose level that caused death in the sighting study was not revisited in the main study..

2. A total of five animals of male sex were used for each dose level investigated. The five animals were made up of one animal from the sighting study dosed at the selected dose level together with an additional four animals.

3. The time interval between dosing at each level was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose was delayed until there was confidence of survival of the previously dosed animals. A period of 3 or 4 days between dosing at each dose level is recommended, if needed, to allow for the observation of delayed toxicity. The time interval may be adjusted as appropriate, e.g., in case of inconclusive response.

f) Observations

Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 hr, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they needed to be removed from the study and humanely killed for animal welfare reasons or were found dead.

Observations included were changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress were humanely killed.

Loss of weight, if more than 20% of initial, or death of animal was considered a positive response at short term outcome (during first 24 hr). For long term outcome death was used as a termination point to stop the test. The duration of observation was determined by the toxic reactions, time of onset and length of recovery period. The times at which signs of toxicity appear and disappear were considered important, especially if there was a tendency for toxic signs to be delayed (7). All observations were systematically recorded, with individual records being maintained for each animal.

10.1.2 Results and discussion

APOE lipoplexes were administered intravenously to the male C57BL/6 mice as per the dosing protocol given in **Table 10.1** for sighting study. During sighting study,

formulations were administered to a single mouse for each dose level and observed for signs of toxicity for 24 hr.

Table 10.1 Sighting Study: Dosing protocol

Group	Dose of lipoplexes equivalent to		
Normal Saline	-		
HDSPE lipoplexes	50 µg/kg of pDNA	100 µg/kg of pDNA	500 µg/kg of pDNA
CDSPE lipoplexes			
G-HDSPE lipoplexes			
G-CDSPE lipoplexes			

All animals were found healthy and no signs of any toxicity were observed. Results for sighting studies are summarized in **Table 10.2**.

Table 10.2 Results of Sighting Study

Group/Formulation	Dose	Observation	
		Toxicological Signs/symptoms#	Mortality
Normal saline	-	None	None
HDSPE lipoplexes	50 µg/kg of pDNA	None	None
	100 µg/kg of pDNA	None	None
	500 µg/kg of pDNA	None	None
CDSPE lipoplexes	50 µg/kg of pDNA	None	None
	100 µg/kg of pDNA	None	None
	500 µg/kg of pDNA	None	None
G-HDSPE lipoplexes	50 µg/kg of pDNA	None	None
	100 µg/kg of pDNA	None	None
	500 µg/kg of pDNA	None	None
G-CDSPE lipoplexes	50 µg/kg of pDNA	None	None
	100 µg/kg of pDNA	None	None
	500 µg/kg of pDNA	None	None

#Observations included were changes in skin and fur, eyes and mucous membranes, respiratory distress, symptoms related to autonomic and central nervous systems such as tremors, convulsions etc., lethargy, and coma.

After performing the sighting study, 100 µg/kg of pDNA and 500 µg/kg of pDNA were considered for the main study. Main test was performed using the dosing protocol show in

Table 10.3. Four animals were administered with the selected doses of pDNA making up total of 5 animals per group including one animal from sighting study.

Table 10.3 MTD Study: Dosing Protocol

Group	Dose of lipoplexes equivalent to	
Normal Saline	-	-
HDSPE lipoplexes	100 µg/kg of pDNA	500 µg/kg of pDNA
CDSPE lipoplexes	100 µg/kg of pDNA	500 µg/kg of pDNA
G-HDSPE lipoplexes	100 µg/kg of pDNA	500 µg/kg of pDNA
G-CDSPE lipoplexes	100 µg/kg of pDNA	500 µg/kg of pDNA

Results for the MTD study are summarized in **Table 10.4**. All groups showed no signs of toxicity after administration of test substance. In all groups, formulation was considered safe at the maximum dose of 500 µg/kg of pDNA administered as the lipoplexes..

As study was not performed on the higher concentrations of pDNA, actual MTD was not reached in the study. The MTD might be considered higher than the evaluated dose. However, the total dose evaluated is considerably higher that would provide the *in vivo* therapeutic efficacy..

