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# Evaluation of the hypolipidemic activity of polyherbal formulation through In-vivo and In-silico studies

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***Abstract*---**Obesity is one of the most important public health issues touching every region of the world. Currently, India is facing the double burden of undernutrition likewise as overnutrition. There are >135 million those that are obese in India. Hyperlipoidaemia is related to risk factors like arteriosclerosis, hypertension, type-II DM, obesity, MI, congestive cardiac failure, angina pectoris, gall bladder diseases, degenerative joint diseases, apnea, and sterility. The present treatment for hyperlipoidaemia is Cholesterol-lowering medications may cause life-threatening serious unwanted effects many facet effects, such as muscle pain, exaggerated glucose levels, constipation, nausea, diarrhea, abdomen pain, cramps, the elevation of liver enzymes. Therefore, the present investigation was designed to investigate the hypolipidemic activity of polyherbal formulation in Wistar rats in an endeavour to determine the traditional use of this Polyherbal formulation and Insilco molecular docking studies. Hypolipidemic effect of polyherbal formulation was studied in the High Cholesterol cocktail diet (HCCD) fed hyperlipidemic rat model and Insilco Molecular docking studies of hHMG Co-A reductase (PBD:1hwk) and analysis of ligand-protein interactions for the prediction of the mechanism of hypolipidemic activity of important phytoconstituents of the polyherbal formulation. The polyherbal formulation to control shows a major reduction in total cholesterol, lipoid, LDL-cholesterol, VLDL-cholesterol and elevates the helpful lipid-like HDL-cholesterol and In silico studies, the ligand-protein interaction analysis revealed that Ellagic Acid, Rutin, Myricetin, Quercetin, Kaempferol, Gingerenone, 6-Shogaol, 6-Gingerol, Gallic Acid, and Alliin molecules shows binding energy near to reference


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drug atorvastatin and occupy similar binding sites as the normal substrate (HMG-CoA) in the catalytic domain.

***Keywords*---**high cholesterol cocktail diet, hHMG CoA reductase, polyherbal formulation, diabetes mellitus, molecular docking.

## Introduction

Obesity is one of the most important public health issues touching every region of the world. Currently, India is facing the double burden of undernutrition likewise as overnutrition. There are >135 million those that are obese in India. Obesity, a prevalent kind of malnutrition, will cause adverse metabolic effects (Hadaye RS, 2020). The prevalence of obesity in India varies due to age, gender, geographical environment, socio-economic status, etc. According to the ICMR-INDIAB study 2015, the prevalence rate of obesity and central obesity are variable from 11.8 to 31.3% and 16.9-36.3% respectively (Rajeev Ahirwar, 2019). Obesity will increase cardiovascular risk through risk factors like exaggerated fasting plasma triglycerides, high LDL cholesterol, low HDL cholesterol, elevated blood glucose, hypoglycaemic agent levels, and high blood pressure (Boudewijn Klop, 2013), (V.Venkata Rajesham, 2018). Hyperlipoidaemia is related to risk factors like arteriosclerosis, hypertension, type-II DM, obesity, MI, congestive cardiac failure, angina pectoris, gall bladder diseases, degenerative joint diseases, apnea, and sterility (K. N. Bharathi, 2010).

The present treatment for hyperlipoidaemia is Cholesterol-lowering medications including Statins - simvastatin, atorvastatin, rosuvastatin, and pravastatin, inhibitor absorption substance Ezetimibe, bile acid sequestrants colesevelam, cholestyramine, and colestipol and nicotinic acid (niacin). Presently available hypolipidemic medications are related to many facet effects, like statins inflicting muscle pain, exaggerated glucose levels, constipation, nausea, diarrhea, abdomen pain, cramps, the elevation of liver enzymes, Cholesterol absorption cholesterol shows side effects like abdomen pain, diarrhea, fatigue, muscle soreness; avoid throughout gestation and lactation, bile acid sequestrants show facet effects like gastrointestinal, like constipation, vomiting, diarrhea, bloating, nausea, gas, heartburn, and niacin shows Facial and neck flushing, itching, stomach upset, increase in glucose. increases in plasma creatinine from 15 to 20% are common in fibrate-treated patients and additional vital increases can occur in patients with underlying nephritic sickness (Gajendra Kumar, 2013). Hence, there has been a search for new safe and effective medication for dyslipidemia. Herbs are used as food and for medicative functions for hundreds of years. research interest has centered on varied herbs that possess a hypolipidemic impact which will be a helpful adjunct in reducing the risks of CVD.

The system of Indian medication is one of every of the oldest systems, and there are several plants mentioned during this system of medicative importance that incorporates traditional knowledge medicines for the treatment of assorted complications. The medicative plants represent a for the most part unknown reservoir of biologically active molecules not only as medication but conjointly as irreproducible templates that might offer a preliminary purpose for a synthetic

analog and an attractive means that may be helpful for a much better understanding of natural processes (Ranjan Kumar Giri, 2021). The present investigation was designed to investigate the hypolipidemic activity of polyherbal formulation in Wistar rats in an endeavour to determine the traditional use of this Polyherbal formulation. The polyherbal formulation contains hydroalcoholic extracts of *A. Sativum, P. Granatum, Z. Officinale*, and *S. Cumini*. All the restorative plants were chosen supported their incontestable antidiabetic, lipid-lowering properties, and insulin-sensitizing mechanisms of individual plants.

## Materials and Methods

### Animals

Healthy Wistar rats of either sex weighing between 150- 180 g were used for study and they were purchased from Shri Venkateshwara Enterprises, Bangalore for experimental purposes. The rats were housed in their cages for seven days before the start of dosing in the experimental room after veterinary examination housed in well-ventilated Polypropylene cages under the standard husbandry condition at 26±2°C, relative humidity 45-55% and light/dark cycle of 12 hrs. The animal was fed with pelletized feed and water was provided at the libitum. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Channabasweshwar Pharmacy College, Latur, Maharashtra, animal studies were performed as per rules and regulations by guidelines of CPCSEA with registration number 713/PO/Re/S/2002/CPCSEA.

### Drugs, Chemicals, and Instruments

The gift sample of hydroalcoholic extract of *Allium Sativum, Punica granatum, Zingiber officinale*, and *Syzygium Cumini* provided by Kisalaya Herbals Limited, Indore, India, Atorvastatin Tablets IP (Atorec-10): Emcure, HDL-cholesterol, LDL-cholesterol, Cholesterol, Triglyceride, Glucose standard kit purchased from Erba diagnostics Mannheim GmbH, Germany manufacturer, Spectrophotometer (UV-1800 Shimadzu), RX-50V Semi-Auto Biochemistry Analyzer, Micro Lab, Ahmedabad, India.

### Development of Polyherbal Formulation

The polyherbal formulation was evolved by combining the dried hydroalcoholic extracts of the plant materials based on the oral glucose tolerance test of individual plant extracts (200 mg/kg each) in normal rats and total antioxidant capacity, advantageous in routine life (nutritional value) and reported activities of plants (Shivakumar S. Ladde, 2021). The polyherbal formulation was made by mixing Hydroalcoholic Extract of *Allium Sativum* (bulb), *Punica granatum* (Fruit), *Zingiber officinale* (Shunt), and *Syzygium Cumini* (Seeds) in the proportion of 1.5:0.5:0.5:1.5

**Evaluation of Hypolipidemic activity of polyherbal formulation (PHF)**
**Experimental Protocol**

The long-term hypolipidemic effect of polyherbal formulation (PHF) was studied in the High Cholesterol cocktail diet (HCCD) fed hyperlipidemic rat model. Male Wistar rats weighing 150-180 g were used in this study. The rats were fed with a High Cholesterol cocktail diet composed of Peanut Oil (100ml), Cholic Acid (10gm), Cholesterol (10gm), Polythiouracil (1gm) for 15 days. (Surendran Surya, 2017), (Manoj Jaybhaye, 2018). The rats are divided into 5 groups each group consists of 6 animals.

