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EFFECT OF COLESEVELAM HYDROCHLORIDE ALONE AND ITS COMBINATION WITH ATORVASTATIN ON EXPERIMENTALLY INDUCED HYPERLIPIDEMIA IN WISTAR RATS

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ABSTRACT

Atherosclerosis is a major cardio effective disorder that affects heart causing hyperlipidemia which blocks the artery, aorta and obstruct the pathway of blood flow which puts life at risk. The modern medication needs to be administered continuously and in higher doses which leads to negative drawbacks. The study aim is to give the Combination therapy treatment as per the drug mechanism of action is known and how bioavailability is also known. Five group of albino wistar rats were being experimented in this case, group I to V i.e. NC, DC, COL, ATS (40mg/kg) and ATS (10mg/kg) + COL is to give the treatment as chrono pharmacology method in combination and in low dose range of atorvastatin 10-20 mg/kg less side effect is observed. Conducting the study is seen that lower dose in combination (bile acid sequestrants and HMG-CO-A inhibitor) was proven that CK-MB levels are decreased in comparison of high dose of Atrorvastatin by Detection of Biochemical Evaluation Parameters like CK-MB, Lipid profile, CBC etc. also Detect the Histopathological parameters by differentiate the slide review of Heart and Aorta of every group according to plan of work and protocol for evaluation of formulation. As further it will come in future work can be done into cell line on molecular base to determine clinical significance.

Keywords: Hyperlipidemia, HFD, Atorvastatin, Colesevelam, Atherosclerosis Parameters

INTRODUCTION

Atherosclerosis is a condition in which the arteries stiffen and narrow. It can obstruct blood flow, putting your health at danger. The word to say atherosclerotic cardiovascular diseases. The plaques or fatty materials are building up in arteries. The composition of plaques or made up of fibrin (a clotting material in the blood), calcium, cellular waste products & fatty substances. It is cardio-pathological condition called arteriosclerotic vascular diseases. In the atherosclerosis, reducing blood flow by this narrow the channel within artery & plaques was build up, the wall of blood vessels thickens. The condition in which an artery wall thickens because to a build-up of fatty substances, such as cholesterol. Because of the build-up of macrophages white blood cells, which is aided by low-density lipoprotein, and because lipids and cholesterol are not adequately removed from macrophages by functioning high-density lipoprotein **[1]**. Every year due to coronary artery diseases 6, 10,000 people passed away in United states of America, it is the major source of killing over 3,70,000 peoples per annum, around 7,35000 people suffers from cardiac arrest per year. Due to plaque rupture 75% of population suffers acute myocardial infarction; it is mainly observe man over 45 years, whereas in woman chances of myocardial infarction is increase after the age of 45year **[2]**. Their effects on low-density lipoprotein (LDL) particles and inflammation may induce atherosclerosis. The causes of atherosclerosis are family history, smoking, hypertension, physical activity, obesity, alcohol & hypertension. The main symptoms of atherosclerosis were seen to diagnosed are Tachycardia, Bradycardia, weakness, sweating, Shortness of breath fainting, chest pain, paralysis & facial numbness **[3]**. By the atherosclerosis there are many complications are also arising. Majorly included those Coronary artery diseases: Unstable angina, Myocardial ischemia, Myocardial infarction, Stroke: Embolic stroke, Thrombotic stroke, Renal artery diseases: Atheroembolic renal disease, Renal artery stenosis, Peripheral artery diseases: Limb claudication, Aneurysms **[4]**. The major risk factors are potentially controllable i.e. Hyperlipidemia, Hypertension, Smoking, Diabetic Mellitus Type 2. High fat diet is the best method to induced hyperlipidemia and many other animals' models are also used for induction in research of hyperlipidemia or atherosclerosis **[5]** . Statins have approval in hyperlipidemia in Mild, Moderate, Severe condition. They have given more potent as per as the clinical trial & have more side effects comes like Rhabdomyolysis, myopathy etc. It belongs

