

**ANTIHYPERLIPIDEMIC STUDIES OF METHANOLIC EXTRACT OF
GOSSYPIUM HERBACEUM: AN *IN SILICO* AND *IN VIVO* APPROACH**

ABSTRACT

Aim: To evaluate anti-hyperlipidemic activity of the whole plant of *Gossypium herbaceum* extract in Wistar Albino Rats.

Methods: The whole plant of *Gossypium herbaceum* were collected and extracted with methanol by soxhlation. It was tested in Triton and Propylthiouracil (PTU) induced hyperlipidemic rat models and antioxidant hydrogen peroxide radical scavenging assay.

Results: Basic phytochemical tests resulted in the presence of flavonoids, terpenoids, steroids, phytosterols, carbohydrates, alkaloids, tannins and phenolic compounds. In rat models of hyperlipidemia induced by Triton and Propylthiouracil (PTU), the anti-hyperlipidemic effect of a methanolic extract of the whole plant of *Gossypium herbaceum* was studied. MEGH (200 and 400 mg/kg, p.o.) treatment greatly decreased the enhanced serum lipids, restored the decreased HDL compared to disease group. Histopathological examinations showed recovery of the damaged liver cells in Propylthiouracil treated group. The extract's capacity to scavenge free radicals caused by hydrogen peroxide was also measured. Ascorbic acid served as the reference. The result demonstrates that the *Gossypium herbaceum* whole plant's methanolic extract has substantial antioxidant and antihyperlipidemic properties. Docking simulation was done to PDB protein of Lecithin cholesterol acyltransferase, HMG-CoA reductase inhibitor and Antioxidant and viewed in discover studio followed by Ramachandran plot.

Conclusion: Methanolic extract of *Gossypium herbaceum* can be used for management of hyperlipidemia and possess antioxidant activities.

Key words: Triton-X-100, Propylthiouracil, hyperlipidemia, antioxidant, *Gossypium herbaceum*, whole plant, rats, Atrovastatin

1. INTRODUCTION

In Western countries and Asia, coronary heart disorders (CHD) constitute the leading cause of death. Ischemic heart disease (IHD) has the highest mortality rate among CHDs [1]. The word "hyperlipidemia" refers to unusually high amounts of lipids in the blood, including triglycerides, lipoproteins, and cholesterol [2]. Currently, approximately 3 million persons in the United States and Europe have been diagnosed with hyperlipidemia, and that number is rapidly increasing. Usually a chronic, progressive illness, hyperlipidaemia necessitates dietary and lifestyle modifications as well as the potential need for additional lipid-lowering drugs. Hyperlipidaemia condition enhances the formation of ROS is of pivotal pathogenetic importance. Propyl thiouracil and Triton X 100 are used in induction of hyperlipidaemia and Atorvastatin and ascorbic acid serves as standard in our present study. Natural cures are now often regarded as inferior or something that people utilise when they can't afford modern medicine because of the way the healthcare system is organized.

Gossypium herbaceum is a bushy shrub that grows height of 2-8 feet, with few branches; stem thick and rigid belongs to family *Malvaceae*. *Gossypium herbaceum* originated in southern Africa but was domesticated there first. From there, cultivated versions of the plant spread to Africa and India in the west and east, respectively [3]. The different parts of *Gossypium herbaceum* are used for anticonvulsant, toxicity studies, hypoglycemic, hypolipidemic activity, antidepressant, antidiabetic activity etc. The aim of our study was to Our study's objectives were to conduct *in-silico* research and evaluate the anti-hyperlipidemic and antioxidant activity of the whole-plant extract of *Gossypium herbaceum* in Wistar albino rats.

2. MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to evaluate anti-hyperlipidemic activity of the whole plant extract of *Gossypium herbaceum* in Wistar Albino Rats

2.1 Plant collection, drying & pulverization:

All parts of *Gossypium herbaceum* was collected and identified. The crude plant material is authenticated by P. Suresh babu, Govt Degree College, Hyderabad, Telangana. The freshly collected parts of the plant were cleared from dirt and then dried under shade for about 15 days and coarsely powdered in a mixer grinder. The powdered material was stored or taken up for extraction process.