**Experimental Protocol**

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| | | |
|---|---|---|
| Group I | : | Normal control rats fed with a normal pellet diet and treated with the vehicle |
| Group II | : | Hyperlipidemic control rats were fed with high-fat diet and treated with the vehicle |
| Group III | : | Hyperlipidemic rats were fed with High Cholesterol cocktail diet and treated with *polyherbal formulation (PHF)* 200 mg/kg |
| Group IV | : | Hyperlipidemic rats were fed with High Cholesterol cocktail diet and treated with *polyherbal formulation (PHF)* 400 mg/kg |
| Group V | : | hyperlipidemic positive control rats were fed with High Cholesterol cocktail diet and treated with Atorvastatin 1.2 mg/kg |

**Treatment Protocol**

All the other groups except Normal Control were administered HCCD followed by Respective treatment. Induction of hypercholesterolemia in rats was confirmed on the $10^{th}$ day by elevated levels of cholesterol in all groups except Normal Rats. The HCCD was discontinued and treatments were given once daily continuously for 28 days, orally. On the $28^{th}$ day, the blood samples were collected by retro-orbital sinus, and the TC, TG, HDL-C, and LDL-C levels were measured using diagnostic kits (ERBA diagnostics Mannheim GmbH, Germany). At the end of the study, the rats were sacrificed, and blood samples were collected for biochemical estimation.

**Evaluation parameters**
**Observational parameter**

Body weight of rat was taken before administration of HCCD and every seven days intervals of experiment and change in body weight, Ponderal Homogeneity Index (iPH) and Ponderal Gain (PG) was calculated (Vikas Kumar K. S.-A., 2018). The ponderal homogeneity index (iPH) and ponderal grain (PG) were calculated by using the following formula

$$iPh = \frac{2BWi}{(BWi + BWh)}$$

$$PG = \frac{(BWf - BWi)}{BWf} X\ 100$$

Where BWi = initial body weight; BWh = highest body weight; BWf = final body weight.

**Biochemical analysis of serum**

The Blood Sugar level, TC, TG, and HDL-C levels were quantified using enzymatic kits. The LDL-Cholesterol level was calculated using the formula of Friedewald et al. LDL-C = TC - (HDLC - TG/5), where TC is the total cholesterol level and TG is the triglycerides level. VLDL= TG/5. The coronary risk index and atherogenic index were calculated using the described formulae (Vikas Kumar P. C.-a., 2016), (Varsha Dhulasavant, 2010), (V.Venkata Rajesham, 2018)

$$\textbf{Atherogenic Index} = \frac{\textbf{Low density lipoprotein cholesteol (LDL)}}{\textbf{High density lipoprotein cholesteol (HDL)}}$$

$$\textbf{Coronary Risk Index} = \frac{\textbf{Total Cholesterol (TC)}}{\textbf{High density lipoprotein cholesteol (HDL)}}$$

**Molecular docking and analysis of ligand-protein interactions**

3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is a rate-controlling enzyme in the biosynthesis of cholesterol and other isoprenoids in the mevalonate pathway. Human HMGR is a single polypeptide that contains 888 amino acids and is divided into three domains: membrane anchor domain (amino acid residues 1–339) is located in the endoplasmic reticulum membrane, catalytic domain (amino acid residues 460–888) is present in the cytoplasm and linker domain (amino acid residues 340–459) connects the membrane anchor domain and the catalytic domain of the protein (Minky Son, 2013). Therefore, Insilco Molecular docking studies of 3-hydroxy-3-methylglutaryl coenzyme A reductase (PBD:1hwk) and analysis of ligand-protein interactions are helpful for the prediction of the mechanism of hypolipidemic activity of important phytoconstituents of the polyherbal formulation.

Molecular docking is one of the foremost used ways in Structure-Based Drug Design Strategies (SBDD) because of its ability to predict, with a considerable degree of accuracy, the conformation of small-molecule ligands inside the

acceptable target binding site. In the present study Molecular docking Studies has allotted to Exploration the mechanism of action and molecular interaction of major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale*, and *S. Cumini* with α-amylase by exploitation PyRx 0.8 Virtual Screening (Fawad Naeem, 2018), (Muhammad Nawaz, 2020), (Leonardo G. Ferreira, 2015). The grid box resolution was adjusted to outline the binding site as Centered at X: 12.8489, Y: 9.8988, Z:6.7761, A grid dimension at X: 15.1651, Y: 18.4252, Z: 12.5556Å.

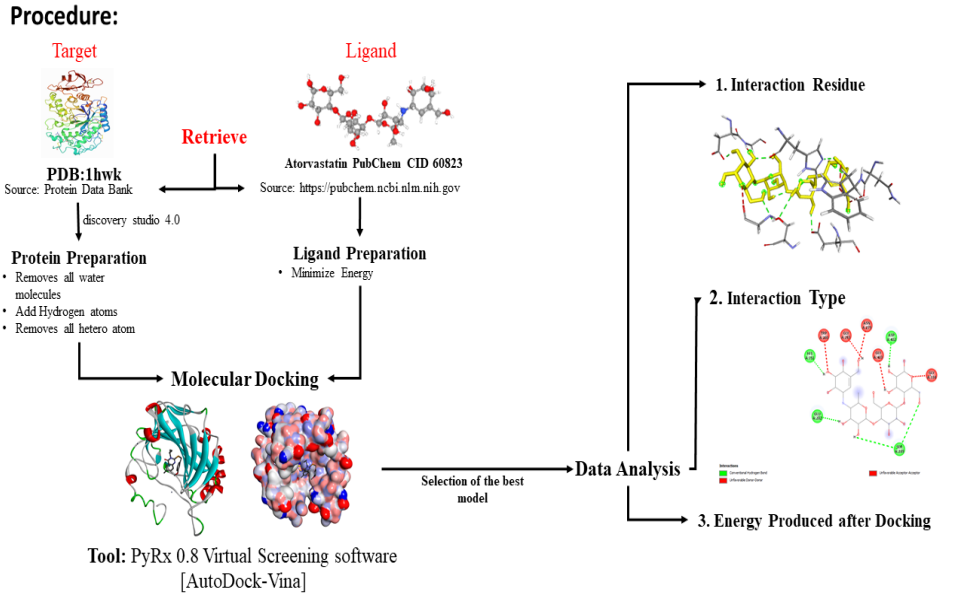


Figure 2.1. Schematic representation of Molecular Docking Studies for HMG CoA (PDB: 1hwk) inhibitory activity

**Statistical analysis**

One-way analysis of variance (ANOVA) followed by the Bonferroni test was carried out and P<0.005 was considered as significant. Hyperlipidemic control group match with Normal control group $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$. Normal control group, a polyherbal formulation, and Atorvastatin treated group matched with Hyperlipidemic control group *p<0.05, **p<0.01, ***p<0.001, ns: not significant.

**Result**

**Evaluation of polyherbal formulation (PHF) on body weight**

The weight change was observed in all groups on the twenty-eighth day after treatment as compared to the initial weight. The Hyperlipidemic control group shows a considerably increased percentage change in weight as compared to the normal control group. Polyherbal formulation treated rats show percentage change weight close to normal rats as shown in table 4.1. The ponderal homogeneity index (iPH) of normal control, polyherbal formulation (200, 400

mg/kg), and atorvastatin showed values of 0.97, 0.96, 0.97, and 0.98 respectively, which were significantly increased as compared to Hyperlipidemic control group rats (0.94). It indicates the body fat mass is reduced by polyherbal formulation concerning normal control group rats (**fig 4.1**). The ponderal grain (PG) was found to be 9.44, 7.59, and 5.77 in polyherbal formulation (200, 400 mg/kg) and atorvastatin respectively. From the results, it indicates that polyherbal formulation 400 mg/kg, and atorvastatin one.5 mg/kg significantly decrease ponderal grain concerning the normal control group (6.25) as compared to the hyperlipidemic control group (12.64).