to the HMG-CoA Reductase Inhibitor class. Myopathy, a common adverse effect of statins, might develop as a result of long-term therapy and high doses of Atorvastatin **[6]**. Colesevelam Hydrochloride is antihyperlipidemic medication that has been authorised by the FDA. Colesevelam can be used alone or combination with an HMG-CoA reductase inhibitor likes ezetimibe and niacin to treat hyperlipidemia. Colesevelam is an FDA approved Bile acid sequestrant that can be used in conjunction with diet and exercise to treat a range of condition. In individuals with primary hyperlipidemia and other illness that produce hyperlipidemia. As per the US Pharmacists, Atorvastatin is used in Moderate and Severe condition. In severe condition there is High dose range of statin like 40-80mg/kg of Atorvastatin and more side effects were diagnosed like myopathy (CK-MB). In moderate condition, there low dose range of statin like 10-20 mg/kg of Atorvastatin and more side effect is also come but not more potent and more effect were shown in Hyperlipidemia. Colesevelam HCL is also used in Primary Hyperlipidemia Treatment dose range is 625 mg/kg. In earlier study of statins and Colesevelam HCl used in combination in hyperlipidemia patients and do also Pre – clinical Trial in Albino wistar rats in 2012 study. As per this study aim is to do given the time therapy management treatment as per the drug mechanism of action were shown and how bioavailability was also shown. Our target is to given the treatment as chrono pharmacology method in combination and in low dose range of atorvastatin 10-20 mg/kg less side effect obtained. It means dose range of Colesevelam hydrochloride 625mg/kg given and bioavailability of this drug is 2 hrs. And atorvastatin 10-20 mg/kg and more effect were shown in resting time and bioavailability is 16-18 hrs. A check in combination therapy considering Low dose of Atorvastatin and Colesevelam which will be similar in terms of effect of high dose of Atorvastatin and to minimize the side effect of myopathy in hyperlipidemia Rats. It was seen by Detection of Biochemical Evaluation Parameters like CK-MB, Lipid profile, CBC etc. Detect also the Histopathological parameters by differentiate the slide review of Heart and Aorta of every group according to plan of work and protocol for evaluation of formulation of Colesevelam HCL was divided into 5 groups **[7, 8]** .

The majority of this global sickness burden is due to atherosclerotic cardiovascular disease (ACD). Triglyceride-lowering drugs known as fibrates include ethyl-chlorophenyl-iso-butyrate, clofibrate, bezafibrate, and fenofibrate. They may help reduce LDL cholesterol, albeit the amount of reduction varies. LDL-lowering drugs

are known as statins, or HMG-CoA reductase inhibitors. Statins work by competitively inhibiting the percentage enzyme HMG-CoA reductase with in cholesterol production pathway, which prevents cholesterol from being synthesised in the liver **[9-10]**.

The objective of this study is To Develop HFD induced Hyperlipidemia. To Study Colesevelam Hydrochloride on Experimentally Induced Hyperlipidemia in Rats. To Investigate Atorvastatin on HFD induced Hyperlipidemia. Combination of Colesevelam hydrochloride & Atorvastatin on Experimentally Induced Hyperlipidemia in Rats. To study low & high dose of Atorvastatin alone & its combination with Colesevelam hydrochloride on creatine kinase in experimentally induced Hyperlipidemia **[11]**. Colesevelam is an FDA-approved bile acid sequestrant that can be used in conjunction with diet and exercise to treat a range of conditions. In individuals with primary hyperlipidaemia and other illnesses that produce hyperlipidaemia, the major indication is to decrease high low-density lipoprotein cholesterol (LDL-C). It can be taken alone or in a three- or four-drug combination with an HMG-CoA reductase inhibitor (statin), ezetimibe, or niacin. Another authorised application is to lower LDL-C levels in heterozygous familial hypercholesterolemia in males and postmenarchal girls aged 10 to 17 years. Only after a proper trial of diet and exercise has failed May Colesevelam be administered as a immunotherapy or in combination with a statin in this circumstance. Finally, improving glycaemic control in type 2 diabetic individuals is an FDA-approved indication **[12-16]**.

Colesevelam is a lipid-lowering polymer that binds to bile acids in the gut and prevents them from being absorbed. The recent past study Colesevelam is an FDA approved antihyperlipidemic drug. When treating hyperlipidemia, Colesevelam should be used in conjuction with restriction of cholesterol and fat intake and exercise and can be used as a monotherapy or combined with an HMG-CoA reductase inhibitor ezetimibe, or niacin. It is approved for the treatment of hyperlipidemia from heterozygous familial hypercholesterolemia in adults and as well as in adolescents (10 to 17 years of age) patients. Colesevelam is also indicatedto improve glycemic control in adults with type 2 diabetes mellitus along with diet and exercise **[17-20]**. The most potent and effective medicines for treating hyperlipidemia are statins. Through anti-inflammatory and other mechanisms, atorvastatin has been proven to be a very effective member of the statin family for reducing blood cholesterol, stabilizing plaque, and avoiding strokes. Atorvastatin reduces cholesterol via blocking 3-