2.2 Preparation of *Gossypium herbaceum* extract

Soxhlet extraction is the process of continuous extraction in which the methanol solvent can be circulated through the extractor for several times. This process involves extraction followed by evaporation of the solvent. The vapours of the solvent are taken to a condenser and the condensed liquid is returned to the drug for continuous extraction

2.3 Experimental animals

Adult wistar albino rats (180 –200 g) were procured from Albino Labs Hyderabad and used for the pharmacological activities. They were kept in polypropylene cages at $25 \pm 2^\circ$ C, with relative humidity 45-55% under 12h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were feed with standard animal feed and water ad libitum.

2.4 Preliminary phytochemical screening

The Methanolic extract of *Gossypium herbaceum* was subjected to preliminary phytochemical screening to identify various phytoconstituents present in *Gossypium herbaceum*

2.5 Acute toxicity testing

Acute toxicity study was carried out in order to check the toxic effects for methanolic extract of *Gossypium herbaceum*. The study was performed as per Organization for Economic Cooperation and Development (OECD). The method is used to evaluate the acute oral toxicity which is up and down procedure (OECD guideline-425). Up and down procedure (OECD guideline-425) acute toxicity studies were carried out as per the OECD 425 guidelines.

2.6 *In-vitro* Anti-oxidant assay

2.6.1 H₂O₂ radical scavenging activity

“Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H₂O₂ can probably react with Fe⁺² and possibly Cu⁺² ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate” [4].

Procedure: “A solution of hydrogen peroxide (2mmol/L) was prepared in phosphate buffer (pH 7.4). Test compounds (10-50 µg/mL) were added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a

blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound

$$\% \text{H}_2\text{O}_2 = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where ‘Abs (control)’ is absorbance of control ‘Abs (sample)’ is absorbance of extract/standard” [5].

2.7 In-vivo Anti hyperlipidaemic activity

2.7.1 Propylthiouracil induced hyperlipidaemia

The proposed method of induction with PTU per oral 10 mg kg⁻¹ bd.wt followed by induction of cholesterol solution in vegetable oil of 400 mg/kg bd.wt dose produced hyperlipidaemia animal models that have cholesterol metabolism disruption. Adult Wistar albino rats were administered with corresponding treatments for 8 days. They were divided into 5 groups with 6 animals per group. Study design of High fat diet induced hyperlipidaemia method, Group –I serves as Control (Normal saline). Group II (disease control), III, IV and V received PTU and cholesterol powder along with respective treatment, Group –III received methanolic extract of *Gossypium herbaceum* (100mg/kg, *p.o*) and Group – IV received *Gossypium herbaceum* (200mg/kg, *p.o*) and atorvastatin (100mg/kg, *p.o*).

“Animals were orally induced with propylthiouracil of 10 mg/kg bd.wt. dosage and 0.01% PTU in drinking water for 7 days. On day 8 test drugs were given to animals orally. One hour after test drugs administration, animals were given a solution of high dosage cholesterol in vegetable oil of 400 mg/kg bd.wt. Serum total cholesterol level was measured in every 1 h after administration of cholesterol for 6 h. After 6 h, a level of total cholesterol in the liver and faeces were measured. This method is simpler and requires less time to get hyperlipidaemia animal model. Serial measurement of serum total cholesterol level in every hour for 6 h gave a cholesterol profile that can explain different drug mechanisms in cholesterol homeostasis” [6].