Table 4.1
Effect of Polyherbal Formulation (PHF) on body weight

| Treatment | Change in body weight (%) | Ponderal homogeneity index (iPH) | Ponderal grain (PG) |
|---|---|---|---|
| Normal Control | 6.85±5.29** | 0.97±0.03** | 6.25±4.46** |
| Hyperlipidemic Control | 14.53±3.11[b] | 0.94±0.02[b] | 12.64±2.34[b] |
| PHF 200 mg/kg | 10.46±2.37ns | 0.96±0.02ns | 9.44±2.34ns |
| PHF 400 mg/kg | 8.22±0.9* | 0.97±0.01* | 7.59±0.77* |
| Atorvastatin 1.5 mg/kg | 6.13±0.93** | 0.98±0.01** | 5.77±0.83** |

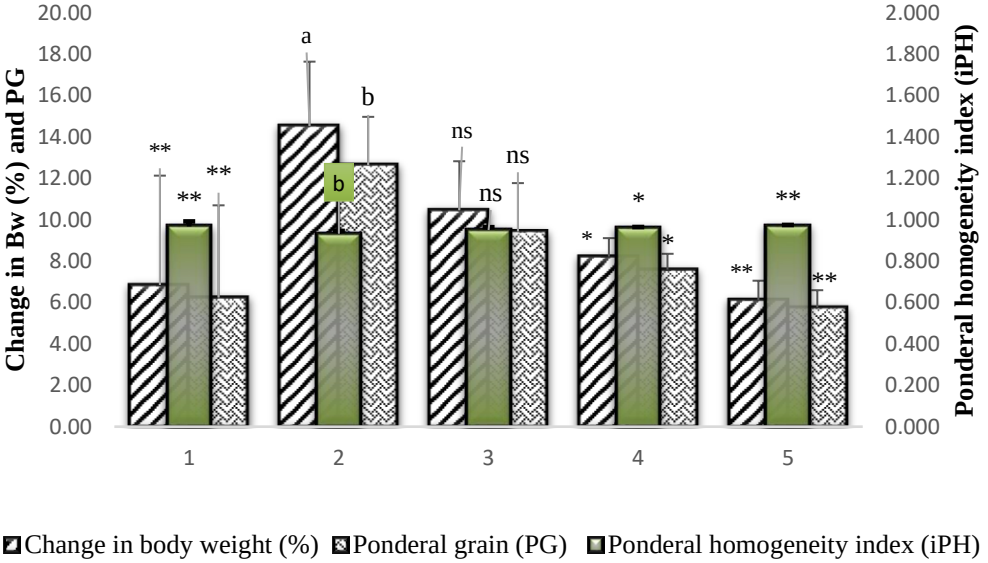


Figure 4.1. Effect of Polyherbal Formulation (PHF) on the percentage change in body weight

**Evaluation of the beneficial effect of polyherbal formulation high cholesterol cocktail diet (HCCD) fed hyperlipidemic rat model**

The result of the serum lipid profile (triglycerides [TG], total-cholesterol [TC], low-density lipoprotein cholesterol [LDLC], very low-density lipoprotein (VLDL-C), and

high-density lipoprotein cholesterol [HDLC]) sowed in (Table 4.2 and 4.3). From the result it shows the TC, TG, LDL-C, VLDL-C was elevated in hyperlipidemic control group rats as compared to normal control group rats, it indicates high cholesterol cocktail diet (HCCD) fed significantly induces hyperlipidemia in rats. In the polyherbal formulation and atorvastatin treated group rats the TC, TG, LDL-C, VLDL-C is significantly decreasing as compared to the hyperlipidemic control group (figure 4.2a, 4.2b,4.3a, 4.3b), however, HDLC was significantly ($P < 0.001$) elevated in PHF 400 mg/kg and atorvastatin treated hyperlipidemic rat groups when compared with the hyperlipidemic control group (figure 4.2c). The results also revealed that the ratio of total cholesterol and HDL cholesterol in polyherbal formulation (200, 400 mg/kg), and atorvastatin is 4.6±0.7, 2.9±0.3, and 2.3±0.3 respectively (figure 4.3c), is reduced as compared to hyperlipidemic control rats (7.5±1.4).

Table 4.2
Effect of Polyherbal Formulation (PHF) on Lipid Profile (TC, TG, HDL-C)

| Treatment | Total Cholesterol (mg/dL) | | Triglyceride (mg/dL) | | HDL-Cholesterol (mg/dL) | |
|---|---|---|---|---|---|---|
| | Initial | On 28th Day | Initial | On 28th Day | Initial | On 28th Day |
| Normal Control | 85±2.59 | 89±2.17*** | 82.75±6 | 89.75±4*** | 44±46.75 | 46.75±0*** |
| Hyperlipidemic Control | 87.25±1.9 | 184.75±4.04$^{c}$ | 85.5±4 | 380±16$^{c}$ | 43±25.25 | 25.25±0$^{c}$ |
| PHF 200 mg/kg | 81±6.79 | 139.25±3.31*** | 84.5±4 | 331±8*** | 42±31.25 | 31.25±0$^{ns}$ |
| PHF 400 mg/kg | 80±3.47 | 120.75±1.71*** | 81.25±4 | 293±6*** | 41.25±42.5 | 42.5±0*** |
| Atorvastatin 1.5 mg/kg | 81.75±2.5 | 110±2.17*** | 80.75±2 | 263.5±6*** | 40.75±49.25 | 49.25±0*** |

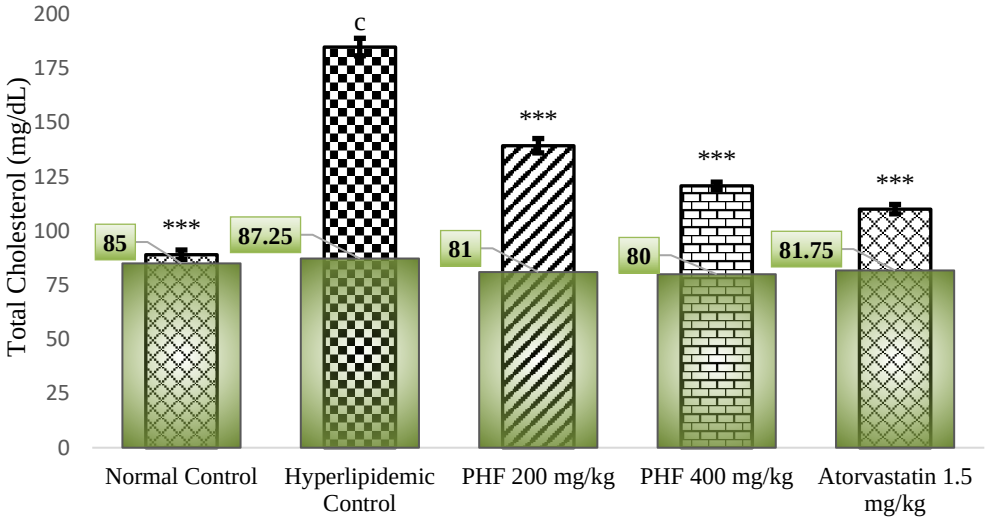


Figure 4.2a. Effect of Polyherbal Formulation (PHF) on Total cholesterol [Initial vs 28$^{th}$ Day]

