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IN-VITRO AND IN-VIVO EVALUATION OF DIRECTLY COMPRESSED TABLETS OF SIMVASTATIN WITH SOY LECITHIN

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ABSTRACT

The study was conducted to carry out comparative *in-vitro* evaluation of two different formulations of Simvastatin tablets containing Soy lecithin (F2) and MCC (F3) to evaluate Soy Lecithin as a direct compression vehicle and a comparative *in-vivo* evaluation to observe potential anti-cholesterol activity with the Soy Lecithin and Simvastatin combination tablets (F2). A blank formulation of tablet was formulated which was F1 containing Soy Lecithin alone. The formulations were subjected to *in-vitro* evaluation. The formulation blends were subjected to pre compression studies and were found to exhibit good flow properties. All the three formulation blends were formulated into tablets by means of direct compression. Magnesium Stearate was added as a lubricant in F3. The prepared tablets of F1, F2 and F3 were subjected to post compression. The results revealed good post compression properties and F2 tablets have shown better release of Simvastatin during dissolution rate study. The study revealed that soy lecithin could function as a substitute DCV in directly compressed tablets. In the *in-vivo* studies, the three formulations of tablets were administered against fructose-induced hyperlipidemic in rats. The results of treated animals have revealed that there was significant decrease respectively in the levels of serum total cholesterol, LDL cholesterol and significant increase in HDL cholesterol in serum when compared to the induced control group. Rats administered with F2 tablets shown the maximum reduction in total cholesterol and LDL Cholesterol and highest elevation in HDL cholesterol. This proved that Soy Lecithin in combination with Simvastatin possesses potential cholesterol lowering effect.

KEYWORDS: Simvastatin, Soy Lecithin, MCC, Direct Compression Vehicle, Potential Cholesterol Lowering agent.

INTRODUCTION

According to the World Health Organization (WHO), cardiovascular disease account for 16.7 million deaths per year. The estimates for the year 2020 maintain cardiovascular disease conditions as the main cause of death, with developing countries contributing more significantly than developed countries.[1] Many of the risk factors like smoking, lack of exercise and consumption of a high fat diet are responsible for causing cardiovascular disease. The majority of risk factors involved in the causation of atherosclerotic diseases are directly or indirectly due to disturbances in the lipid and lipoprotein metabolism. Evidence from studies both in animals and humans indicates that progression can be slowed if elevated serum concentration of the atherogenic lipoprotein and triglycerides are reduced, which in turn prevents coronary heart disease.[2] Cardiovascular disease includes numerous problems, many of which are related to a process called atherosclerosis. Atherosclerosis is a condition that develops when a substance called plaque builds up in the walls of the arteries. This build ups narrow down the arteries, making it harder for blood to flow through. If a blood clot forms, it can stop the blood flow. This can cause a heart attack or stroke.[3] One of the most common cardiovascular disease is atherosclerosis which is a leading cause of death in the developed countries. Atherosclerosis is a disease of the large arteries and is the primary cause of heart disease and stroke.[4] Atherosclerosis is mainly caused by hyperlipidemia.[5] It is generally believed that reduction of total cholesterol and low density lipoprotein cholesterol (LDL) reduces atherosclerosis in animals and clinical cardiovascular events in humans.[6,7] Statins are first choice medication for reducing LDL-C values, and clinical trials have demonstrated beyond doubt that lowering LDL-C with statins considerably diminishes the risk for cardiovascular disease in a wide range of patients.[8]

Statins, among the most commonly prescribed drugs worldwide, are cholesterol-lowering agents used to manage and prevent cardiovascular and coronary heart diseases. The commonly known pharmacological activity of statins relies on a potent inhibition of the endogenous mevalonate pathway, which leads directly to the biosynthesis of cholesterol and isoprenoids.[9] Statins are potent inhibitors of cholesterol biosynthesis. In clinical trials, statins are beneficial in the primary and secondary prevention of coronary heart disease.[10] Overview of all published randomized trials of statin drugs demonstrates large reductions in

cholesterol and clear evidence of benefit on stroke and total mortality. There was also a large and significant decrease in CVD mortality.[11] In patients without established cardiovascular disease but with cardiovascular risk factors, statin use was associated with significantly improved survival and large reductions in the risk of major cardiovascular events.[12]

Simvastatin belongs to the class statins. Simvastatin's molecular formula is C25H38O5 and its molecular weight is 418.56622. It is a derivative of lovastatin and potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA Reductases), which is the rate-limiting enzyme in cholesterol biosynthesis. Due to the induction of hepatic LDL receptors, it increases breakdown of LDL cholesterol. At the maximal recommended dose of 80 mg/day, simvastatin produces an average reduction in low-density lipoprotein cholesterol (LDL-C) of 47% with reduction of very LDL-C, triglycerides and apolipoprotein B, and a moderate increase in high-density lipoprotein cholesterol.[13] Many studies were reviewed and Simvastatin was proven to reduce lipid levels that is useful in reducing fatal coronary heart disease, nonfatal myocardial infarctions, fatal and nonfatal strokes, all cardiovascular deaths, or mortality to any cause and thus proving the efficacy of statins in prevention of CVD.[14] Simvastatin is indicated for the treatment of hypercholesterolemia and for the reduction in the risk of cardiac heart disease mortality and cardiovascular events.[15]

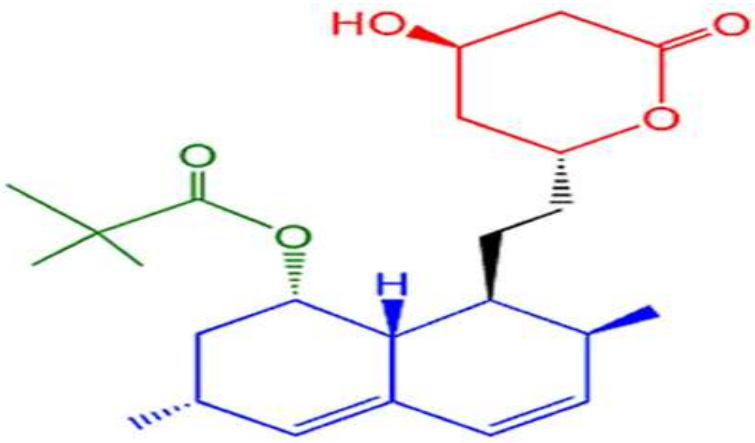

Figure 1: Chemical Structure of Simvastatin

The limitations of statins are regarding its side effects. The U.S. Food and Drug Administration (FDA) released an advice on January 2014 on statin risk reporting that "statin benefit is indisputable, but they need to be taken with care and knowledge of their side effects". One of the most common complications is myopathy, ranging from common but clinically benign myalgia to rare but life-threatening rhabdomyolysis. This class side effect