2.7.2 Triton induced hyperlipidaemic rat model

“Hyperlipidaemia was induced in Wistar albino rats by single *intra peritoneal* injection of freshly prepared solution of Triton X-100 (100 mg/kg bd.wt) in physiological saline solution after overnight fasting for 18 h. The animals were divided into four groups of containing five rats each group. Group I was given standard pellet diet, water (1% acacia). Group II was given a single dose of Triton X-100 administered at a dose of 100 mg/kg, bd.wt, *i.p*. After 48 hrs of Triton injection, this group received a daily dose of 1% acacia (*p.o*) for 7 days. Group III and Group IV was administered METP at doses 200 mg/kg bd.wt and 400 mg/kg bd.wt for 7 days. Group V was administered standard atorvastatin 10 mg/kg bd.wt, *i.p*

for 7 days. On the blood was collected by retro orbital sinus puncture. The collected samples were centrifuged for 10mins. Then serum samples were collected and used for estimation of various biochemical experiments” [7].

2.8 *In silico* analysis

2.8.1 Molecular docking

“The mechanism of binding of drug with the target protein is called docking. Docking can be used to find inhibitors for specific target proteins and thus to design new stable drugs from docking results. Docking can be calculated by binding energy (energy release during protein and ligand interaction). In this project, mCule software was used for docking” [8].

2.8.2 Structure based drug design

Initially the protein downloaded from PDB was prepared by selecting any one chain. Water molecules present in the chains are removed. Attributes selected. Later docking was performed by mCule online software *i.e* protein-ligand docking performed for protein 6MVD, 3CCZ and 1URM.

2.8.3 mCule docking results

Docking indicates that some of our compounds have good binding ability with LCAT activator (PDB ID: 6MVD), HMG-CoA reductase inhibitor (PDB ID: 3CCZ) and Antioxidant (PDB: 1URM)

2.8.4 Ramachandran plot

“Ramachandran plot has been generated from PROCHECK validation server which was used to access the quality of the model by looking into the allowed and disallowed regions of the plot” [9].

2.9 Statistical analyses

The Results were expressed as the $m \pm S.E.M$. The significance of the results was calculated using ANOVA and Dunnett’s test and results were deliberated statistically noteworthy when significant $p < 0.0001$, $p < 0.001$, $p < 0.01$, ns- non significant.

3. RESULTS

Animal models were used to test the anti-hyperlipidemic effects of a methanolic extract of *Gossypium herbaceum*, and *in-vitro* antioxidant assays were used to check its antioxidant capacity. Below is a list of every result that this study's findings produced.

3.1 Preparation of methanolic extract of *Gossypium herbaceum*

By using the soxhlation method, a methanolic extract of *Gossypium herbaceum* was prepared. About 7.3% w/w of the extract's yield as a percentage was obtained.

3.2 Preliminary phytochemical analysis

The initial phytochemical screening of the methanolic extract of *Gossypium herbaceum* revealed the presence of proteins, alkaloids, steroids, terpenoids, tannins and phenolic compounds, and flavonoids.

3.3 Acute toxicity studies

The following limit test was chosen for the current toxicity study: *Gossypium herbaceum* methanolic extract was tested on female mice at a dose of 2000 mg/kg bd.wt. *p.o.* *Gossypium herbaceum* methanolic extract did not show any indicators of toxicity and death even up to 2000 mg/kg bd.wt. Even after 14 days of surveillance, all animals were secure. Accordingly, the working dose was determined to be 1/20th, or 100 mg/kg bd.wt, based on limit experiments.

3.4 *In vitro* Antioxidant assay

3.4.1 Hydrogen peroxide radical scavenging activity

The IC₅₀ values for the MEGH and the reference medication ascorbic acid in the hydrogen peroxide radical scavenging assay were 32 and 38 µg/mL, respectively. It is obvious from this data that MEGH had strong antioxidant properties.