Table 10.4 Results for Main test

Group	Observations		
	Change in weight of animals	Toxicological Signs/symptoms#	Mortality
Normal Saline	-	None	None
HDSPE lipoplexes	**	None	None
CDSPE lipoplexes	**	None	None
G-HDSPE lipoplexes	**	None	None
G-CDSPE lipoplexes	**	None	None

#Observations included were changes in skin and fur, eyes and mucous membranes, respiratory distress, symptoms related to autonomic and central nervous systems such as tremors, convulsions etc., lethargy, and coma.
*.** indicates statistically insignificant change in weight after 14 days with $p<0.005$.*

Considering the fact that the formulations is targeted to the cells inhabiting the liver cells, the locally expressed quantity of the APOE protein will be sufficiently high to provide therapeutic activity high enough to induce formation of ApoE protein. This provide higher plasma levels for longer period of time reducing the frequencies of administration and at doses lower enough than used in this study. Based on these acute toxicity studies, the developed formulations and synthesized lipids can be considered safe for *in vivo* administrations..

10.2 *In vivo* performance study

In vitro study of the therapeutic activity of a gene delivery system demonstrates the ability of the system to act on the target cells. However, the delivery system when administered in vivo, is going to face several hurdles before it reaches the therapeutic site. *In vitro* simulation studies such as serum stability studies, effect of electrolytes, etc. may give preliminary idea on the potential of the delivery system. Thus, performing in vivo studies in animals more close idea to the actual performance of the gene delivery system can be achieved.

Selection of a model and choice of animal species depend on several factors such as disease condition simulation; differences between animal and human physiology; animal size, gender and age; ease and time for osteoporosis induction etc.

In the present investigation, male C57BL/6 mice model has been used for investigation of therapeutic potential of the APOE gene delivery system. Atherosclerosis induced mice were given the intravenous dose of APOE lipoplexes and aorta was isolated for estimation of remission/decrease in lesion area along with monitoring the plasma lipid profile.

10.2.1 Diet induced atherogenesis

Materials

Table 10.5 Atherogenic diet composition

Reagent/Chemical/Ingredient	*Source*
Pelleted chow for rats	Amrut Feed, Pranav Agro, India
Cholesterol	Spectrochem, India
Coconut oil	HiMedia, Mumbai, India
Cholic acid	HiMedia, Mumbai, India
2-Thiouracil	HiMedia, Mumbai, India
Casein	SD Fine Chemicals, India
Sudan Red IV	HiMedia, Mumbai, India
Hydroxypropylcellulose (HPC)	Gift sample from Wockhardt Ltd., Aurangabad India

Test compound (MCR-788)	Synthesized in Pharmaceutical Chemistry Laboratory of Pharmacy Dept., The M. S. University of Baroda, Gujarat, India
Kits for Total cholesterol, triglyceride and HDL-C estimation	Coral Clinical Systems, Mumbai, India
Solvents (diethyl ether, methanol, isopropyl alcohol)	SD Fine Chemicals, India

Atherogenic Diet Composition

The composition of the diet was as follows: Cholesterol (2%), Coconut oil (1%), Cholic acid (1%), Propylthiouracil (0.5%), Casein (16.5%) and powdered chow and hydroxypropyl cellulose (HPC) binder (q.s. to 100%). After accurately weighing all the ingredients casein, cholic acid and propylthiouracil were ground in a mortar pestle until a homogenous solid mixture was formed. This dry mixture was added to a large vessel and mixed with required amount of powdered chow. Next, cholesterol was dissolved in appropriate volume of diethyl ether and this ethereal solution was properly mixed with the powder mass. The ether was allowed to evaporate for 2-3 hours. Once the ether evaporated, required amount of coconut oil was mixed properly. For every kilogram of diet to be prepared 800 ml of 1% HPC solution was prepared by stirring for about half an hour. This solution was added at once to the dry mass and vigorously kneaded to evenly mix all the HPC. The consistency achieved was such that the lumps formed neither crumbled nor were too sticky. The lumps were hand cured to form suitably shaped lumps which looked like pelleted chow. The final pellets were dried overnight in a hot-air oven at 45-50°C. This step improves long term storage and prevents growth. The pellets were stored in a perforated bag at 2-8°C. The formed pellets were not stored for more than 7 days.