appears to be dose dependent, with higher doses of statins for example simvastatin carrying a higher overall risk. Hence, to minimize statin-associated myopathy, clinicians should take into consideration a series of factors that potentially increase this risk such as drug-drug interactions, female gender, advanced age, diabetes mellitus, hypothyroidism and vitamin D deficiency. Whenever it is appropriate to stop statin treatment, the recommendations are to stay off statin until resolution of symptoms or normalization of creatine kinase values. Afterwards, clinicians have several options to treat dyslipidemia, including the use of a lower dose of the same statin, intermittent non-daily dosing of statin and initiation of a different statin, alone or in combination with non-statin lipid-lowering agents.[16] Out of all the dosage forms available, tablets are the most commonly used dosage forms. The ease of manufacturing, convenience in administration, accurate dosing, and stability compared to oral liquids, tamper-proofness compared to capsules, and safety compared to parenteral dosage forms makes tablets a popular and versatile dosage form.[17] In this study, the tablet dosage forms were chosen as suitable formulations as they were proven to have many beneficial advantages over other dosage forms. Tablet dosage forms are simple, economical in manufacturing, most stable and most convenient. Besides that, it is very easy to mask the taste of bitter active ingredients and it provides an accurate dosage to the patient as compared to other dosage forms. Furthermore, simvastatin have been produced and marketed as tablets thus far further proving the advantages, suitability and stability of the tablet dosage forms over other dosage forms in Simvastatin formulation.

There are mainly three ways to compress tablets namely wet granulation, dry granulation and direct compression. In this study, direct compression method was used to compress the tablets. The main advantage of direct compression is it is cost effective since the direct compression requires fewer unit operations leading to reduced production cost of tablets due to less equipment, lower power consumption, less space, less time and less labor. Besides that, this method ensures stability of formulation especially for moisture and heat sensitive APIs, since it eliminates wetting and drying steps and increases the stability of active ingredients. Furthermore, there is less chance of changes in dissolution profiles to occur in tablets made by direct compression on storage than in those made from granulations. Moreover, faster dissolution is another advantage as tablets prepared by direct compression disintegrate into API particles instead of granules that directly come into contact with dissolution fluid and exhibits comparatively faster dissolution than API prepared by wet granulation. In addition, there will less wear & tear of punches as the high compaction

pressure involved in the production of tablets by slugging or roller compaction can be avoided by adopting direct compression. The next advantage is lesser contamination and microbial growth. This is because the ingredients were processed for a shorter period of time owing to lesser chance for contamination and also the absence of water in granulation significantly minimizes the chances microbial growth in direct compression tablets.[18] In this study, the tablets were produced via direct compression method due to its vast advantages.

In this study, the direct compression vehicle (DCV) used were Soy Lecithin and Microcrystalline Cellulose (MCC). Soy Lecithin is a naturally occurring emulsifier that can be found in soybeans.[19] Lecithin is made by purifying phospholipids of the soybeans. It is used for a variety of purposes such as an emulsifying agent, wetting and instantizing agents, viscosity modifier, extrusion aid, separating agent, and as nutritional supplement. Lecithin contains phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidyl-inositol and phophatidic acid which contributes to its unique hydrophobic and hydrophilic surface active properties. In pharmaceuticals, formulations made with phospholipids have several advantages like enhanced bioavailability of drugs with low aqueous solubility or low membrane penetration potential, improvement or alteration of uptake and release of drugs, protection of sensitive active agents from degradation in the gastrointestinal tract, reduction of gastrointestinal side effects of non-steroidal anti-inflammatory drugs and even masking the bitter taste of orally administered drugs.[20] A study concluded that crushed soya nuggets which consists of soy lecithin can act as a novel nutraceutical additive/excipient for simvastatin tablets providing bulk to the tablets.[21] Soy lecithin has also been proven to be a good and suitable Direct Compression Vehicle in formulating direct compression tablets.[22]

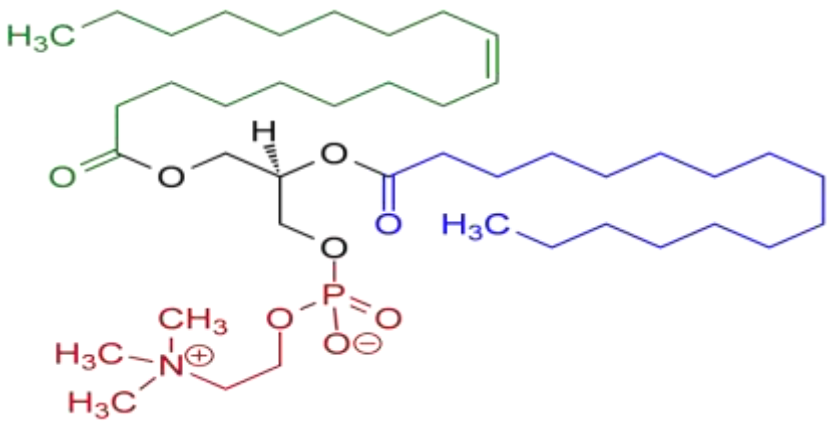

Figure 2: Chemical Structure of Soy Lecithin

Recent studies suggest that a lecithin enriched diet can modify the cholesterol homeostasis and lipoprotein metabolism. Lecithin diet modifies the cholesterol homeostasis in the liver, increasing the activity of HMG-CoA reductase and cholesterol 7-alpha-hydroxylase, and decreasing the microsomal ACAT activity.[23] One of the most spectacular properties of lecithin is its ability to reduce the excess of LDL cholesterol. It also promotes the synthesis in the liver of great amount of HDL, the beneficial cholesterol.[24] A study conducted demonstrated that lecithin induced a striking reduction in the plasma levels of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) cholesterol as well as an increase in the level of high density lipoprotein (HDL).[25] Furthermore, another study demonstrated the inhibition of cholesterol absorption in diets which are rich in phosphatidylcholine. This study suggests that the high degree of saturation of acyl groups of the soybean phosphatidylcholine decreases the cholesterol intestinal absorption.[26] In addition, another study claimed that bile acid secretion with high levels of cholesterol and phospholipids is encouraged by lecithin-rich diets when compared with diets without lecithin.[20] Therefore, reduction of plasma LDL cholesterol by soy lecithin is assumed to relate to reduction of cholesterol uptake from the digestive tract. The increase of HDL is presumably linked to the action of the enzyme lecithin: cholesterol acyltransferase, which transfers an unsaturated fatty acid moiety from lecithin onto cholesteryl esters in HDL, being subsequently degraded in the liver.[27] It is found that the liver plays a major role in the reduction of plasma cholesterol, the increased biliary lipid being provided by both HDL and the hepatic microsomal pools of plasma cholesterol and cholesterol in soy lecithin diet.[28] To top that, another study investigating the impact of administration of soy lecithin as a supplement on cholesterol levels revealed that lecithin administration in hypercholesterolemic patients may reduce cholesterol concentrations by increasing biliary secretion.[29] Another study revealed that soy lecithin may increase HDL cholesterol by a mechanism independent of its polyunsaturated fatty acid content, whereas the PUFA content of both soy lecithin and corn oil may alter the LDL cholesterol level.[30] One study revealed that cholesterol-lowering efficacy of the American Heart Association Step I diet can be enhanced with the addition of soy lecithin without reducing plasma HDL-C levels.[31] It is concluded that dietary soy lecithin but not egg lecithin decreased the plasma cholesterol concentration in hamsters via a mechanism other than decreasing cholesterol absorption.[32] A study revealed that soy lecithin taken up to 2,000 mg/kg administrated once orally were safe in male and female rats.[33] Therefore, the soy lecithin cholesterol-lowering activity was tested in this study.