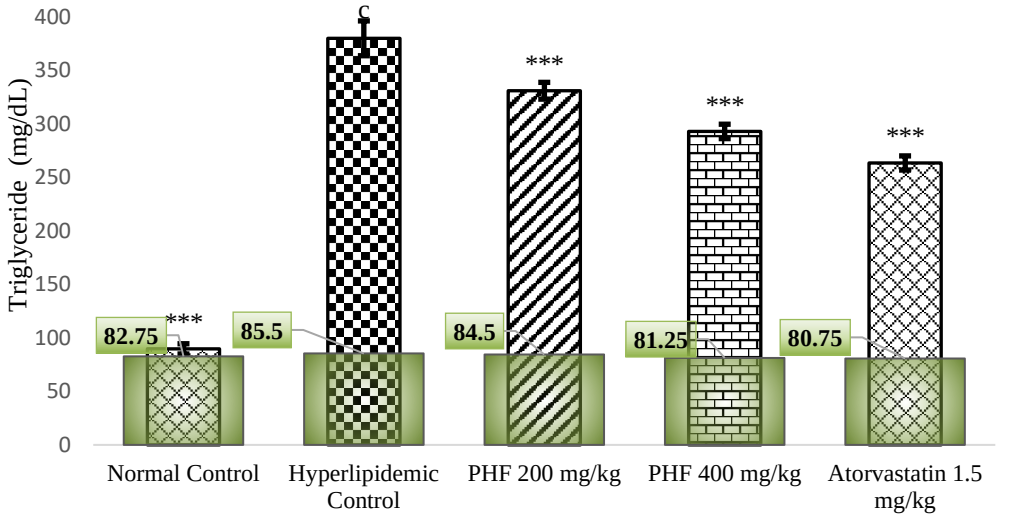


Figure 4.2b. Effect of Polyherbal Formulation (PHF) on Triglyceride [Initial vs 28th Day]

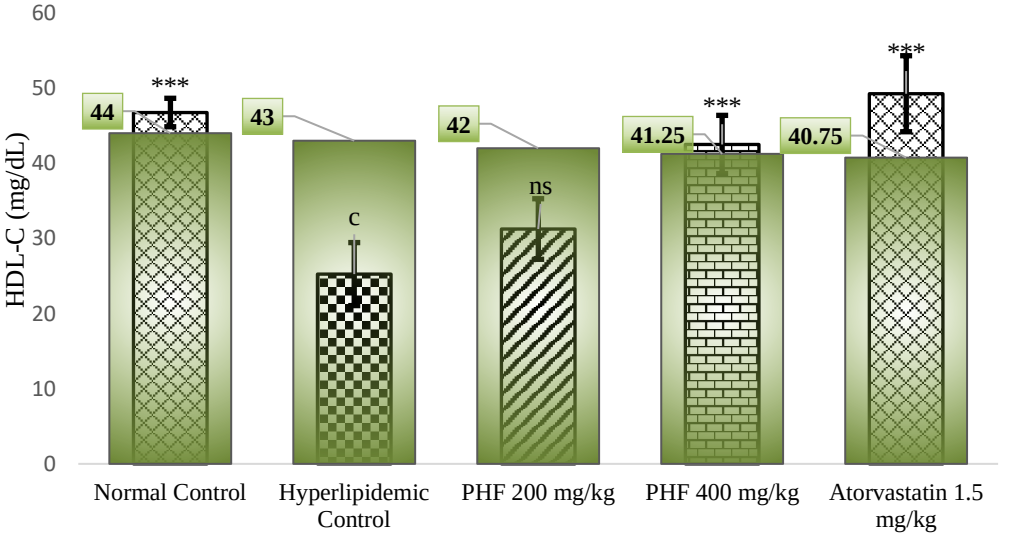


Figure 4.2c. Effect of Polyherbal Formulation (PHF) on HDL-Cholesterol [Initial vs 28th Day]

Table 4.3
Effect of Polyherbal Formulation (PHF) on Lipid Profile (VLDL-C, LDL-C, HDL-C)

| Treatment | VLDL-C(mg/dL) | | LDL-C(mg/dL) | | TC: HDL-C | |
|---|---|---|---|---|---|---|
| | Initial | On 28th Day | Initial | On 28th Day | Initial | On 28th Day |
| Normal Control | 16.55±1.04 | 18±1*** | 57.55±3.47 | 60.2±2.8*** | 1.94±0.15 | 2±0.2*** |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hyperlipidemic Control | 17.1±0.91 | 76±3.3[c] | 61.35±4.08 | 235.5±11.2[c] | 2.04±0.18 | 7.5±1.4[c] |
| PHF 200 mg/kg | 16.9±0.69 | 66.2±1.6*** | 55.9±9.29 | 174.2±7.1*** | 1.95±0.28 | 4.6±0.7*** |
| PHF 400 mg/kg | 16.25±0.67 | 58.6±1.4*** | 55±3.91 | 136.9±5.7*** | 1.95±0.17 | 2.9±0.3*** |
| Atorvastatin 1.5 mg/kg | 16.15±0.45 | 52.7±1.4*** | 57.15±3.86 | 113.5±6.6*** | 2.02±0.16 | 2.3±0.3*** |

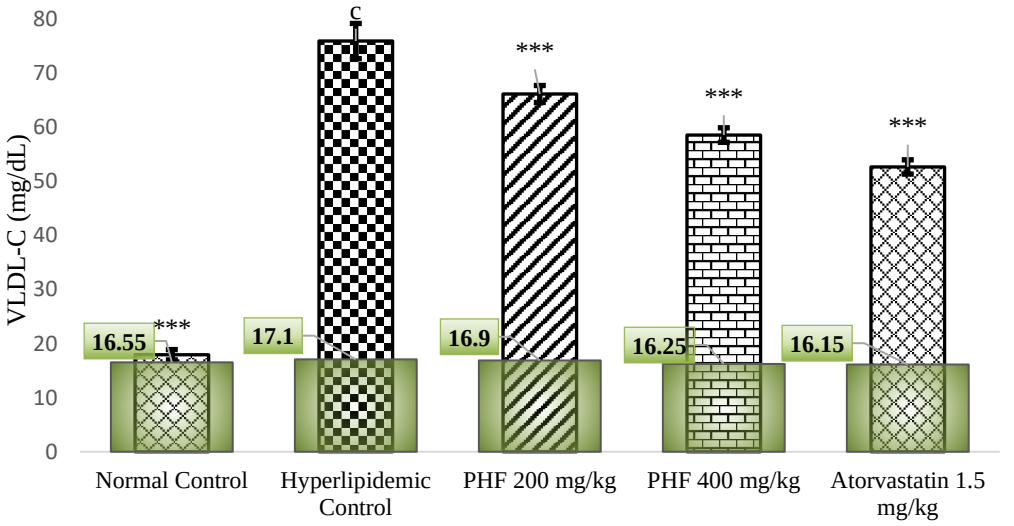


Figure 4.3a. Effect of Polyherbal Formulation (PHF) on VLDL-Cholesterol [Initial vs 28th Day]

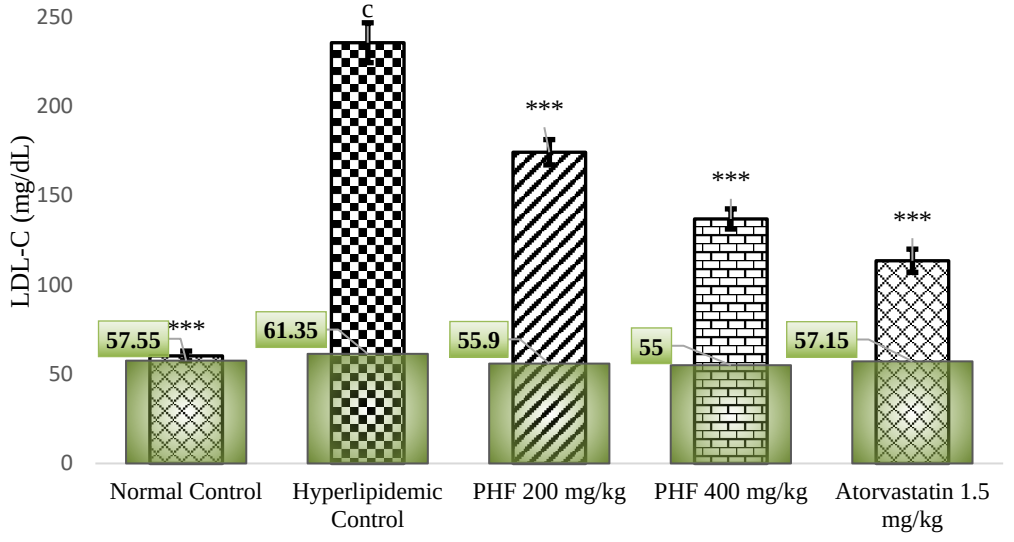


Figure 4.3b. Effect of Polyherbal Formulation (PHF) on LDL-Cholesterol [Initial vs 28th Day]