10.2.1.1 Method

Mice fed high fat diet with induced atherosclerosis were used as an animal model for evaluating the therapeutic activity of the developed gene delivery system. Study protocols were approved by IAEC (Institutional Animal Ethical Committee, Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, India). C57BL/6

mice of 5-6 weeks age were selected. Animal care was observed according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India). The animals were divided in several groups (Table 10.5). The control group received normal chow diet, while other groups received HFD. The plasma lipid profile was estimated at initiation of diet. After 3 months of feeding the lipid profile was again measured and 2 animals from each group receiving HFD were sacrificed for determination of aortic lesions. Following detection of lesions, one group was given oral solution of atorvastatin (20mg/kg) each day. Whereas, the other groups were given IV dose of APOE lipoplexes (equivalent to 50 μg pDNA per mice per week). At the end of 1-month and 3 months of treatment, lipid profile was measured and 4 animals were sacrificed each time from each of treatment group and aorta were sectioned.

Table 10.6 Animal groups for atherogenesis induction and treatment

Group	Condition	Treatment	No of animal per group
Control group	None	None	8
Positive control	HFD induced atherogenesis	None	10
Treatment I	HFD induced atherogenesis	Oral solution of Atorvastatin	10
Treatment II	HFD induced atherogenesis	gHDSPE lipoplexes	10

Collection of Serum:

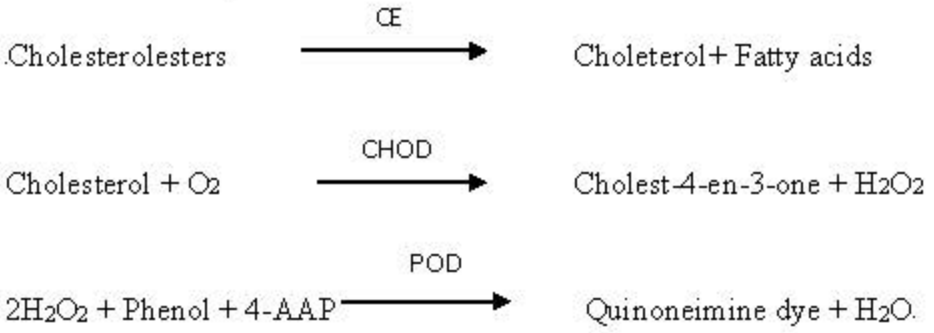
The blood samples were withdrawn from the retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature and centrifuged at 4000 rpm for 20 minutes. The serum obtained was kept at 4^0 C until use.

10.2.2 Estimation of total cholesterol: [i]
(CHOP-PAP Method)

Total cholesterol in serum was estimated using kit obtained from Reckon Diagnostics Ltd.

Principle: [ii]

.Cholesterol esters are hydrolysed by Cholesterol Esterase (CE) to give free Cholesterol and Fatty acids. In sunsequent reaction, Cholesterol Oxidase (CHOD) oxidizes the 3-OH group of free Cholesterol to liberate Cholest-4-en-3-one and Hydrogen Peroxide. In presence of Peroxidase (POD), Hydrogen Peroxide couples with 4 Aminoantipyrine (4-AAP) and Phenol to produce Red Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of Total Cholesterol concentration in the sample..



Procedure:

.Three test tubes were labeled as Blank, Standard and Test, and as per the following table different reagents were used and further process was carried out..

Solution	Blank	Standard	Test
Unknown	-	-	10µL
Cholesterol standard	-	10µL	
Cholesterol Reagent	1000µL	1000µL	1000µL

.Solutions were mixed well and incubated at 37° C for 10 minutes. Absorbance of standard and test were measured against blank at 505 nm and results were calculated as per following formula.

Calculation:

Cholesterol concentration (mg/dL) = Absorbance of Test / Absorbance of Std × 200

10.2.3 Estimation of HDL cholesterol:[22]
(PEG-CHOD-PAP Method)

HDL cholesterol in serum was estimated using kit obtained from Span Diagnostics Ltd.