Fructose or fruit sugar, is a simple ketonic monosaccharide found in many plants, where it is often bonded to glucose to form the disaccharide sucrose. It is one of the three dietary monosaccharides, along with glucose and galactose that are absorbed directly into the bloodstream during digestion. It is soluble in water, alcohol, or ether. It is used as a preservative and an intravenous infusion in parenteral feeding. The molecular formula is C6H12O6 and its molecular weight is 180.15588.[34]

D-Fructose was used to induce hypercholesterolemia in the rats for this study. Recent studies have found that high fructose diets enhance hepatic secretion of VLDL and may decrease its plasma clearance, which frequently results in modest hypercholesterolemia and hypertriglyceridemia.[35,36,37,38] Besides that, it is found that a diet rich in fructose resulted in a marked hypercholesterolemia.[39] Furthermore, fructose intake impacted on lipid metabolism in apolipoprotein AI-CIII-AIV transgenic mice which caused hypertriglyceridemia and hypercholesterolemia.[40] In addition, D-Fructose was used successfully to increase cholesterol levels in Male Wistar rats in two separate studies.[41]

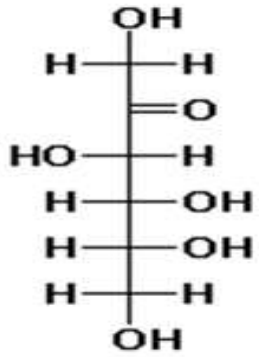

Figure 3: Chemical Structure of D-Fructose

In this study, three different formulations (F1, F2 & F3) of Simvastatin tablets were formulated by direct compression using Soy Lecithin (SL) and Microcrystalline Cellulose (MCC) as the direct compression vehicle (DCV). The formulations were subjected to *in-vitro* comparative evaluation studies which are pre and post compression parameters. Besides that, the comparison of the *in-vivo* cholesterol lowering effect of the prepared formulations in Fructose-induced hypercholesterolemic rats were conducted.

Materials: Soy Lecithin was purchased from Radiant Code Sdn. Bhd., Simvastatin was obtained as a gift sample from CCM Pharmaceuticals, Microcrystalline Cellulose, Magenesium Stearate, D- Fructose and Male Wistar albino rats were supplied by AMU.

Methods

Formulation Methods

Formulation of tablets using direct compression [22]

The tablet formulations F1, F2 and F3 were prepared by direct compression. The drug and excipients were mixed together by geometric dilution and passed through a 500 micron sieve. The mixture was compressed by using a tablet compressing machine.

Table I: The ingredients and their respective weights for F1, F2 & F3

Formulation	Simvastatin(mg)	Microcrystalline Cellulose (mg)	Soy Lecithin (mg)	Magnesium Stearate (mg)
F1	-	-	300	-
F2	80	-	220	-
F3	80	217	-	3

Evaluation Methods

Evaluation of Direct Compression Tablets[22]

Pre and post compression studies were determined. Each parameter was repeated three times. The mean values and standard deviations were obtained from the results. The results were tabulated and compared.

Pre compression studies

Pre-compression studies were conducted on Soy Lecithin powder, Simvastatin with Soy lecithin blend and Simvastatin with Microcrystalline Cellulose blend. The flow properties of all the blends were determined and compared.

1. Angle of repose

For angle of repose (θ), the blends were poured through the walls of a funnel, which is fixed at a position, using a retort stand, such that its lower tip was at a height of exactly 2.0 cm above hard surface. The blends were poured till the time when the upper tip of the pile surface touches the lower tip off the funnel. The radius was calculated. The tan-1 of the (height of the pile/radius of its base) was calculated as the angle of repose.

$\tan \theta = h/r$;where h is height of powder cone, r is radius of powder cone

2. Bulk density and tapped density

The blends were weighed and poured gently through a glass funnel into a graduated cylinder. The volume of the blends were noted and the bulk density was calculated. Using the same

blend in the cylinder, the blends were then tapped using the tapped density apparatus for 100 taps. The tapped density was calculated. The bulk density (BD) and tapped density (TD) were calculated using the formula below.

Bulk density = weight of the blend/ untapped volume

Tapped density = weight of the blend/tapped volume

3. Hausner's ratio and compressibility index

Hausner's ratio (HR) and Carr's compressibility index (CI) were calculated according to the two standard equations given below

HR = TD/BD; CI = (TD - BD)/TD × 100

4. Calibration Curve

10 mg of Simvastatin was taken in a conical flask and dissolved in 10 ml of methanol. 10 ml of this solution was taken and diluted up to 100 ml with phosphate buffer (pH 6.8). The aliquots of 1, 2, 3, 4, & 5 ml solution were prepared in 10 ml of phosphate buffer (pH 6.8). The absorbance was measured at 238 nm by using UV visible spectrophotometer & the graph of concentration (µg/ml) versus absorbance was plotted as standard calibration curve.[42]

Post Compression Studies

Post Compression Studies were conducted on the directly compressed tablets of F1, F2 and F3. Results were obtained and compared.

1. Uniformity of weight

Twenty tablets were taken randomly from each group and their individual weights were determined on a digital weighing balance. The average weight of the tablets was calculated from the collective weight. The weights were compared for uniformity.

2. Friability

The friability of randomly picked twenty tablets from each group was measured using a Roche Friabilator. Twenty pre-weighed tablets were rotated at 25 rpm. for 100 rotations. The tablets were then reweighed after removal of fine powders and the percentage of weight loss and the friability were calculated using the formula below.

Friability = [(Initial weight - Final weight) / Initial weight)] ×100 [%]

3. Hardness

Twenty random tablets were selected from each group. Hardness of the prepared tablets in unit kg was determined by using the Electrolab Tablet Hardness tester and the values were compared.

4. Disintegration time

Disintegration time was measured according to the USP 701. One tablet was placed in each of the 6 tubes of the basket in 900mL of water and the temperature was maintained at 37 ± 2°C. The disintegration time of 6 individual tablets were observed and recorded.

5. DISSOLUTION

Dissolution rate was studied by using USP type II apparatus rotated at 50 rpm. Tablets were placed in pH 7.0 buffer solution consisting of 0.01 M phosphate buffer with 0.5 % Sodium Dodecyl Sulfate prepared by dissolving 30 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6000 ml of water.[43] The volume of the dissolution medium was 900 ml and the temperature was maintained at 37 ± 0.5 °C. 10 ml samples were withdrawn from the vessel at specific time intervals and the same volume (10 ml) of the dissolution medium was rapidly replaced.

The absorption of the solution was checked out by UV spectroscopy at 238 nm and Simvastatin content was determined from the standard curve.[44] The dissolution rate was studied for both formulations (F2 & F3) and the results were compared.

***in-vivo* studies**

The male Wistar Albino Rats were procured by AMU animal House. Animal Ethical Approval was requested and AMU Animal Ethical Committee provided approval for the study with the AEC approval number AMU/AEC/FOP/2014/38.