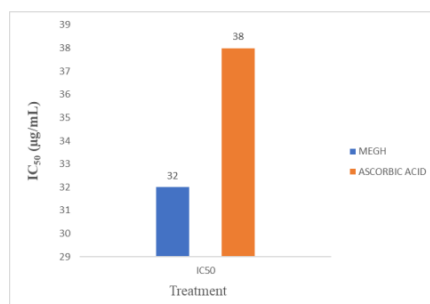


Figure 1: Hydrogen peroxide radical assay of MEGH and Ascorbic acid

Almost every molecule present in a living cell is susceptible to damage by the highly reactive free radical hydrogen peroxide, which is generated in the biological system. The radicals have the ability to combine with the DNA's nucleoside and break the strand. Additionally, because these species extract hydrogen from unsaturated fatty acids, they are thought to be quick lipid peroxidation initiators. The ability of MEGH to scavenge ROS and quench hydroxyl radicals appears to be directly related to preventing the spread of the lipid peroxidation process. The presence of phenolic, flavonoids in MEGH may be the cause of its lowering power activity.

3.5 *In vivo* Anti hyperlipidemic activity

In both Triton- and Propylthiouracil-induced hyperlipidemic rat models, the antihyperlipidemic efficacy of a methanolic extract of the herb *Gossypium herbaceum* was investigated. Below are all of the outcomes of this research.

3.5.1 Propylthiouracil induced hyperlipidaemic rat model

Table 1: Anti-hyperlipidaemic activity for methanolic extract of *Gossypium herbaceum* on Propylthiouracil induced hyperlipidaemic rat model

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal	98.1 ±	90.5 ±	37.7 ±	90.2 ±	22.5 ±
Control	4.14	6.96	1.64	1.56	0.79
Disease	98.1 ±	236.5 ±	17.4 ±	156 ±	70.4 ±
Control	4.14	13.5**	0.52**	2.77**	2.89**
MEGH (100 mg/kg)	127.1 ±	193.3 ±	22.1 ±	131.5 ±	50.8 ±
	3.75**Bb	4.64**Ab	0.68**Ab	3.45**Bb	1.78**Bb
MEGH (200 mg/kg)	127.1 ±	165.5 ±	25.2 ±	121.6 ±	42.7 ±
	3.75**Bb	1.77**Ba	0.69**Aa	1.72**Bb	0.95**Ba
Atorvastatin (10 mg/kg)	77.7 ±	126.7 ±	30.83 ±	102.1 ±	34.2 ±
	0.86**B	3.45**B	1.7**B	2.28**B	0.83**B

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. (* = p<0.0005, ** = p <0.0001) when compared to control group, (A = p<0.0001, B= p<0.001), when compared to disease control group, (a =p<0.0001, b =p<0.001) when compared to standard group.

The propylthiouracil induced hyperlipidaemic rat model showed significant increase in serum biochemical parameters like TC, TG, LDL, VLDL levels and decrease in serum HDL when compared to normal control group. The MEGH treated group at 100 mg/kg showed significant decrease in TC, TG, LDL, VLDL and increase in HDL when compared to disease control group. The MEGH treated group at 200 mg/kg decreased TC, TG, LDL, VLDL and increased HDL. The standard group showed significant decrease in TC, TG, LDL, VLDL and increase in HDL.