Principle:

Low Density Lipoprotein (LDL) Cholesterol, Very Low-Density Lipoproteins (VLDL) Cholesterol and Chylomicron fractions were precipitated by addition of Polyethylene Glycol 6000 (PEG). After centrifugation, the High-Density Lipoprotein (HDL) Cholesterol fraction remains in the supernatant and is determined with CHOP-PAP method as described in Total Cholesterol estimation[6,7,8,9]..

Procedure:

Other cholesterol were precipitated using precipitating reagent.

Step: 1 HDL-Cholesterol separation

Serum sample which was treated as per following procedure.

Solution	Test
Serum sample	200 µL
Precipitating Reagent	200 µL

Mixed well and kept at Room Temperature (15-30° C) for 10 minutes. Centrifuge for 15 minutes at 2000 rpm clear supernatant was separated. The supernatant was used for HDL-Cholesterol estimation.

Step: 2 HDL-Cholesterol estimation.

Three test tubes were labeled as Blank, Standard and Test, and as per the following table different reagents were used and further process was carried out.

Solutions	Blank	Standard	Test
Supernatant from step 1	-	-	100 µL
HDL-C Standard	-	100 µL	-
Cholesterol Reagent	1000 µL	1000 µL	1000 µL

Solutions were mixed well and incubated at 37° C for 10 minutes. Absorbance of standard and test were measured against blank at 505 nm and results were calculated as per following formula.

Calculation:

HDL-Cholesterol concentration (mg/dL) = Abs of Test / Abs of Std × 50

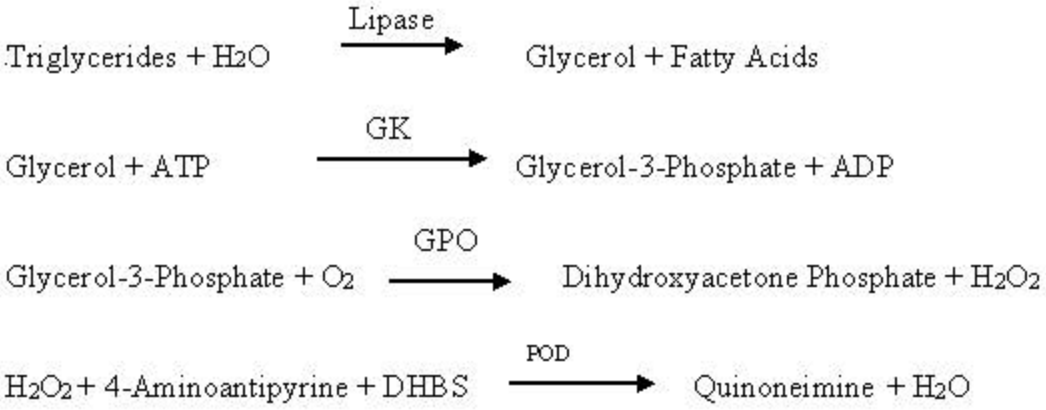
10.2.4 Estimation of Triglycerides:[iv]

(GPO Method)

Triglycerides in serum was estimated using ENZOPAK Kit.

Principle:

Lipase hydrolyses triglycerides sequentially to Di & Monoglycerides and finally to glycerol Glycerol Kinase (GK) using ATP as PO_4 source converts Glycerol liberated to Glycerol-3-Phosphate (G-3-Phosphate). G-3-Phosphate Oxidase (GPO) oxidizes G-3-Phosphate formed to Dihydroxy acetone phosphate and hydrogen peroxide. Peroxidase (POD) uses the hydrogen peroxide formed to oxidize 4-Aminoantipyrine and chlorophenol to a purple coloured complex. The absorbance of the coloured complex is measured at 520nm[v]..



Procedure:

Three test tubes were labeled as Blank, Standard and Test, and as per the following table different reagents were used and further process was carried out.