Male Wistar albino rats (200 – 250g) were housed in rodent cages under standard conditions (temperature 24 ± 2 ^{o}C, humidity 60 ± 10%, light from 7 AM to 7 PM) with free access to water and standard rat pellets. 25% D-Fructose solution in water was prepared by dissolving 25g of D-Fructose in 100ml of drinking water was substituted with drinking water to induce the rats. After acclimation for a week in the laboratory environment, the rats were randomly assigned to one of the five different experimental groups lasting for 21 days.

Table II: Animal Treatment Grouping

Animal Grouping	Description	Quantity
Group 1	Control	6
Group 2	Induced Control	6
Group 3	F1 treatment group: A standard rat pellet diet + 25% D-Fructose in drinking water with a F1 tablet crushed and mixed with water directly administered to the rats OD.	6
Group 4	F2 treatment group: A standard rat pellet diet + 25% D-Fructose in drinking water with a F2 tablet crushed and mixed with water directly administered to the rats OD.	6
Group 5	F3 treatment group: A standard rat pellet diet + 25% D-Fructose in drinking water with a F3 tablet crushed and mixed with water directly administered to the rats OD.	6

*F1 tablets: Soy Lecithin

*F2 tablets: Simvastatin + Soy Lecithin

*F3 tablets: Simvastatin + Microcrystalline Cellulose

Tablets were crushed, mixed with water and administered orally via a feeding needle directly to the rats following the dose of 10mg/kg/day of simvastatin. Administration was performed at 6p.m. daily for 21 days. Blood samples were collected on day 21 (the final day) of the experiment.

Determination of Total Cholesterol, LDL-Cholesterol and HDL-cholesterol in blood samples collected from the rats.

The rats were fasted for 14 hours. They were then exposed to chloroform till they faint and then blood was collected through the retro-orbital plexus vein of each rat on Day 21. The blood samples were then centrifuged promptly (3000 g, 15 min) at room temperature. Separated top layer of serum from each sample will be used for the analysis.[45] Cholesterol test kits were used for the determination of total cholesterol, LDL-cholesterol and HDL-cholesterol. Total Cholesterol was determined with the CHOLESTEROL liquicolor using the "Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing Factor" method. LDL cholesterol was determined with the LDL CHOLESTEROL liquicolor using the "Direct Homogenous Test for the Determination of LDL-Cholesterol Enzymatic Colorimetric Test" method. HDL cholesterol was determined with the HDL CHOLESTEROL liquicolor using the "Direct Homogenous Test for the Determination of HDL-Cholesterol Enzymatic Colorimetric Test" method.

RESULTS

Table III: Pre compression Studies

PARAMETERS	F1	F2	F3
Angle of Repose (^{0})*	27.0127 ± 0.432	27.8476 ± 0.367	29.6237 ± 0.854
Bulk Density (g/ml)*	0.4310 ± 0.0236	0.4425 ± 0.0342	0.4273 ± 0.0452
Tapped Density (g/ml)*	0.5102 ± 0.0148	0.5155 ± 0.0116	0.5263 ± 0.0126
Hausner's Ratio*	1.1838 ± 0.1347	1.1650 ± 0.2363	1.2317 ± 0.2314
Carr's Compressibility Index (%)*	15.5233 ± 0.2345	14.1610 ± 0.2146	18.8106 ± 0.3620

*n=3

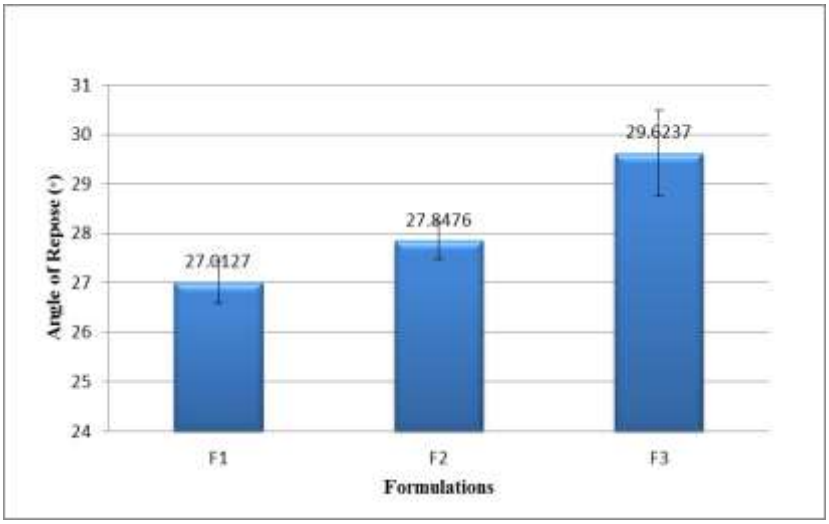

Figure 4: Angle of Repose

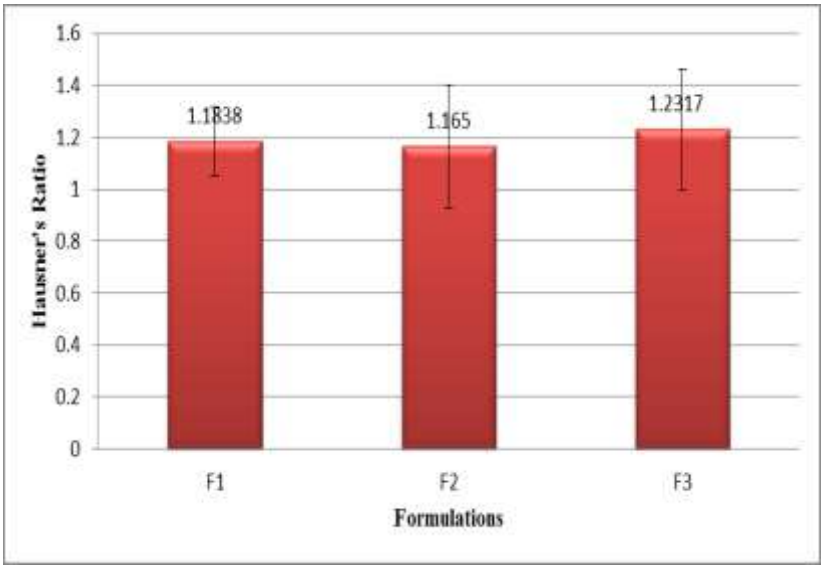

Figure 5: Hausner's Ratio

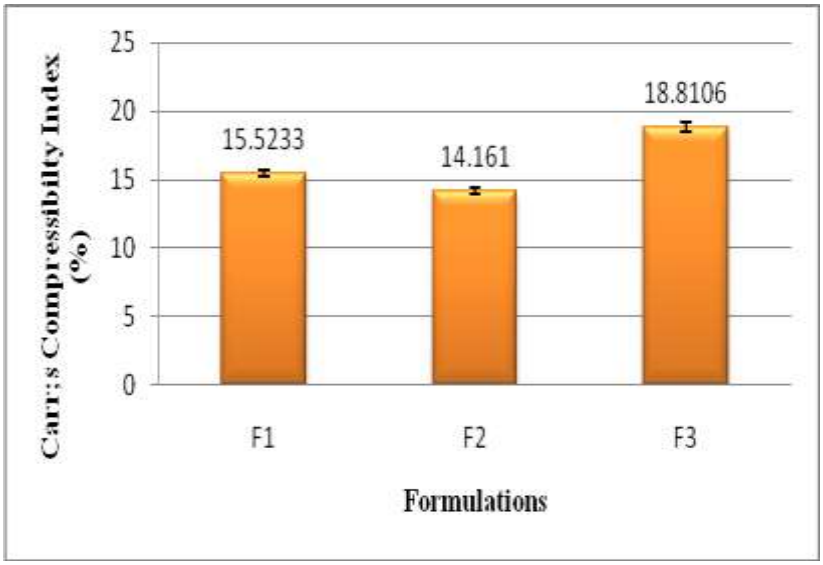

Figure 6: Carr's Compressibility Index (%)