3.5.2 Histopathology of Liver

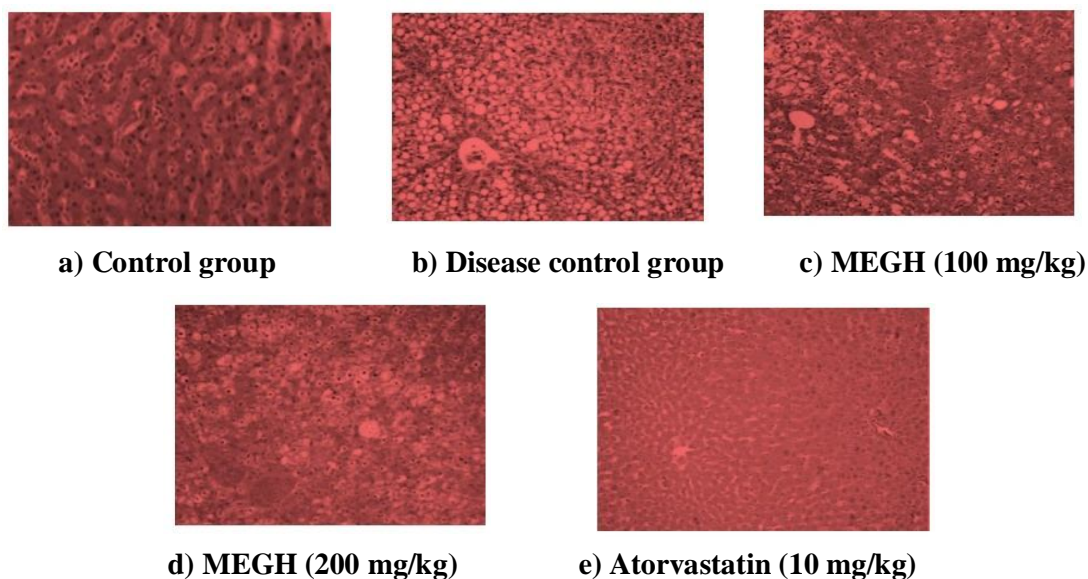


Fig 2: Histopathology of liver in Propylthiouracil induced hyperlipidemic rat model

The control group displayed the criteria listed below, including a bile duct that appeared normal and no fibrosis or inflammation around the liver's portal region. Both sinusoids and kupffer cells are healthy. No signs of fibrosis or fatty alteration. Hepatocytes in the hyperlipemic group disease control displayed the Cord pattern. In the liver's periportal region, there are only a few periportal lymphocytes in a localised area of fibrosis. Cytoplasmic fatty alteration and fibrosis are both present. Rat liver histopathology after MEGH 100 mg/kg treatment showed considerable sinusoidal space dilatation, hepatic haemorrhages, a small number of periportal lymphocytes in a localised location, and Hepatocytes in the rat liver treated with MEGH 200 mg/kg had a mild cord pattern, according to histopathology mild haemorrhage and sinusoidal space dilatation. The kupffer cells are healthy. Rat liver histopathology following atorvastatin 10 mg/kg treatment revealed. There was no sign of fat deposition in the sinusoids, Kupffer cells, or hepatocytes, which all appeared normal.

3.5.3 Triton X-100 induced hyperlipidemic rat model

Table 2: Anti-hyperlipidemic activity for methanolic extract of Gossypium herbaceum on Triton X-100 induced hyperlipidemic rat model

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal	178.2±	124.5±2.4	56±3.01	98±0.30	24.6±2.18

Control	1.84				
Disease	291 ±	241.3 ±	15.9 ±	177 ±	72.63 ±
Control	2.91*	6.24*	1.2*	4.65*	3.21*
MEGH (100 mg/kg)	260.3 ±	193.7 ±	25.08 ±	121.8 ±	58.5 ±
	3.03*Ab	1.24*Ab	0.45*Ab	3.63*Ab	1.57*Ab
MEGH (200 mg/kg)	250.2 ±	169.2 ±	28.08 ±	112.9 ±	55 ±
	1.24*Ab	4.06*Aa	0.87*Ab	2.95**Ab	1.43*A
Atorvastatin (10 mg/kg)	220.98 ±	149.6 ±	40.3 ±	73.2 ±	38.3 ±
	2.7*A	1.39**A	0.73*A	1.53*A	3.27**A

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. (* = p < 0.0001, ** = p < 0.0005) when compared to control group, (A = P = 0.001) when compared to disease control group, (a = P = 0.0005, b = P = 0.0001) when compared to standard group.

The Triton X-100 induced hyperlipidemic rat model showed significant increase in serum biochemical parameters like TC, LDL, VLDL levels and decrease in HDL level when compared to normal control group. The MEGH treated group at 100 mg/kg showed significant decrease in TC, TG, LDL, VLDL and increase in HDL when compared to disease control group. The MEGH treated group at 200 mg/kg decreased TC, TG, LDL, VLDL and increased HDL. The standard group showed significant decrease in TC, TG, LDL, VLDL and increase in HDL.