Solutions	Blank	Standard	Test
Working Reagent (ml)	1.0	1.0	1.0
Standard (ml)	-	0.05	-
Sample (ml)	-	-	0.05

Mixed well and incubated at 37°C for 20 minutes.			
Distilled water (ml)	1.5	1.5	1.5

Solutions were mixed well and incubated at 37° C for 10 minutes. Absorbance of standard and test were measured against blank at 520 nm and results were calculated as per following formula

Calculation:

Triglyceride concentration (mg/dL) = Absorbance of Test / Absorbance of Std. × 200

10.2.5 Estimation of LDL Cholesterol:

Estimation of LDL cholesterol depends on the amount of total cholesterol, triglycerides and HDL cholesterol.

Estimation of LDL Cholesterol was done using Friedewald formula.

LDL Cholesterol = Total cholesterol – (Triglycerides/5 + HDL cholesterol)

10.2.6 Histopathology of atherosclerotic lesions:

Mice were euthanized humanely. The heart and the arterial tree were perfused with saline solution under physiological pressure. The aortas were isolated and paraffin embedded. Cross sequential sections 5 mm thick were prepared and stained with hematoxylin–eosin. Images of the aortas were captured with a Nikon digital camera and the atherosclerotic lesions were quantified using the image analysis software OPTIMAS 6.2..

Statistical analysis:

Statistical analyses of the results was carried out using Prism 6.0 software using ANOVA with Tukey's multiple comparison test considering the $p < 0.05$ as a significant difference for other estimations. All graphical presentations show mean values ± standard deviation (SD). Morphometric evaluations show mean values ± standard error (SE).

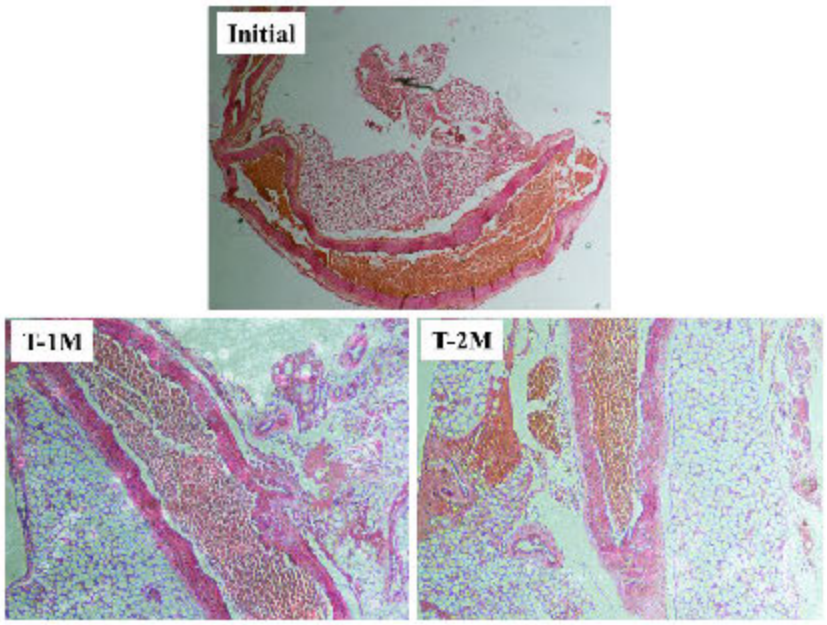
10.2.7 Results and discussion:

Healthy male mice were selected for the experiment. The effect of high fat diet on the physiological changes was evident from the increase in weight of the mice along with lipid profile. At the end of one month the aorta isolated from the mice showed minimal amount of lesions and hence the feeding was continued for two more months. The feeding of diet led to atheromatous lesion in the aorta at the end of three months. After the induction of atherosclerosis, treatment was initiated that led to reduction in total lipid amount in all the three treatment groups. The data are presented in as seen in case of lipoplexes of HDSPE, the lipid profile was more favorable than compared to the oral solution of statin. However, the highest reduction in the total cholesterol and LDL level was observed in case of targeted lipoplexes.