Table IV: Post Compression Studies: Weight Variation

Parameters	F1	F2	F3
Weight (mg)*	298.5 ± 1.342	298.9 ± 7.782	303.4 ± 7.303
Range of Weight Variation (%)*	-0.96 to +0.54	-5.26 to +4.95	-3.93 to +5.02

*n=3; Mean ± Standard Deviation

Table V: Post Compression Studies: Friability, Hardness and Disintegration Time

Parameters	F1	F2	F3
Friability (%)*	0.572 ± 0.24	0.699 ± 0.13	0.735 ±0.32
Hardness (kg)*	7.15 ± 0.39	6.25 ± 0.19	6.64 ± 0.27
Disintegration Time (s)*	55.2 ± 2.9	49.7 ± 4.2	60.7 ± 2.7

*n=3; Mean ± Standard Deviation

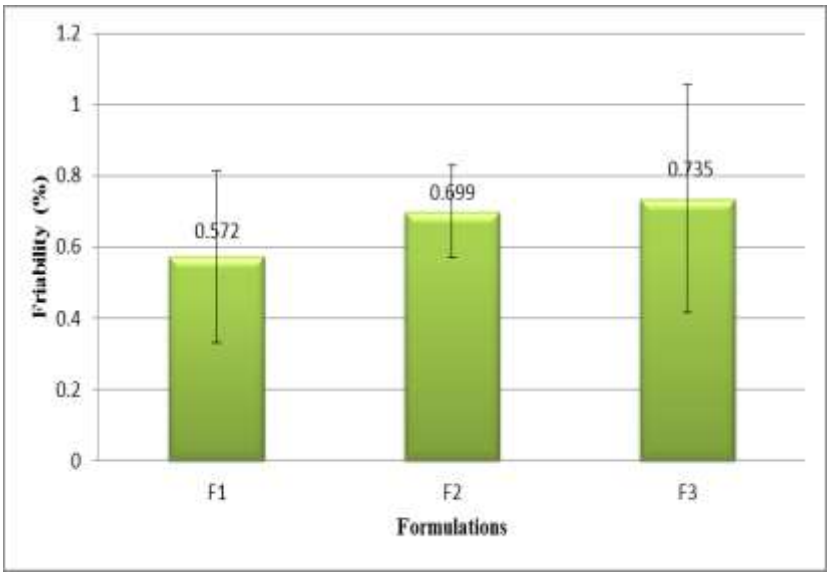

Figure 7: Friability (%)

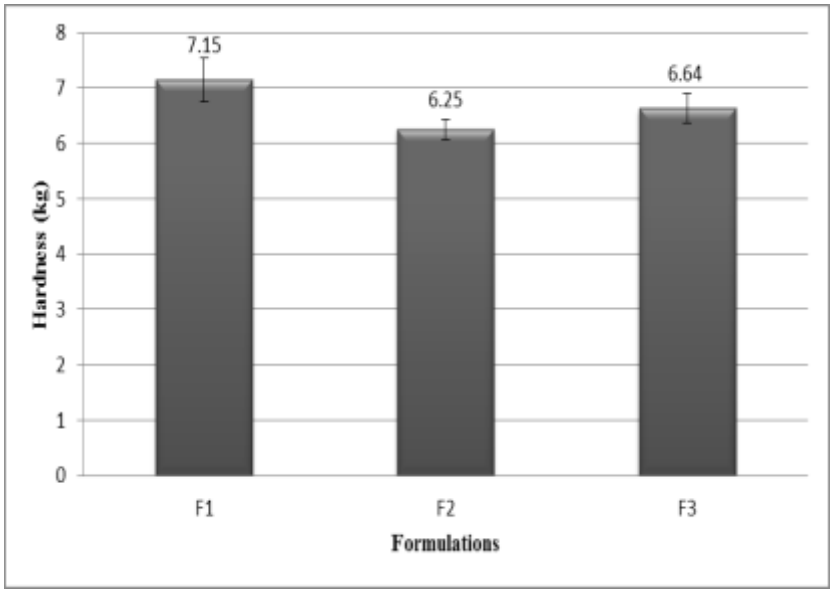

Figure 8: Hardness (kg)

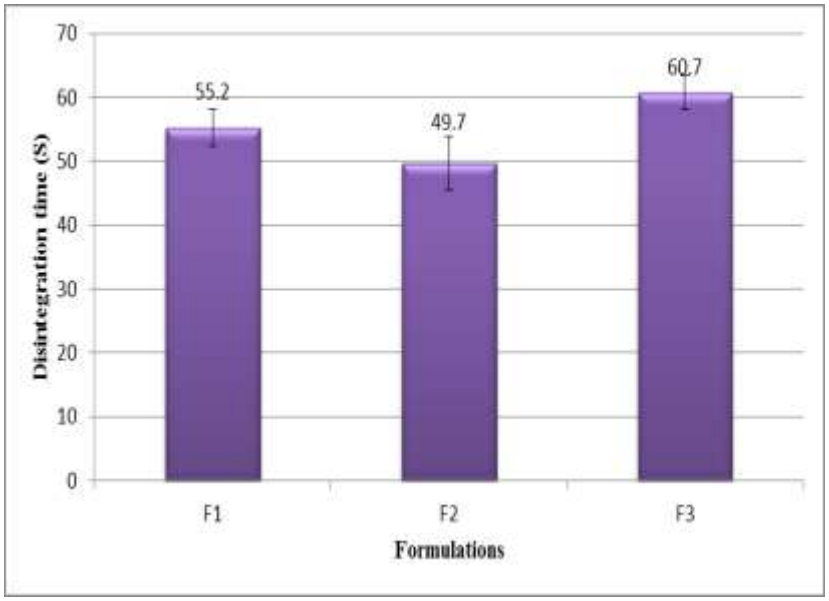

Figure 9: Disintegration Time (S)

Table VI: Observations of Calibration Curve

Concentration (µg/ml)	Absorbance at 238 nm
0	0.00
10	0.210
20	0.415
30	0.612
40	0.809
50	1.038