3.6 *In silico* analysis

3.6.1 Molecular docking

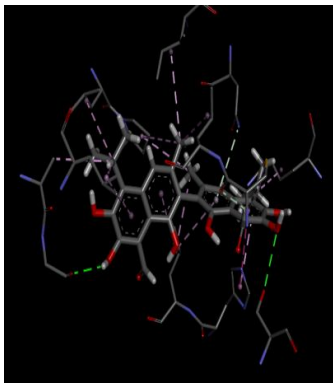
Table 3: Docking score of chemical constituents and amlodipine with protein 6MVD, 3CCZ, 1URM.

Compounds	6MVD	3CCZ	1URM
Cyanidin	-6.9	-7.5	-5.6
Delphinidin	-7.1	-6.7	-5.5
Flavon-3-ol	-7.7	-6.8	-6.0
Catechin	-7.3	-7.4	-5.6
Gallocatechin	-7.4	-6.5	-5.4
Epicatechin	-7.3	-7.4	-5.6

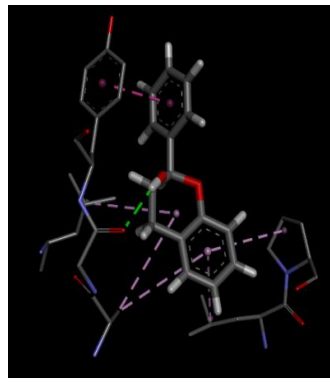
Epigallocatechin	-7.4	-6.5	-5.5
Gossypol	-8.1	-6.8	-6.0
Lactic acid	-4.1	-3.5	-3.1
Palmitic acid	-5.6	-4.4	-3.7
Stearic acid	-5.9	-4.0	-3.4
Atorvastatin	-4.7	-6.9	-5.5

G score = glide score, the more negative the Glide score, the more favourable the binding.