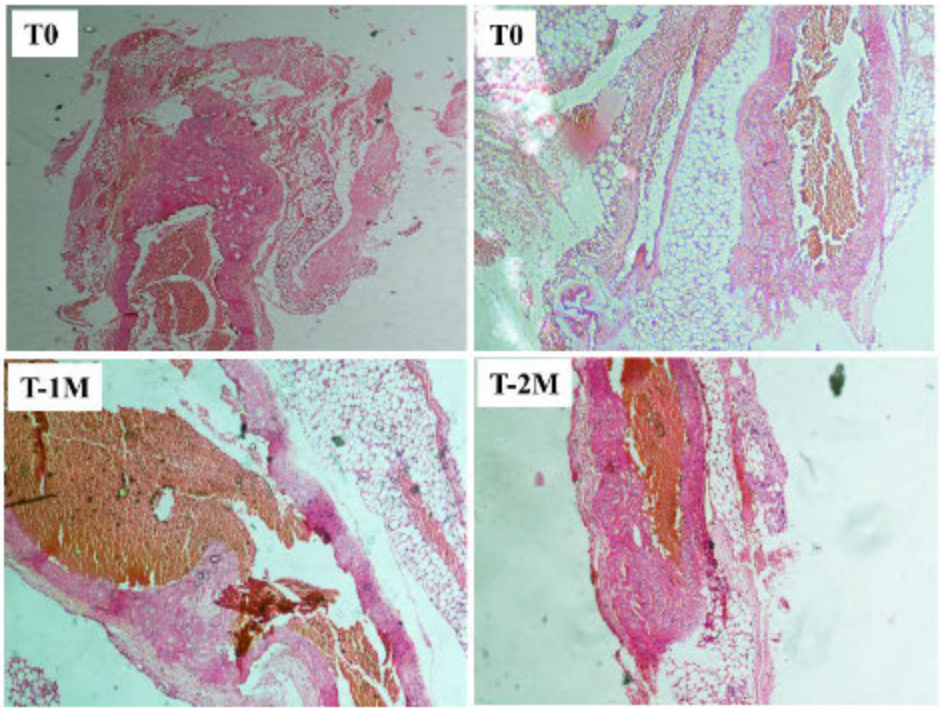
Table 10.7 Lipid profile in mice prior to treatment

Parameter	Control group	HFD (for initiation of lesions)	
	Initial weight	1 M	3 M
Body weight (grams)	21.56±1.4	26.95±1.9	32.63±2.2
Total cholesterol (mg/dl)	320±21	439±30	659±38
HDL (mg/dl)	15.6±1.1	12.3±0.9	11.2±1.5
Triglycerides (mg/dl)	95±6	98±4	105±5
LDL (mg/dl)	285.4±15	407.1±38.2	627.2±42