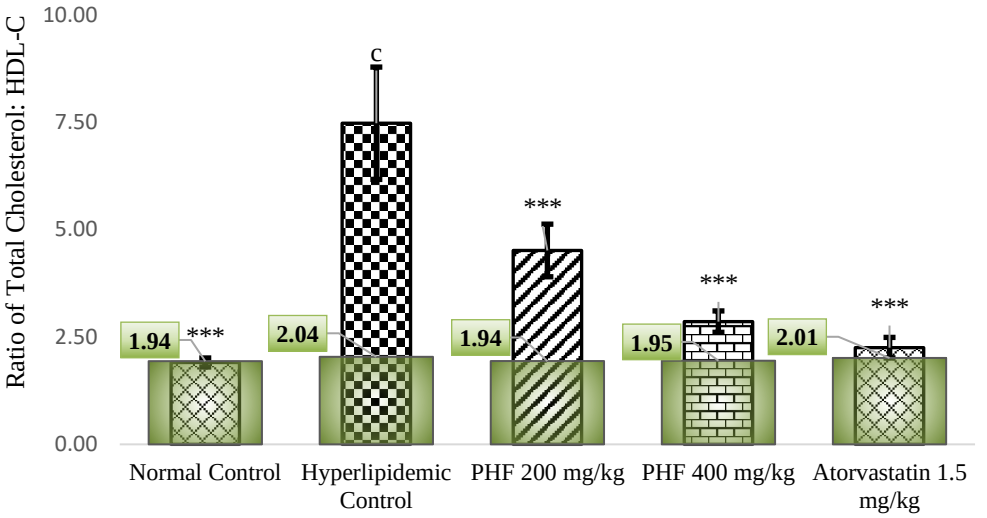


Figure 4.3c. Effect of Polyherbal Formulation (PHF) on Ratio of TC: HDL-Cholesterol [Initial vs 28th Day]

**Evaluation of the effect of Polyherbal Formulation (PHF) on Atherogenic index, Coronary risk index, and blood sugar level (BSL) in high cholesterol cocktail diet (HCCD) fed hyperlipidemic rat model**

Atherogenic index and coronary risk index were calculated in normal control and hyperlipidemic control rats and the results shown in table 4.4 indicates that Polyherbal formulation (200, 400 mg/kg) and Atorvastatin 1.5 mg/kg significantly lowers the atherogenic index 5.26, 3.25, and 2.34 respectively as compared to the hyperlipidemic control group (9.57), however polyherbal formulation (200, 400 mg/kg) and atorvastatin 1.5 mg/kg also significantly lowers the coronary risk index. On the other side polyherbal formulation (200, 400 mg/kg) reduces significantly blood sugar level 137.5 and 128.00 respectively as compared to hyperlipidemic control group rats (29.3.5).

Table 4.4
Effect of Polyherbal Formulation (PHF) on Atherogenic index, Coronary risk index, and blood sugar level (BSL)

| Treatment | Atherogenic index | | Coronary risk index | | BSL (mg/dL) | |
|---|---|---|---|---|---|---|
| | Initial | On 28th Day | Initial | On 28th Day | Initial | On 28th Day |
| Normal Control | 1.32±0.2 | 1.3±0.2*** | 1.94±0.15 | 1.91±0.11*** | 79±3.17 | 82.5±5.9*** |
| Hyperlipidemic Control | 1.44±0.2 | 9.57±2c | 2.04±0.18 | 7.48±1.31c | 78.5±2.65 | 293.5±5.1c |
| PHF 200 mg/kg | 1.35±0.3 | 5.66±0.9*** | 1.95±0.28 | 4.52±0.62*** | 80.25±4.6 | 137.5±6*** |
| PHF 400 mg/kg | 1.35±0.2 | 3.25±0.4*** | 1.95±0.17 | 2.86±0.25*** | 79.5±4.21 | 128±2.6*** |

| | | | | | | |
|---|---|---|---|---|---|---|
| Atorvastatin 1.5 mg/kg | 1.41±0.2 | 2.34±0.4*** | 2.02±0.16 | 2.26±0.24*** | 82.5±4.44 | 168±2.2*** |

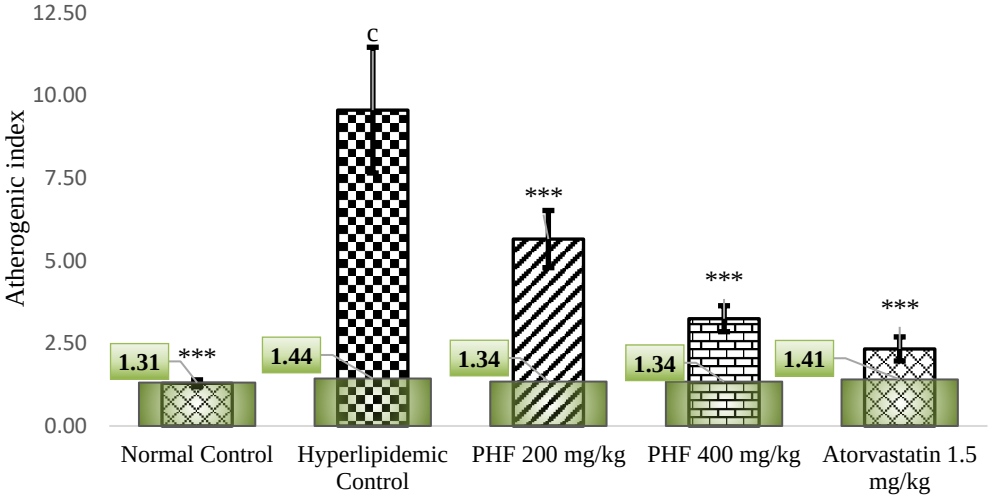


Figure 4.4a. Effect of Polyherbal Formulation (PHF) on Atherogenic index [Initial vs 28th Day]

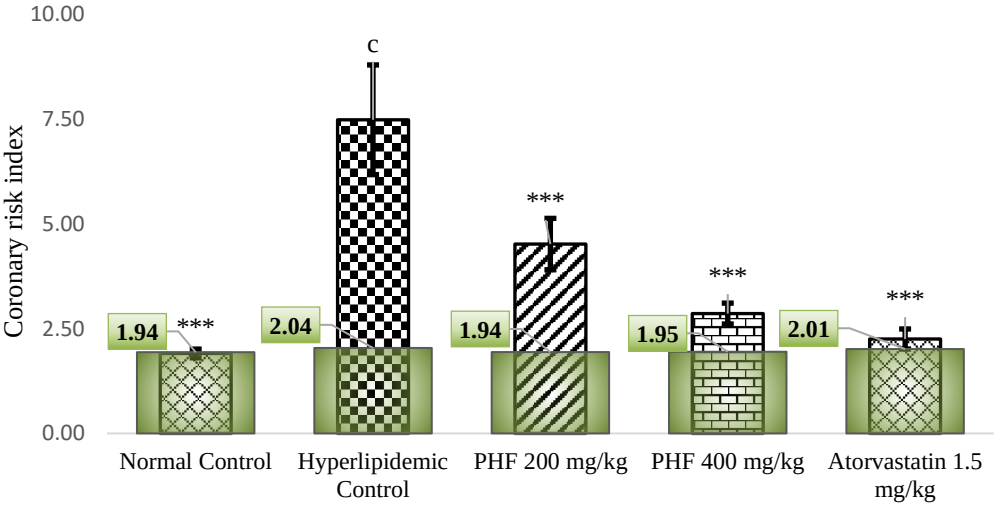


Figure 4.4b. Effect of Polyherbal Formulation (PHF) on Coronary risk index [Initial vs 28th Day]