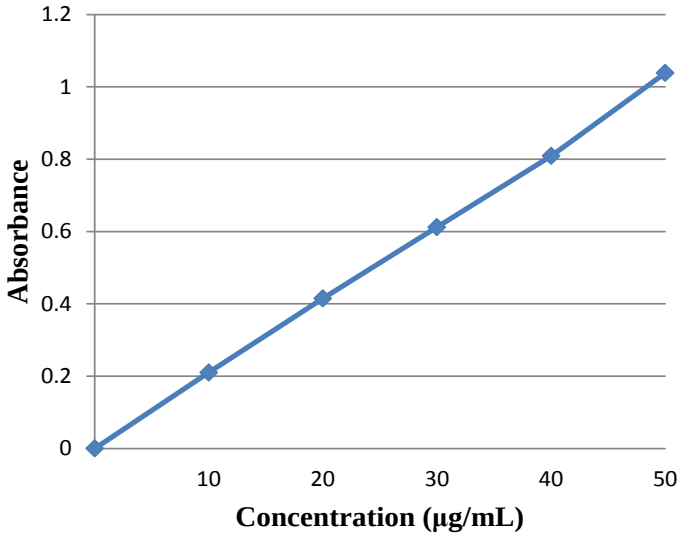

Figure 10: Absorbance vs Concentration graph

Table VII: Dissolution Profile of F2

Time Interval (hour)	Absorbance	Concentration (µg/ml)	Concentration in 900ml (mg/ml)	Percentage of drug release (%)
0	0	0	0	0
5	0.24823 ± 0.00081	12.2	11.0	13.8
10	0.57942 ± 0.00078	28.4	25.6	32.0
15	1.03008 ± 0.00115	50.5	45.4	56.8
20	1.36507 ± 0.00125	66.9	60.2	75.3
25	1.62373 ± 0.00111	79.6	71.6	89.5
30	1.78087 ± 0.00059	87.3	78.6	98.3

Table VIII: Dissolution Profile of F3

Time Interval (hour)	Absorbance	Concentration (µg/ml)	Concentration in 900ml (mg/ml)	Percentage of drug release (%)
0	0	0	0	0
5	0.19187 ± 0.0004	9.4	8.5	10.6
10	0.43248 ± 0.00117	21.2	19.1	23.9
15	0.73020 ± 0.00145	35.8	32.2	40.3
20	1.09959 ± 0.00193	53.9	48.5	60.6
25	1.34020 ± 0.00111	65.7	59.1	73.8
30	1.48838 ± 0.00651	73.0	65.7	82.2

Table IX: Percentage of Drug Release from F2 and F3

Time (mins)	Percentage of Drug Release from F2 (%)	Percentage of Drug Release from F3 (%)
0	0	0
5	13.8	10.6
10	32.0	23.9
15	56.8	40.3
20	75.3	60.6
25	89.5	73.8
30	98.3	82.2

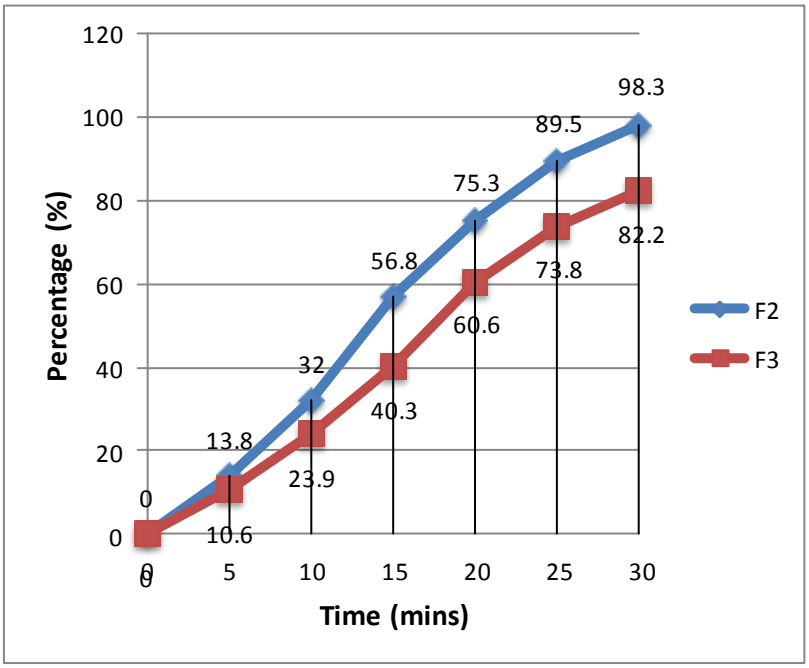

Figure 11: Percentage of Drug Release from F2 and F3 (%) *in-vivo* studies

Table X: Total Cholesterol, LDL Cholesterol and HDL Cholesterol levels (mg/dl)

Parameters/Groups	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)
G1 (Control)	88.17 ± 3.97	32.17 ± 2.32	40.50 ± 2.74
G2 (Induced Control)	131.17 ± 4.02	87.67 ± 1.75	20.17 ± 3.76
G3 (F1 treated)	104.83 ± 6.37	59.83 ± 3.43	28.83 ± 2.32
G4 (F2 treated)	89.00 ± 5.10	49.83 ± 2.32	38.33 ± 2.58
G5 (F3 treated)	96.00 ± 6.07	53.17 ± 2.48	34.00 ± 1.79

*n=6

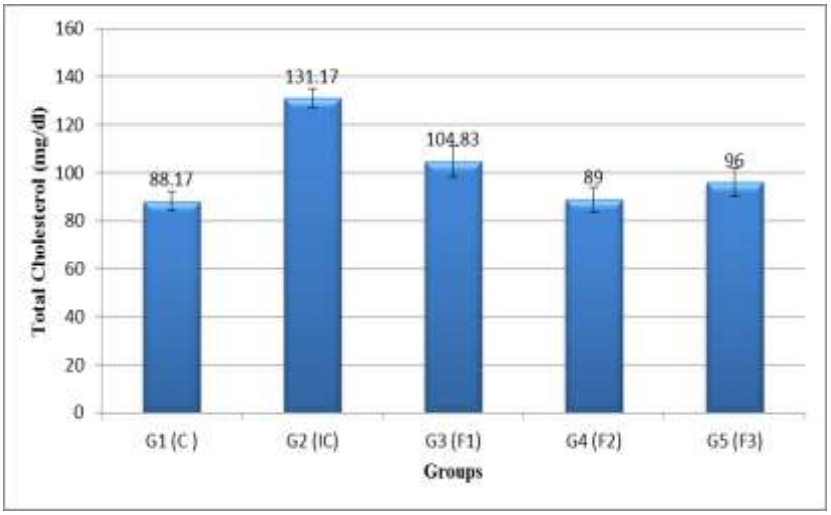

Figure 12: Total Cholesterol Levels (mg/dl)