hydroxy-3-methylglutaryl coenzyme. A (HMG CoA) reductase, which prevents the synthesis of the cholesterol molecule's primary building component. Severe side effects, such as liver damage and skeletal muscle abnormalities, such as myopathy and rhabdomyolysis, might occasionally restrict the use of these hypolipidemic agents for lipid-lowering treatment **[21-25]**. As a result, a safe and effective treatment for reducing cholesterol levels would be of great interest. A diet rich in plant foods has been found to decrease the development of some chronic illnesses such as atherosclerosis in several epidemiological studies seeking for alternative treatments. Garlic, gum guggul, and capsicum are among the plant foods that have been shown to have hypocholesterolaemia properties in clinical and experimental investigations **[26-30]**.

The aim of the study is to evaluate the effect of low and high dose of atorvastatin alone and its combination with Colesevelam HCL on lipid profile and CK-MB in experimentally induced hyperlipidaemia. In induction model, HFD individually inducers of hyperlipidemia in long duration of time more than 48 Days. The composition of High fat diet in induction of 48 days is Folic acid (1%), Cholesterol (2%), Dalda ghee (30%) and Rat chow (68%).

HIGH FAT DIET INDUCED HYPERLIPIDEMIA [31]

Procedure - This model closely resembles hyperlipidaemia in humans. This approach involves mixing a large amount of cholesterol with vegetable oil and administering it to all groups except the control group. Following the chronic treatment with high fat, the second group is given a conventional medicine, the third group is given a test sample, and the fourth group is given merely a regular diet as a control group. Collect the blood sample by an appropriate technique under minor anaesthetic at the conclusion of the 30th day. Animals are sacrificed, and organs such as the heart, liver, aorta, pancreas, spleen, and kidney are isolated, weighed, and submitted to histopathological tests **[32-33]**.

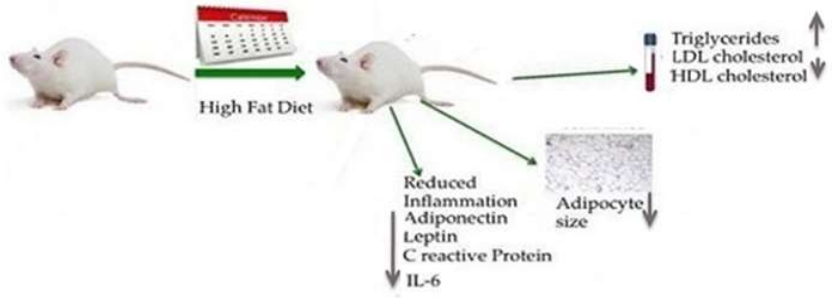

Figure 1: Mechanism of action of HFD in hyperlipidemia Model

MATERIAL and METHOD

Drugs

Colesevelam Hydrochloride (Ally amine Hydrochloride) was procured from Genzyme Europe, The Netherlands.

Atorvastatin (Dimethyl Fumarate) was procured from Zydus PVT. LTD., Gujarat.

Animals

Healthy Albino wistar rats (Female) weighing 250-300g were used for the study. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of Pharmacology department, Parul institute of pharmacy and research and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) **[33-34]**.

Protocol No. 984/PO/Re/S/06/CPCSEA

Permitted Animals: 30

Animals were procured from Zydus Cadilla Research Center, Ahmedabad

Housing

Albino wistar rats were allowed for acclimatization for seven days on pelleted standard rat food with water and housed in a group of 3 rats per cage under well-controlled standard conditions of temperature (22±2ºC), humidity (55±5%) and 12hrs light conditions and 12hrs dark condition cycle in animal house. Animal were given High fat diet and fed regularly with water through polypropylene water bottles with SS spout ad libitum **[34-36]**.

Induction of Atherosclerosis in rats by High Fat Diet

Protocol for evaluation of formulation for treatment of Colesevelam Hydrochloride was divided into 5 groups of six animals each.

Normal Control Group (NC)

Group-1: Received vehicle for 28 days (p.o.)