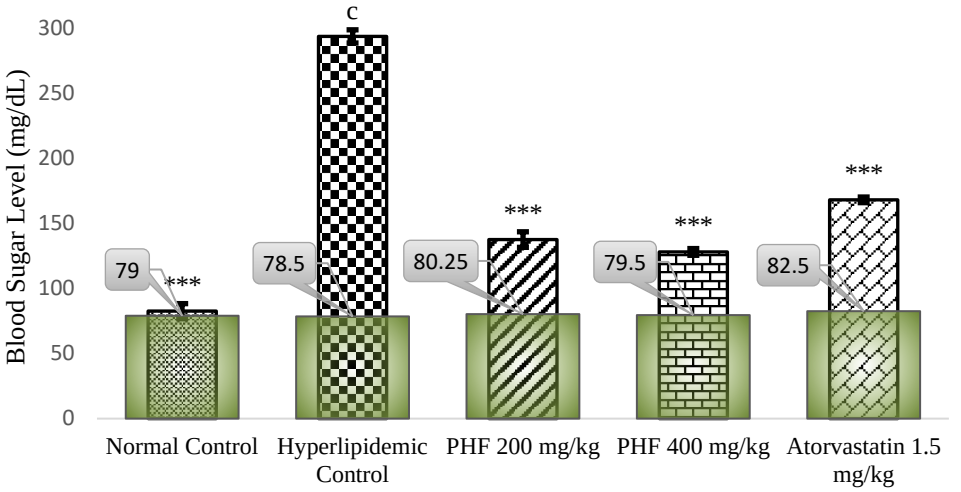


Figure 4.4c. Effect of Polyherbal Formulation (PHF) on Blood sugar level [Initial vs 28th Day]

**Molecular Docking Studies**

In silico screening is an excellent approach for the screening of libraries of compounds in a very short time and therefore it minimizes the arduous work. Molecular docking may be a widely used technique to predict binding interactions between the 3D conformations of assorted ligands and receptor proteins that facilitate improvement and lead toward drug development (Furqan Ahmad Saddique, 2021). In the molecular docking experiment, most of the phytoconstituents of selected plants show binding energy near to standard reference drug atorvastatin with -6 KJ/mol (Table 4.5). Among the selected phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale,* and *S. Cumini* show binding energy Ellagic Acid (-9.8), Rutin (-9.2) have docking scores from -8.1 to -9.8 KJ/mol were ranked the most effective hypolipidemic molecule. The ligands Myricetin (-7.9), Quercetin (-7.5), Kaempferol (-7.4), Gingerenone (-7.2), 6-Shogaol (-7), 6-Gingerol (-6.3), Gallic Acid (-5.9), Alliin (-5) were also considered important due to good binding energy values in the range of having docking scores from -5.0 to -8.0 KJ/mol. The reference compound Atorvastatin formed a hydrogen bond with GLY560, MET655, ASN658, ASN810, Pi-alkyl bond with ALA525, CYS561, and Pi-Sulfur bond with CYS526. The Alliin (-5), Allicin (-4.1), DATS (-3.7), DADDS (-3.6), and DAS (-3.5) the phytoconstituents of *A. Sativum* showed hydrogen bond interactions with MET655, GLY765, VAL805, GLY806, GLY808, Pi-Alkyl bond with MET655, MET657, Ellagic Acid (-9.8), Kaempferol (-7.4), and Gallic Acid (-5.9) the phytoconstituents of *P. Granatum* showed hydrogen bond interactions with MET655, GLY656, MET657, GLY765, ASP767, VAL805, Pi-Anion bond with GLU559, ASP767, Pi-Sigma bond with MET655, GLY807, and Pi-Alkyl bond with MET655, GLY806, Gingerenone (-7.2), 6-Shogaol (-7), and 6-Gingerol (-6.3) the phytoconstituents of *Z. Officinale* showed hydrogen bond interactions with ASN658, GLY806, GLY807, GLY808, Pi-Anion bond with GLU559, Pi-Sigma bond with MET655, and Pi-Alkyl bond with LEU562, ALA654, MET655, MET657, ALA759, and Rutin (-9.2), Myricetin (-7.9), Quercetin (-7.5),

and Ursolic Acid (-2.2) the phytoconstituents of *S. Cumini* showed hydrogen bond interactions with ALA525, THR558, ASN658, ASP690, LYS691, GLY765, VAL805, Pi-Anion bond with GLU559, Pi-Sigma bond with GLY807, Pi-Alkyl bond with ALA654, MET655, MET657 amino acid residue of human 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) respectively.

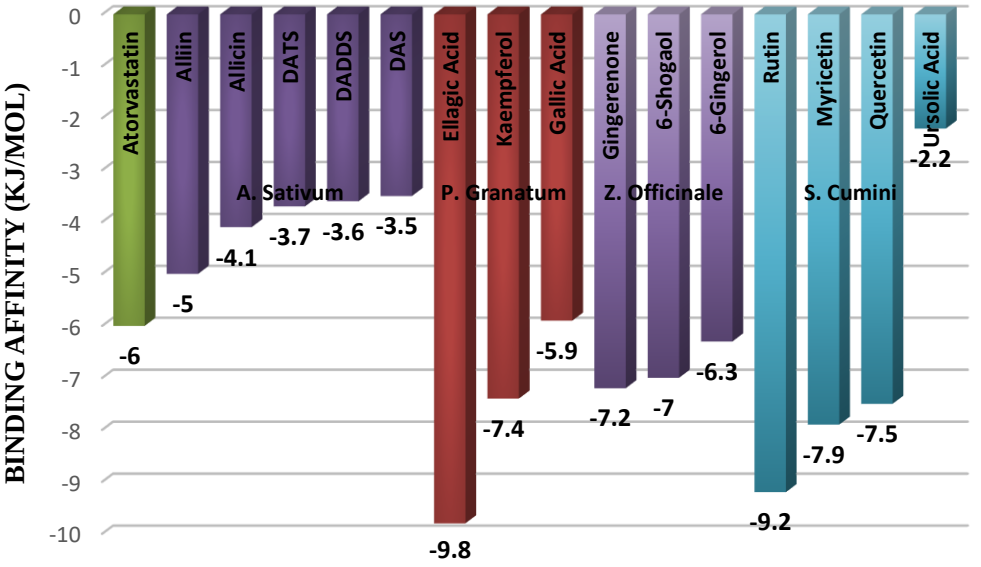


Figure 4.5a. Binding energy of interactions between major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale, and S. Cumini* with hHMG CoA reductase (PDB: 1hwk) inhibitory activity