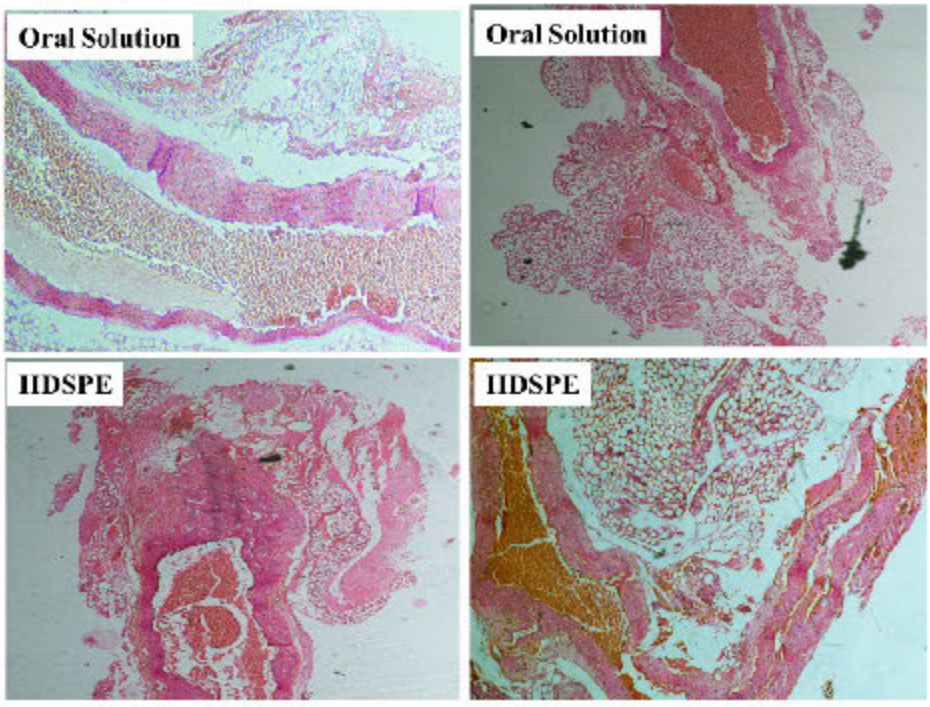
Control – Normal chow diet



Positive Control – No treatment



Treatment -1 Month



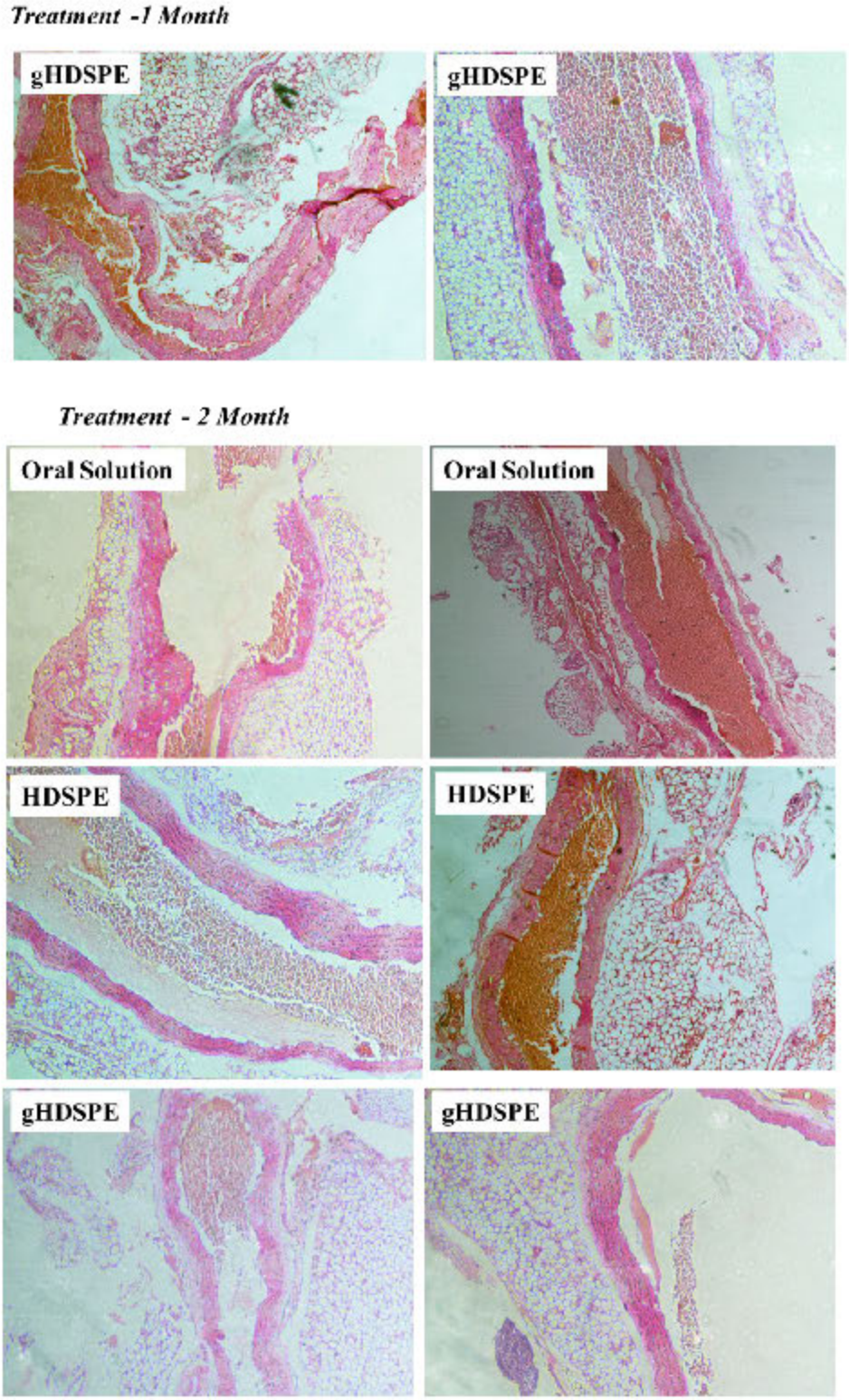


Figure 10.1 Histopathologic evaluation of aorta isolated from mice at different time points for various treatment groups (T0- initial time point; T-1M: at one month; T-2M: at 2 month)