Diseases Control Group (DC)

Group-2: Each animal were given of High fat Diet per day for 48 days

Test Control Group

Group-3 (COL): Diseases control + Colesevelam HCl (100 mg/kg,p.o/day) For 28 days

Group-4 (ATS 40mg/kg): Diseases control + Atorvastatin (6.5 mg/kg,p.o./day) For 28 days

Group-5 (ATS 10mg/kg+ COL): Diseases control + Colesevelam HCl (100 mg/kg,p.o./day) for 28 days followed by Atorvastatin (1.6 mg/kg,p.o/day) for 28 days

Principle: The composition of HFD induced hyperlipidemia in 48 days is rat chow (68%), Folic acid (1%), Cholesterol (2%), Dalda ghee (30%). Parameters which have determined by blood collection method with different routes such as Retro orbital **[37-39]**.

Blood Collection

Blood was collected via retro-orbital route. The amount of blood collected was up to 1.5 ml. The blood was collected in EDTA vacuum tubes and Eppendorf was sent to Parul Sevashram Hospital's Central Laboratory, Vadodara for pathological evaluation.

The blood was collected before and after induction of High Fat Diet. After induction blood was collected at an interval of 7 days for the period of treatment. Final blood collection was done after 28 days of treatment **[40-41]**.

Histopathological Evaluation

Animals were sacrificed to obtain heart. The organs were washed with pH 7 buffer solution and then were kept in 10% formalin solution (buffered) for 24 hours. After 24 hours the organs were sent for histopathological evaluation to Vadodara clinical Laboratories (VCL), Vadodara.

Biochemical parameters Evaluation

Lipid profile, CK-MB, CBC, LFT are pathologically diagnosed by the therapeutic process **[41-49]**.

RESULT

INDUCER WEIGHT CHART (0 to 48 days) (Figure 2)

Here,

- **12 day, 2- 24day, 3-36 day, 4- 48 day**

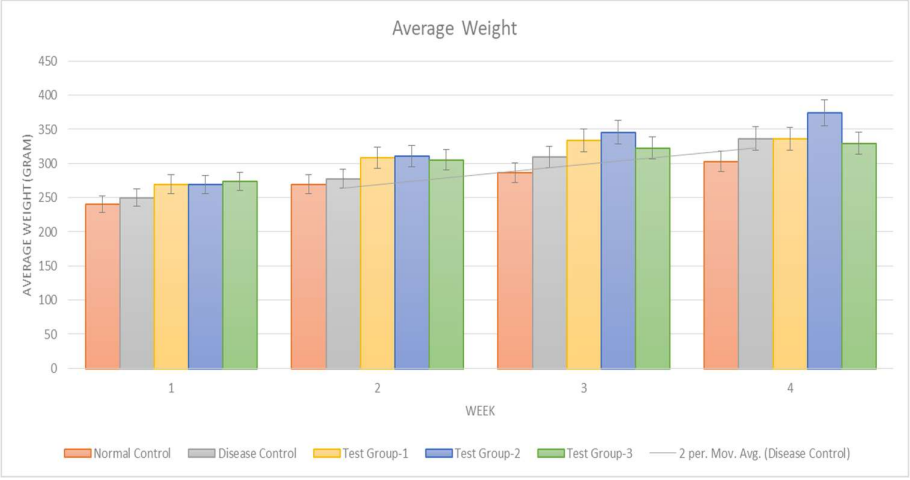

Figure 2: Inducer weight Chart

NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

Biochemical parameter

1. **Lipid Profile (HDL, LDL, VLDL, Total Cholesterol, Triglyceride) (Table 1)**

LDL

Hyperlipidaemia LDL Level at 28 day

✓ day, 2- 14 day, 3- 28 day **(Figure 3)**

Table 1: Hyperlipidaemia LDL Level at 28 day

Group	Treatment	LDL (Mean± SEM)
I	NC	19.18±1.91
II	DC	119.50±4.64
III	COL	45.25±3.27
IV	ATS (40mg/kg)	27.11±3.17
V	ATS (10mg/kg) + COL	24.68±3.06***

All Values are expressed as Mean ± SEM (n=6), where p<0.003 when compared to treated group ANOVA followed by Dunnett's test