Table 4.5
Binding energy of interactions between major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale, and S. Cumini* with human HMG CoA reductase (PDB: 1hwk) inhibitory activity

| S. No | Drug | Chem. Constituents | PubChem CID | Binding Affinity (KJ/mol) |
|---|---|---|---|---|
| 1 | Atorvastatin | - | CID_60823 | -6 |
| 2 | *A. Sativum* | Alliin | CID_121922 | -5 |
| | | Allicin | CID_65036 | -4.1 |
| | | DATS | CID_16315 | -3.7 |
| | | DADDS | CID_16590 | -3.6 |
| | | DAS | CID_11617 | -3.5 |
| 3 | *P. Granatum* | Ellagic Acid | CID_5281855 | -9.8 |
| | | Kaempferol | CID_5280863 | -7.4 |
| | | Gallic Acid | CID_370 | -5.9 |
| 4 | *Z. Officinale* | Gingerenone | CID_5281775 | -7.2 |
| | | 6-Shogaol | CID_528179 | -7 |

| | | | | |
|---|---|---|---|---|
| | | 6-Gingerol | 4<br>CID_442793 | -6.3 |
| 5 | *S. Cumini* | Rutin | CID_528080<br>5 | -9.2 |
| | | Myricetin | CID_528167<br>2 | -7.9 |
| | | Quercetin | CID_528034<br>3 | -7.5 |
| | | Ursolic Acid | CID_64945 | -2.2 |

Table 4.6
Binding affinity and binding interactions of Atorvastatin, major phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale, and S. Cumini* with hHMG CoA reductase

| S. No | Ligand | Bond Type | Interacting Amino Acid Residue |
|---|---|---|---|
| 1 | Atorvastatin (-6) | H-bond | GLY560, MET655, ASN658, ASN810 |
| | | Pi-Alkyl | ALA525, CYS561 |
| | | Pi-Sulfur | CYS526 |
| | | CH-Bond | ALA525, CYS561, ASN658 |
| 2 | *A. Sativum* | | |
| | Alliin (-5) | H-bond | VAL805, GLY806, GLY808 |
| | Allicin (-4.1) | H-bond | GLY765 |
| | | Pi-Alkyl | MET655 |
| | DATS (-3.7) | H-bond | GLY808 |
| | | Pi-Alkyl | MET655, MET657 |
| | DADDS (-3.6) | H-bond | MET655 |
| | DAS (-3.5) | Pi-Alkyl | MET655 |
| 3 | *P. Granatum* | | |
| | Ellagic Acid (-9.8) | H-bond | GLY656, MET657, GLY765 |
| | | Pi-Anion | ASP767 |
| | | Pi-Sigma | MET655, GLY807 |
| | | Pi-Alkyl | MET655 |
| | Kaempferol (-7.4) | H-bond | MET655, GLY765, VAL805 |
| | | Pi-Anion | GLU559 |
| | | Pi-Alkyl | MET655, GLY806 |
| | | Amide Stacked | MET655, GLY806 |
| | Gallic Acid (-5.9) | H-bond | GLY765, ASP767 |
| 4 | *Z. Officinale* | | |
| | Gingerenone (-7.2) | Pi-Anion | GLU559 |
| | | Pi-Sigma | MET655 |
| | | Pi-Alkyl | ALA654, MET655, MET657 |
| | | CH-Bond | GLU559 |
| | 6-Shogaol (-7) | H-bond | GLY808 |
| | | Pi-Sigma | MET655 |
| | | Pi-Alkyl | LEU562, ALA759 |
| | | CH-Bond | GLN766 |
| | 6-Gingerol (-6.3) | H-bond | ASN658, GLY806, GLY807, GLY808 |

| | | | |
|---|---|---|---|
| | | Pi-Anion | GLU559 |
| | | Pi-Alkyl | MET655 |
| | *S. Cumini* | | |
| | Rutin (-9.2) | H-bond | ALA525, THR558, ASN658, ASP690, LYS691, GLY765 |
| | | Pi-Anion | GLU559 |
| | | Pi-Alkyl | ALA654, MET657 |
| | | CH-Bond | GLU559, ASN658, ASP767, GLY807 |
| 5 | Myricetin (-7.9) | H-bond | ASN658, ASP690, GLY765, VAL805 |
| | | Pi-Anion | GLU559 |
| | | Pi-Sigma | GLY807 |
| | | Pi-Alkyl | MET655, MET657 |
| | Quercetin (-7.5) | H-bond | THR558, ASN658, LYS691, GLY765 |
| | | Pi-Anion | GLU559 |
| | | Pi-Sigma | GLY807 |
| | | Pi-Alkyl | MET655, MET657 |
| | Ursolic Acid (-2.2) | H-bond | ASN658 |

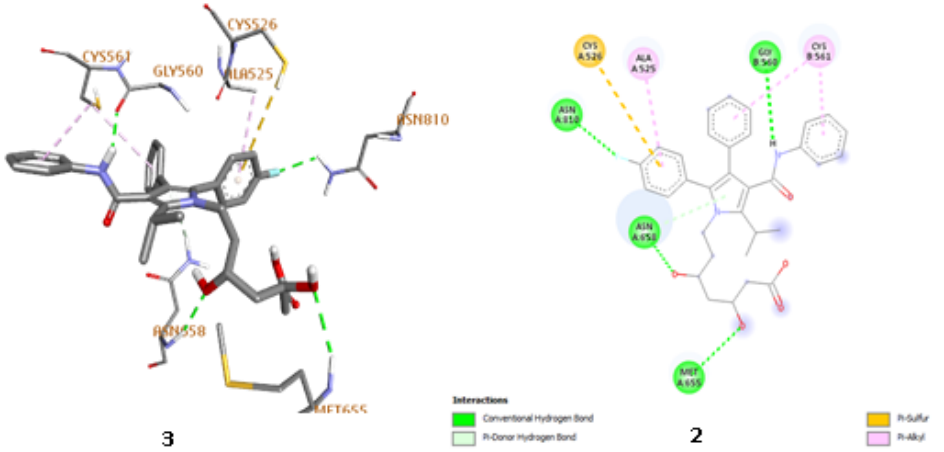


Figure 4.6a. Binding cavity of hHMG CoA Reductase with Atorvastatin (Binding Affinity (KJ/mol) -6.0)

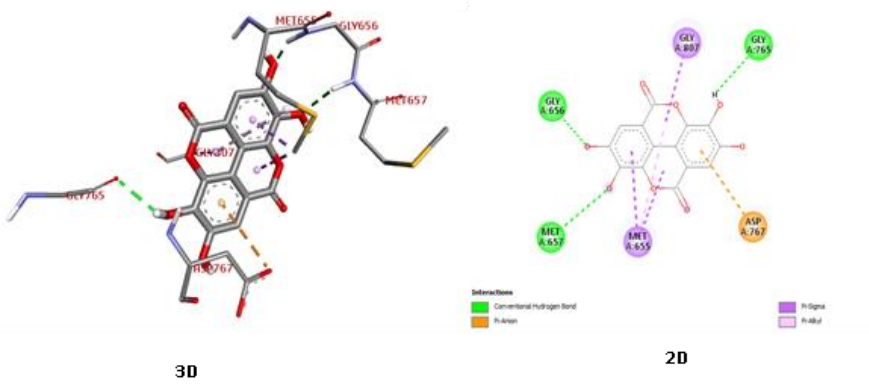


Figure 4.6b. Binding cavity of hHMG CoA Reductase with Ellagic acid (Binding Affinity (KJ/mol) -9.8)

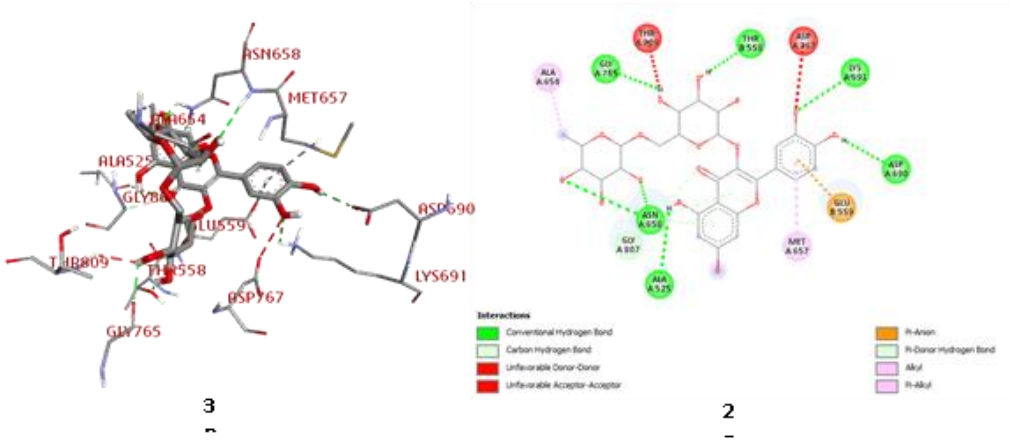


Figure 4.6c. Binding cavity of hHMG CoA Reductase with Rutin (Binding Affinity (KJ/mol) -9.2)