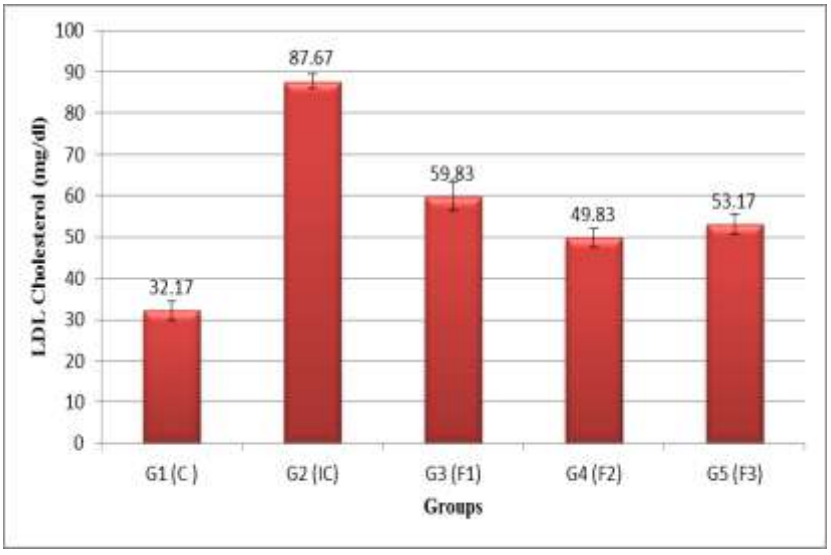

Figure 13: LDL Cholesterol Levels (mg/dl)

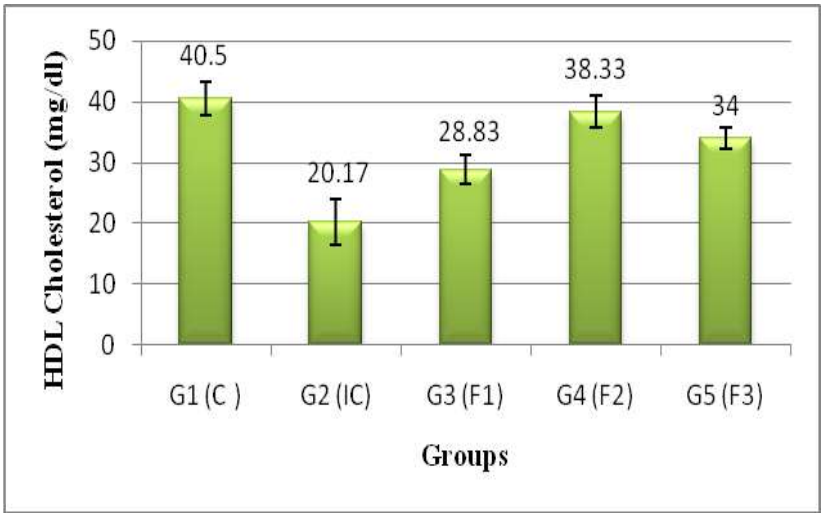

Figure 14: HDL Cholesterol Levels (mg/dl)

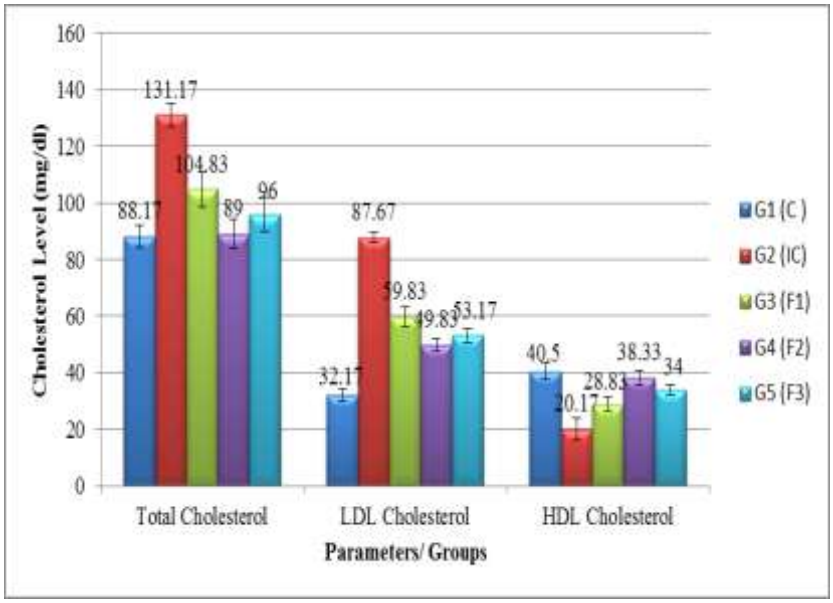

Figure 15: Total Cholesterol, LDL Cholesterol and HDL Cholesterol levels (mg/dl)

Table XI: Difference in Total Cholesterol, LDL Cholesterol and HDL Cholesterol

Total Cholesterol (mg/dl)				
Parameters	Group 2	Group 3	Group 4	Group 5
Formula	A of G2 – A of G1	A of G2 – A of G3	A of G2 – A of G4	A of G2 – A of G5
Difference in Total Cholesterol Levels (mg/dl)	43	26.34	42.17	35.17
LDL Cholesterol (mg/dl)				
Parameters	Group 2	Group 3	Group 4	Group 5
Formula	B of G2 – B of G1	B of G2 – B of G3	B of G2 – B of G4	B of G2 – B of G5
Difference in LDL Cholesterol Levels (mg/dl)	55.5	27.84	37.4	34.5
HDL Cholesterol (mg/dl)				
Parameters	Group 2	Group 3	Group 4	Group 5
Formula	C of G1 – C of G2	C of G3 – C of G2	C of G4 – C of G2	C of G5 – C of G2
Difference in HDL Cholesterol Levels (mg/dl)	20.33	8.66	18.16	13.83

DISCUSSION

The study was conducted to carry out comparative *in-vitro* and *in-vivo* evaluation of different formulation of Simvastatin tablets containing Soy lecithin and MCC and to evaluate Soy Lecithin as a direct compression vehicle (DCV). The main aim of this study was to observe the synergistic anti-cholesterol activity of Soy Lecithin with Simvastatin as a combination in formulated tablets (F2). In the study, three formulations of tablets were formulated which are coded as F1 which consists of only Soy Lecithin, F2 which consists of Simvastatin and Soy

Lecithin as DCV and F3 which consists of Simvastatin, MCC as DCV and Magnesium Stearate as a lubricant. All the three formulations were subjected to pre and post compression studies. All materials used for the study were mentioned in Table I and the animal grouping system was mentioned in Table II. The chemical structures of simvastatin,soy lecithin and D-Fructose were represented in Figures 1,2 & 3 respectively.