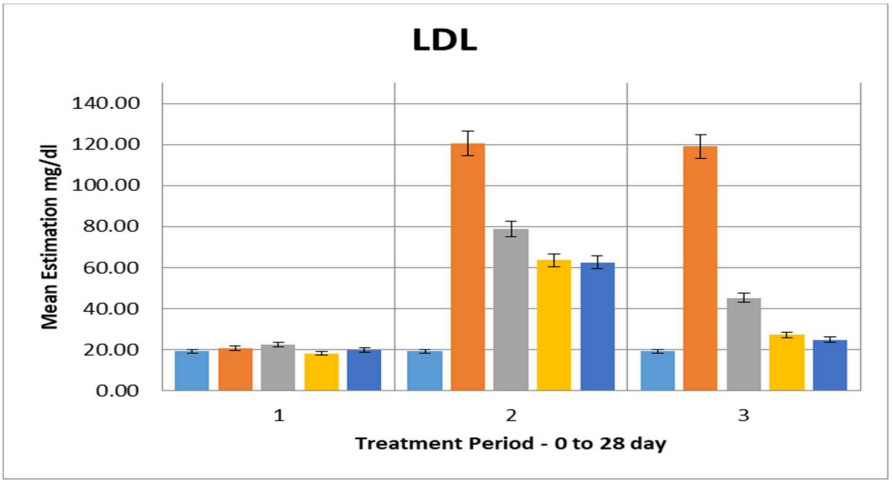

Figure 3: LDL factor levels

NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

2. HDL (Table 2)

Table 2: Hyperlipidaemia HDL Levels at 28 days

Group	Treatment	HDL (Mean± SEM)
I	NC	46.46±1.88
II	DC	42.55±1.60
III	COL	72.03±1.81
IV	ATS (40mg/kg)	66.25±1.79
V	ATS (10mg/kg) + COL	64.43±1.83***

All Values are expressed as Mean ± SEM (n=6), where p<0.003 when compared to treated group ANOVA followed by Dunnett's test

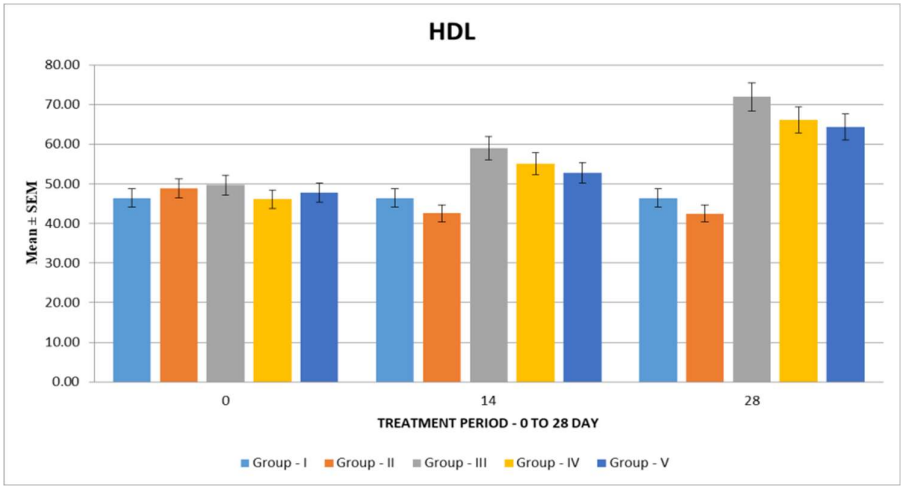

Figure 4: HDL factor levels

NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V).

Table 3: Hyperlipidaemia Triglyceride Levels at 28 day

Group	Treatment	Triglycerides (Mean± SEM)
I	NC	125.52±3.93
II	DC	205.52±21.18
III	COL	148.57±3.67
IV	ATS (40mg/kg)	130.3±3.24
V	ATS (10mg/kg) + COL	128.6±1.91****

All Values are expressed as Mean ± SEM (n=6), where p<0.004 when compared to treated group ANOVA followed by Dunnett's test