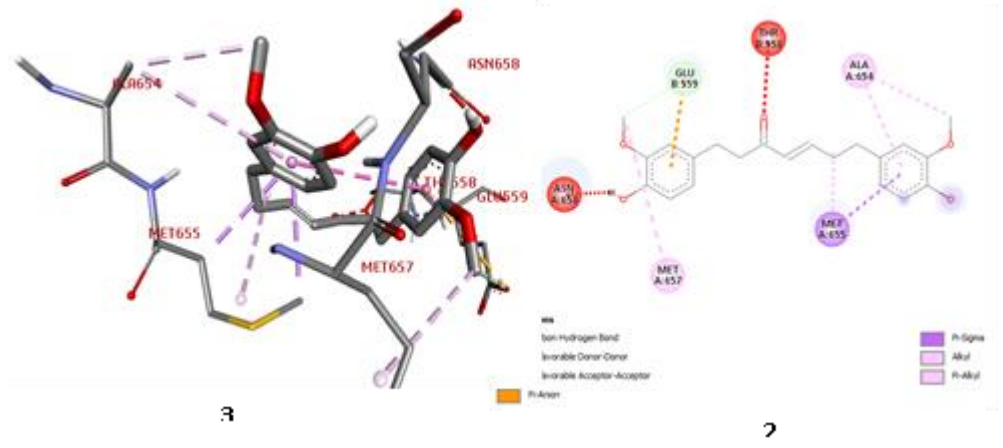


Figure 4.6d. Binding cavity of hHMG CoA Reductase with Gingerone (Binding Affinity (KJ/mol) -7.4)

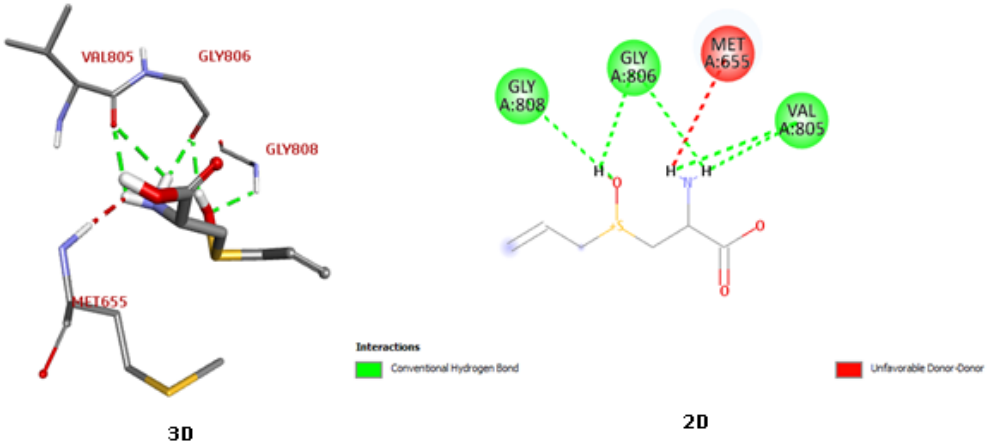


Figure 4.6e. Binding cavity of hHMG CoA Reductase with Alliin (Binding Affinity (KJ/mol) -5)

**Discussion**

Obesity is related to various comorbidities like CVD, sort a pair of diabetes, high blood pressure, certain cancers, and sleep disorder. obesity is an associate degree freelance risk issue for CVD and CVD risks are documented in obese youngsters (Paul Poirier, 2006). Obesity conjointly contributes to the burden of disease within the community, thanks to its association with many chronic conditions like hyperlipidemia, non-insulin-dependent diabetes, high blood pressure, and coronary artery disease. obesity has become one of the leading preventable causes of mortality, through the vision of public health (G Dinç, 2006). In the present study, the polyherbal formulation shows improvement in the Ponderal homogeneity index and Ponderal grain in high cholesterol cocktail diet-fed hyperlipidemic rat model, which indicates that the polyherbal formulation has been helped to regulate weight in obesity. The polyherbal formulation to control shows a major reduction in total cholesterol, lipoid, LDL-cholesterol, VLDL-cholesterol and elevates the helpful lipid-like HDL-cholesterol, it indicates that polyherbal formulation has helpful effects to lowers the chance of obesity is related to cardiovascular disease, type 2 diabetes, hypertension, certain cancers, etc. it is also found that the polyherbal formulation considerably lowers the blood sugar level, that indicates that the polyherbal formulation has helpful effects within the population have a diabetes-related metabolic disorder, where obesity is a significant risk factor.

The atherogenic index of plasma could be a novel index composed of triglycerides and high-density lipoprotein cholesterol. it has been accustomed quantify blood lipid levels associate degreed is usually used as an optimum indicator of dyslipidemia and associated diseases (e.g., vas diseases) (Xiaowei Zhu, 2018). It is defined as logarithm [log] of the quantitative relation of plasma concentration of TG to HDL-C and is powerfully related to CVD risks. The atherogenic index calculation estimates the values of the “zone of atherogenic risk” (Myat Su Bo, 2018). The atherogenic indices are thought of as a helpful predictor for the hardening of the arteries and CV diseases in stable patients with chronic

preventive pneumonic diseases (Tuba Tulay Koca, 2019). Here the polyherbal formulation considerably lowers the atherogenic index and coronary index as compared to hyperlipidemic control groups, it indicates the polyherbal formulation conjointly lowers the risk of cardiovascular disease and atherosclerosis associated with obesity.

In silico studies, the ligand-protein interaction analysis revealed that Ellagic Acid, Rutin, Myricetin, Quercetin, Kaempferol, Gingerenone, 6-Shogaol, 6-Gingerol, Gallic Acid, and Alliin molecules shows binding energy near to reference drug atorvastatin and occupy similar binding sites as the normal substrate (HMG-CoA) in the catalytic domain and may form hydrogen bonds, electrostatic interactions, and hydrophobic interactions with the residues at the binding site of hHMGR (Figure 4.6a-e). However, *in-vitro* study needs further investigation to know the exact mechanism of polyherbal formulation for its use in the treatment of obesity-associated with numerous comorbidities.

## Conclusion

The hyperlipidemia was induced in Wistar rats by high cholesterol cocktail diet (HCCD) fed. The selected animals were then administered with polyherbal formulation (200, 400mg/kg, body weight, p. o) and atorvastatin (1.5 mg/kg, body weight, p.o) for 28 days. The animals were then sacrificed after 28 days, and the blood was collected for various biochemical estimations. Hyperlipidemic control rats exhibited elevated serum glucose, serum cholesterol, serum triglycerides, and decreased serum HDL-cholesterol, whereas the extract administered rats exhibited decreased serum glucose, serum cholesterol, serum triglycerides, and increased serum HDL-cholesterol. Also, in Insilco studies the phytoconstituents of *A. Sativum, P. Granatum, Z. Officinale* and *S. Cumini* like Ellagic Acid, Rutin, Myricetin, Quercetin, Kaempferol, Gingerenone, 6-Shogaol, 6-Gingerol, Gallic Acid, and Alliin molecules shows human HMG coenzyme A reductase (PBD:1hwk) inhibitory activity. Hence this polyherbal formulation proves beneficial effects for the treatment of obesity-associated with numerous comorbidities.

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