The pre compression parameters of F1, F2 and F3 blends were determined and compared and the results were shown in Table III. The repose angle of F1, F2 and F3 were found to be 27.0127^{o}, 27.8476 ^{o} and 29.6237 ^{o} respectively and where compared in Figure 4. The values shown that all three formulation blends have good flow properties ranging from 20^{o} to 30^{o}.[46] The Hausner's ratio and Carr's compressibility index were calculated by using the values of bulk density and tapped density, the Hausner's ratio for F1, F2 and F3 were found to be 1.1838, 1.1650 and 1.2317 respectively and the results were compared in Figure 5. The Hausner's ratio values for all the three formulations were found to be below 1.25 which reveals that they have a good flow property (46). The Carr's compressibility index for F1, F2 and F3 were found to be 15.5233%, 14.1610% and 18.8106% respectively and were compared in Figure 6. The values revealed that F1 and F2 have good flow properties and F3 has a fair flow property.[46]

The post compression studies were conducted and the results were tabulated and compared among which the results of weight variation test of F1, F2 and F3 were found ranging between -0.96% to +0.54%, -5.26% to +4.95% and -3.93% to +5.02% respectively and were represented in Table IV. All the three formulations passed the weight variation test as all the results were found to be in the standard range.[46]

The friability test was carried out and the friability values of F1, F2 and F3 were found to be 0.572%, 0.699% and 0.735%. These were acceptable and within the range of 0.5 to 5.0% . The friability values were compared in Figure 7. The hardness test was conducted and the values of F1, F2 and F3 were found to be 7.15kg, 6.25kg and 6.64kg. The hardness values were within the range 4-10kg.[46] The hardness values were compared in Figure 8. The disintegration test was conducted and the values for F1, F2 and F3 were found to be 55.2s, 49.7s and 60.7s. The results of disintegration test revealed that all the three formulations have fast disintegration and all values were found to be under standard limits (46). The disintegration time readings were compared in Figure 9. The results of Disintegration, Hardness and Friability were tabulated in Table V.

The results of determined dissolution test were recorded by using USP type II apparatus and the dissolution profiles of F2 & F3 were represented in Table VII and Table VIII respectively. The concentration in µg/ml were obtained from the Calibration Curve which is shown in Figure 10. The percentage drug release for F2 and F3 tablets were shown in Table IX and they were compared in Figure 11. Dissolution studies were carried out for 30 minutes and it was observed that the release of Simvastatin for F2 and F3 were 98.3% and 82.2% respectively. The release rate of Simvastatin from the formulated tablets of F2 which are compressed with Soy Lecithin as DCV were found to exhibit rapid and high when compared with the formulated tablets of F3 compressed with MCC. From the comparative drug release rate study, it can be considered that the tablets of Simvastatin compressed with Soy Lecithin exhibit faster and higher rate of dissolution and are more efficient in terms of release rate of Simvastatin from directly compressed tablets.

In *in-vivo* studies, three parameters were evaluated which are Total Cholesterol, LDL Cholesterol and HDL cholesterol. The Cholesterol levels obtained were shown in Table X and compared in Figure 15. For the study, 30 wistar albino male rats were selected and separated randomly into 5 groups of 6 animals in each. The *in-vivo* study was carried out for 21 days. Group 1 (G1) was the Control Group where no interventions were carried out and Group 2 (G2) was the Induced Control Group in which the rats were fructose-induced with D-Fructose to result in elevated cholesterol levels but were not subjected to intervention. Group 3 (G3) was the F1 treatment group where the rats were induced and treated with F1 (Soy Lecithin) once daily as a dose. Group 4 (G4) was the F2 treatment group where the induced rats were treated with F2 (Soy Lecithin with Simvastatin) once daily as a dose. Group 5 (G5) was the F3 treatment group in which the induced rats were treated with F3 (Simvastatin with MCC) once daily as a dose. On the 21st day of the study, the blood samples were withdrawn from all the rats and the serum Total Cholesterol, LDL cholesterol and HDL cholesterol levels were measured in terms of mg/dl by using the commercial cholesterol test kits which are Total Cholesterol Liquicolor, LDL Cholesterol Liquicolor and HDL Cholesterol Liquicolor.

The total cholesterol test was carried out and the values for G1, G2, G3, G4 and G5 were found to be 88.17, 131.17, 104.83, 89.00 and 96.00mg/dl respectively were represented in Figure 12. The results of Total Cholesterol test revealed that in G2 there was an increase of 43mg/dl of Total Cholesterol from G1 and was represented in Table XI. This shows that D-

Fructose has the capability of increasing Total Cholesterol in rats. The values of Total Cholesterol reduction for G3, G4 and G5 from G2 were found to be 26.34mg/dl, 42.17mg/dl and 35.17mg/dl respectively and were represented in Table XI. This shows that F2 tablets produced the most Total Cholesterol lowering effect, followed by F3 tablets and then F1 tablets.

The LDL cholesterol test was carried out and the values for G1, G2, G3, G4 and G5 were found to be 32.17, 87.67, 59.83, 49.83 and 53.17mg/dl respectively was represented in Figure 13. The values revealed that for G2 there was an increase of 55.5mg/dl of LDL Cholesterol from G1 and was represented in Table XI. This shows that D-Fructose has the capability of increasing LDL Cholesterol in rats. The values of LDL Cholesterol reduction for G3, G4 and G5 from G2 were found to be 27.84mg/dl, 37.4mg/dl and 34.5mg/dl respectively and were represented in Table XI. This shows that F2 tablets produced the most LDL Cholesterol lowering effect, followed by F3 tablets and then F1 tablets.

The HDL cholesterol test was carried out and the values for G1, G2, G3, G4 and G5 were 40.50, 20.17, 28.83, 38.33 and 34.00mg/dl respectively and were represented in Figure 14. The values revealed that for G2 there was a decrease of 20.33mg/dl of HDL Cholesterol from G1 and was represented in Table XI. This shows that D-Fructose has the capability of decreasing HDL Cholesterol in rats and was considered suitable for the study. The values of HDL Cholesterol elevation for G3, G4 and G5 from G2 were found to be 8.66mg/dl, 18.16mg/dl and 13.83mg/dl respectively and were represented in Table XI. This shows that F2 tablets produced the most HDL Cholesterol elevating effect, Followed by F3 tablets and then F1 tablets.

The three studies revealed that F2 containing Simvastatin compressed with Soy Lecithin treatment group showed the most reduction in Total Cholesterol and LDL Cholesterol and most elevation in HDL cholesterol when compared with the other two treatment groups.

CONCLUSION

This study revealed that soy lecithin passed all the pre and post compression evaluation tests and it can be a suitable choice of a direct compression vehicle (DCV). The flow property of the prepared blend of Soy Lecithin was comparable to that of the prepared blend of Microcrystalline Cellulose. The batch of tablets containing Simvastatin and soy lecithin (F2) resulted in a faster disintegration and dissolution profiles as compared to the Simvastatin

tablets containing MCC (F3). There have been studies that exhibited Soy Lecithin's anti-cholesterol activity as daily supplements. In this study, the soy lecithin's activity was tested by combining it with an anti-cholesterol drug Simvastatin to evaluate the potential cholesterol lowering effect. It was proven through the animal study as the rats treated with the F2 tablets containing soy lecithin and Simvastatin showed significant reductions in Total Cholesterol, LDL cholesterol and significant elevation in HDL Cholesterol as compared to the other two groups treated with F1 tablets containing Soy Lecithin and F3 tablets containing Simvastatin and MCC. Soy lecithin was concluded to have the ability to take on two roles that is as a direct compression vehicle (DCV) in tablet formulation and also as a cholesterol lowering agent. This study revealed that the soy lecithin can be used as a direct compression vehicle and also as a potential cholesterol lowering component in combination with Simvastatin in tablet formulations.

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