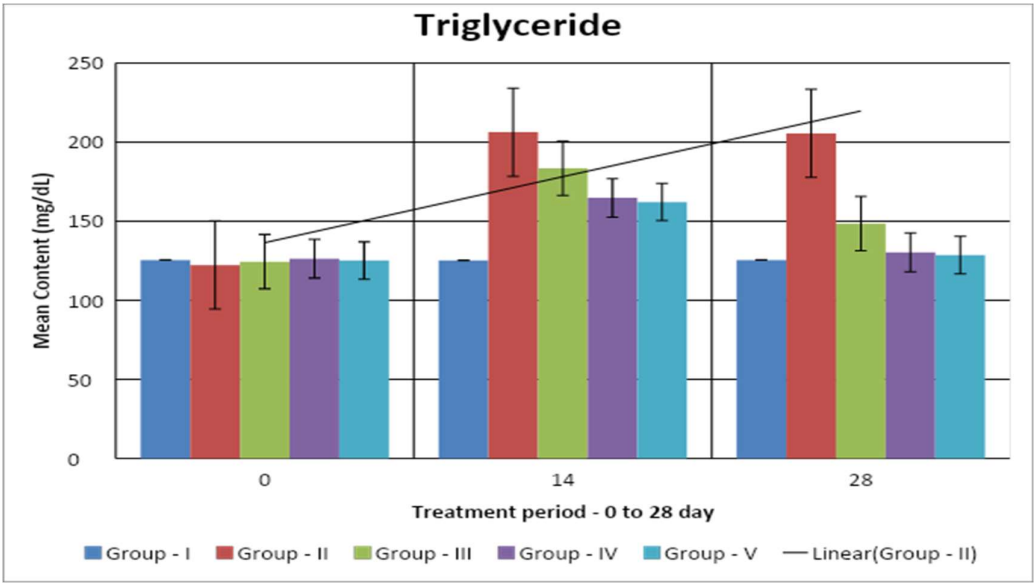

Figure 5: Triglyceride factor levels
NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

4. VLDL (Table 4 and Figure 6)

Table 4: Hyperlipidemia VLDL Levels at 28 day

Group	Treatment	VLDL (Mean± SEM)
I	NC	25.5±2.23
II	DC	40.55±3.38
III	COL	43.3±1.47
IV	ATS (40mg/kg)	34.32±1.08
	ATS (10mg/kg) + COL	31.5±1.22***

All Values are expressed as Mean ± SEM (n=6), where p<0.003 when compared to treated group ANOVA followed by Dunnett's test

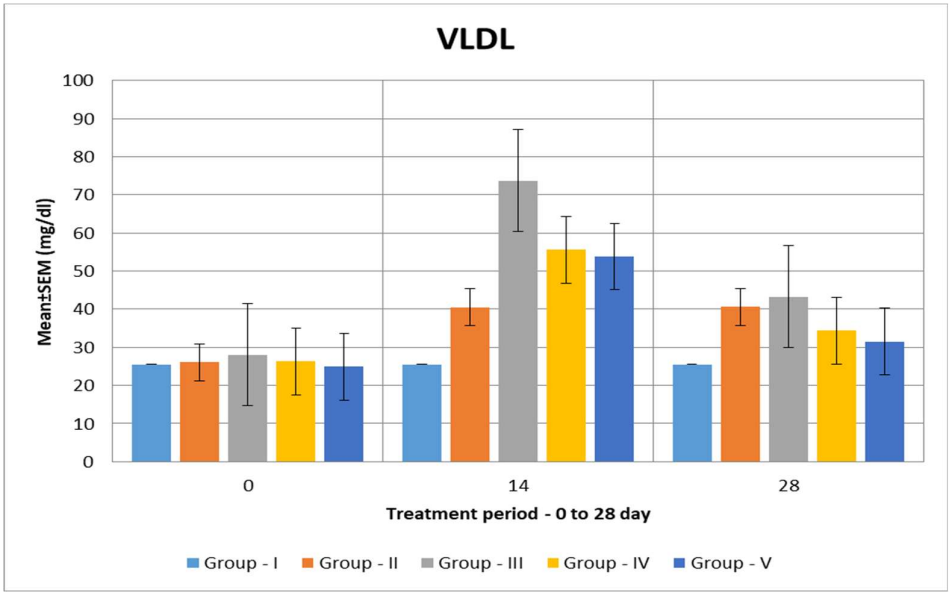

Figure 6: VLDL factor levels
NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

5. Total Cholesterol (TC) (Table 5 and Figure 7)

Table 5: Hyperlipidaemia TC Levels at 28 day

Group	Treatment	TC (Mean± SEM)
I	NC	84.76±1.28
II	DC	198.77±7.35
III	COL	122.57±8.17
IV	ATS (40mg/kg)	102.57±3.02
V	ATS (10mg/kg) + COL	99.52±1.22****

All Values are expressed as Mean ± SEM (n=6), where p<0.004 when compared to treated group ANOVA followed by Dunnett's test