Figures indicate the lesion in the transverse section of aorta for the various treatment group. The Figure represents the aortic lesion area for the targeted and non-targeted lipoplexes. As evident at the end of treatment period of 2 months, the greatest efficacy was shown by the targeted formulation, which may be due to higher accumulation of ApoE inside the liver parenchymal cells leading to higher production of ApoE protein that could have increased the metabolism of Triglyceride and lipoprotein remnants from the plasma.

Table 10.8 Lipid profile in mice during treatment

Parameter	Control group	HFD	During treatment period in mice having atherosclerotic lesions					
	At end of 2 M	3M	Oral Atorvastatin		HDSPE I.V.		gHDSPE I.V.	
			1M	2M	1M	2M	1 M	2M
Body weight (grams)	24.68±1.9	32.63±2.2	33.59±2.1	31.63±1.2	34.26±1.8	35.1±2.5	35.4±2.3	34.6±0.9
Total cholesterol (mg/dl)	331±19	659±38	464±33	381±25	406±36	304±26	386±31	294±19
HDL (mg/dl)	15.5±1.8	11.2±1.5	12.3±1.0	13.2±1.5	13.6±1.1	15.9±1.4	14.1±1.0	16.1±0.6
Triglycerides (mg/dl)	96±9	105±5	102±4	98±4	100±0.6	94±1.5	99±1.1	93±0.8
LDL (mg/dl)	296.3±12	627.2±42	431.7±26.1	348.2±20.9	372.4±18.9	269.3±18.4	352.1±17.6	259.4±10.7

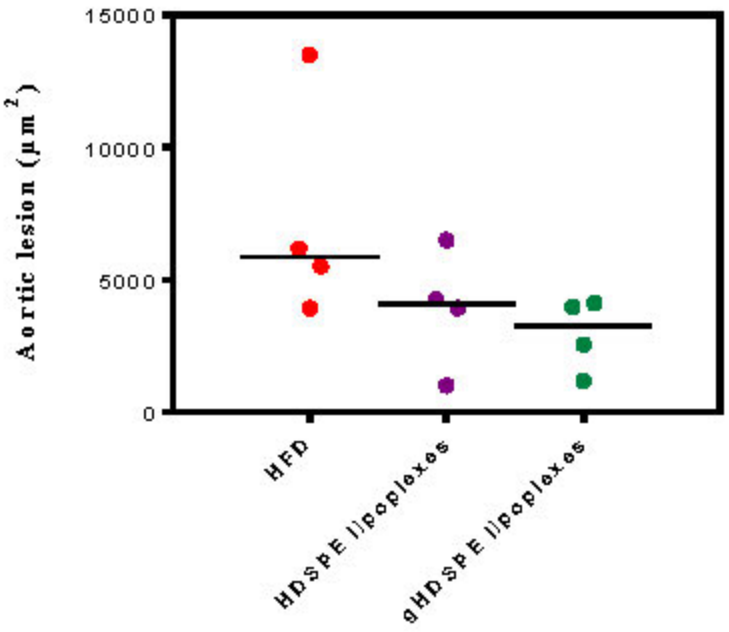


Figure 10.2 The areas of atherosclerotic lesions in untreated (HFD) and treated (HDSPE lipoplexes and gHDSPE lipoplexes) animals (n = 4 in each group) are shown in the graph (overlapping dots are not shown) at end of 2 months. The line indicates the mean value.

Thus, the targeted system performed effectively and have shown efficacy in the animal model for effective remission of the disease based on the treatment duration of 2 months.

10.3 References

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