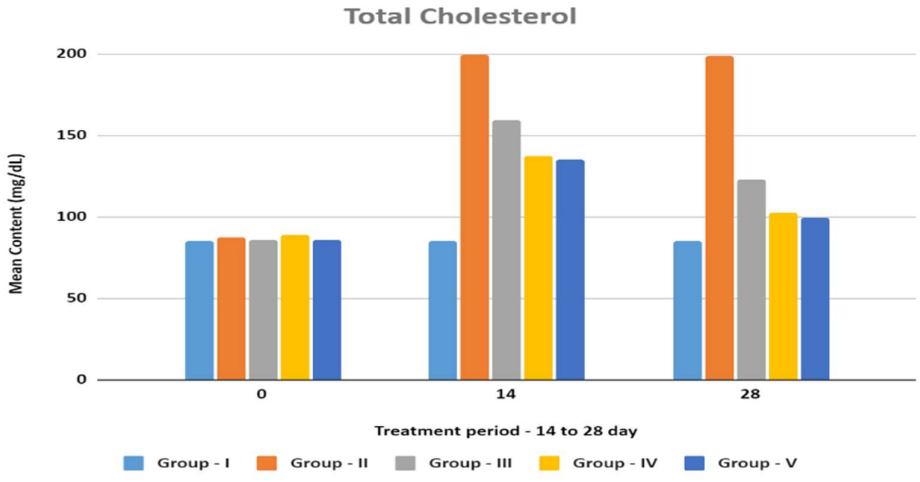

Figure 7: TC factor levels
NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

6. CREATININE KINASE MYOPATHY MARKER (CK-MB) (Table 6 and Figure 8)

Table 6: Hyperlipidaemia CK-MB Levels at 28 days

Group	Treatment	CK-MB (Mean± SEM)
I	NC	46.55±4.16
II	DC	54.80±3.10
III	COL	56.53±0.67
IV	ATS (40mg/kg)	75.63±1.84
V	ATS (10mg/kg) + COL	73.00±1.12****

All Values are expressed as Mean ± SEM (n=6), where p<0.04 when compared to treated group ANOVA followed by Dunnett's test.

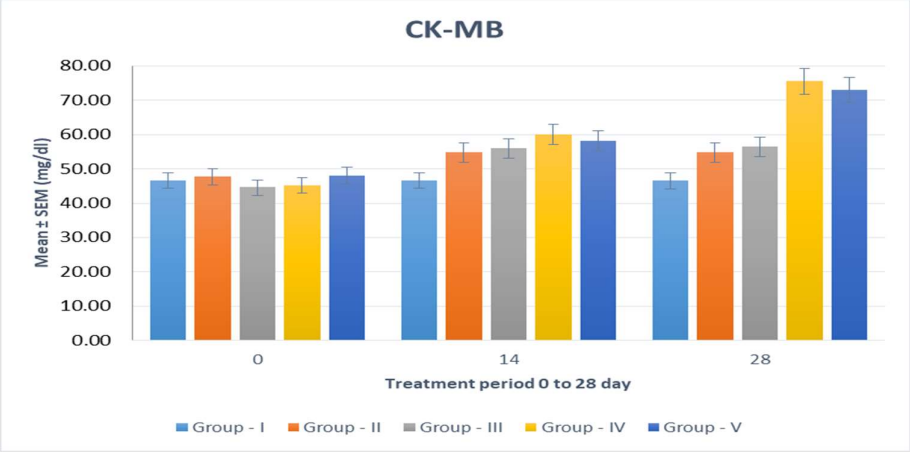

Figure 8: CK-MB factor levels

NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

7. HEART WEIGHT

- Measurement of heart weight after 28 days of treatment by digital weighing balance **(Figure 9)**.

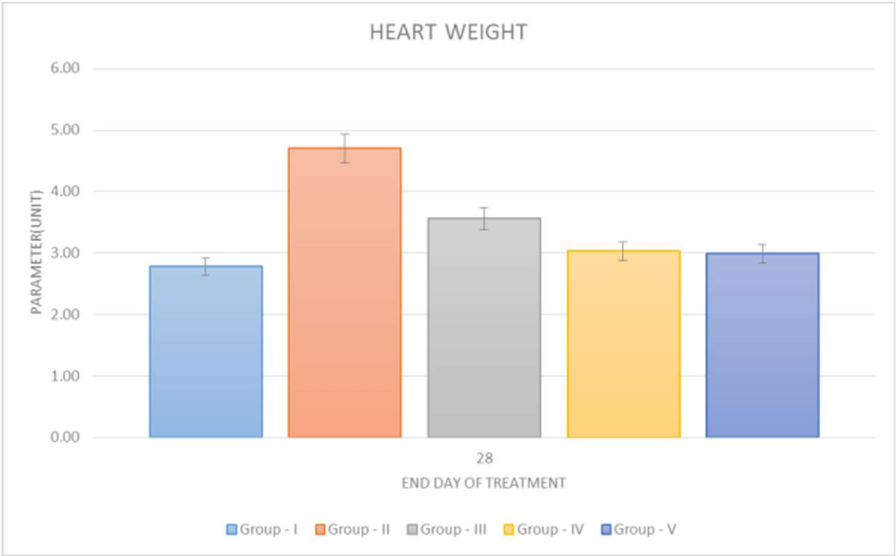

Figure 9: Heart weight measurement

NC- Normal Control (Group-1), DC- Diseases Control (Group-2), Col- Colesevelam 625mg/kg (Group – III), ATS-/Atorvastatin 40mg/kg (Group – IV), ATS + COL-Colesevelam HCL 625mg/kg + Atorvastatin 10mg/kg (Group – V)

DISCUSSION

The animals of group (II – V) were given orally HFD for induction of hyperlipidemia. After induction for 48 days there was an increase level of Hyperlipidemia. This confirms the induction of Hyperlipidemia (Atherosclerosis). Before initializing of treatment biochemical estimation was performed and was compared to Normal control Group (Group-I). Normal control Group (Group-I) was considered as base line for biochemical analysis.

Initial increases in Hyperlipidemia was as per expectation as the HFD model is very potent and generate very predictable data making it very suitable choice for studying Atherosclerosis (Hyperlipidemia).

After 14 day of treatment the biochemical analysis data demonstrate that Test Group – I (Group-III) to Test Group – III (Group-IV to V) have lipid profile decreases but HDL was increased and increased Creatine kinase i.e. myopathy marker of Test Group-II (Group-IV). Analysis of Creatine Kinase Parameter i.e. Myopathy marker the slightly decreases CK-MB in test Group – I and Over Slightly decreases CK-MB of Test Group – III as compare to Test Group – I and III.

After 28 day it is evident that Group-III and Group V have decreases the CK-MB as well the Group- IV have increased the CK-MB i.e. myopathy and Rabidomyolysis. Although every treatment group have slightly decreased Lipid Profile (LDL, VLDL, TC, and TG) or hyperlipidemia but HDL was increases.

Moreover, in Test Group – II lipid profile or hyperlipidemia was decreases and CK-MB was increases.

After 28 day of treatment in Test Group- III LDL, VLDL, TC, TG have decreases but HDL was increases and CK-MB slightly decreases as compared to Test Group- IV.

The major cause of this critical situation is acute myocardial infraction which leads to cardiac arrest, so it is necessary to treat the disease and control its progression with much more effectiveness.

Previous study data did not show any significant or clinical values to combination study of statins class of drug with bile-acid sequestrants (Colesevelam). In this study we trailed the same test series of concentrations with statins in combination with Colesevelam but in a time dependent manner to further asses the study and further evaluate the clinical significance of the study by means of chrono pharmacology.

CONCLUSION

Atherosclerosis Marker such as: LDL, VLDL, TG, TC, HDL and CK-MB Should have been measured to truly ascertain soy's true Hyperlipidemia protection. As current clinical scenarios suggest that higher dose Atorvastatin 40mg/kg which is currently

used in treatment of hyperlipidemia which causes elevated level of CK-MB and causes myopathy. In this study we administered Combination therapy by combining Colesevelam and Atorvastatin (low concentration 10 mg/kg) and administered orally. So the result showing low dose of atorvastatin and Colesevelam HCL significantly decreases LDL, HDL, VLDL, TG, cholesterol but no adverse effect on CK-MB compared with DC. Low-dose of atorvastatin and Colesevelam HCL combination decreases same as high dose of atorvastatin but no any adverse effect changes in CK-MB. The whole experiment and data obtained shows that the combinational low dose therapy is more significant and cause less adverse effect than that of standard treatment of high dose atorvastatin that is currently being used in treatment. In future aspects the combinational therapy can be more exploited and can show more clinical significance than current mode of treatment.

Conclusively, the observed cholesterol reducing action of the High dose of Atorvastatin and Colesevelam Alone and in combination Atorvastatin and Colesevelam indicate the hypolipidemic activity.

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