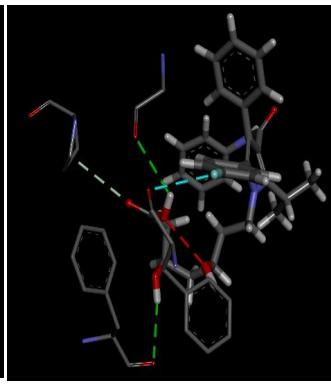
1) 6MVD



a) Gossypol -8.1

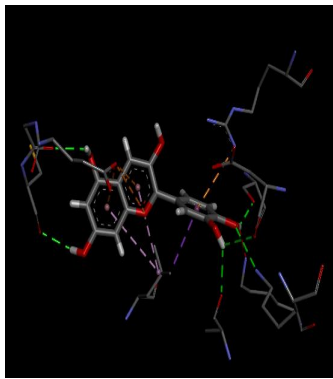


b) Flavon-3-ol -7.7

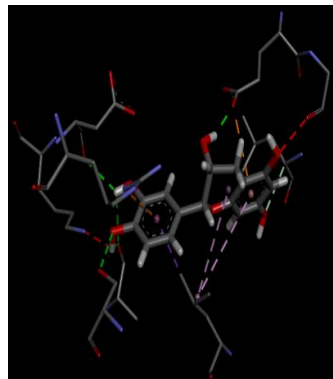


c) Atorvastatin -4.7

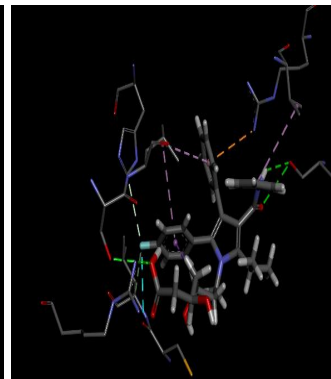
2) 3CCZ



a) Cyanidin -7.5

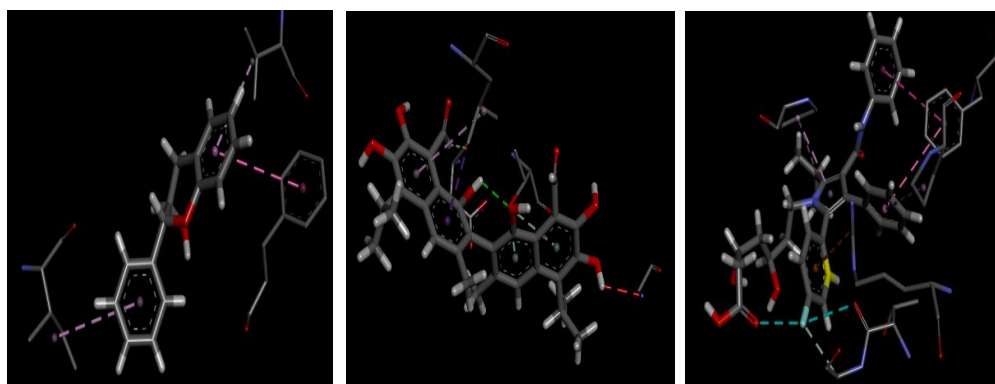


b) Catechin -7.4



c) Atorvastatin -6.9

3) 1URM



a) Flavon-3-ol -6.0

b) Gossypol -6.0

c) Atorvastatin -5.5

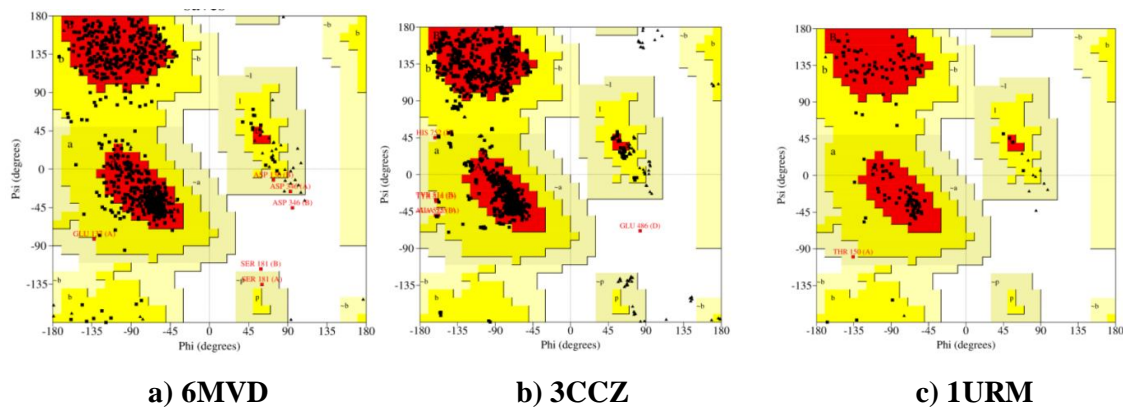
Figure 3: Hydrophobic bond interactions of ligands with 6MVD, 3CCZ, 1URM protein

3.6.2 Ramachandran plot Analysis

Proteins 6MVD, 3CCZ, and 1URM were studied using a Ramachandran plot to determine the presence of amino acids in various regions of each protein. The results are shown in table 4 and in the image below.

Table 4: Ramachandran plot status with protein with 6MVD, 3CCZ and 1URM.

Residues	6MVD	3CCZ	1URM
Most favourable region (%)	86.1	91.1	89.4
Additional allowed regions (%)	12.99	8.5	9.8
Generously allowed regions (%)	0.6	0.4	0.8
Disallowed regions (%)	0.3	0.1	0.0



a) 6MVD

b) 3CCZ

c) 1URM

Figure 4: Ramachandran plot of protein 6MVD, 3CCZ and 1URM protein

4. DISCUSSION

Increased levels of the triglyceride-rich lipoproteins, chylomicrons and VLDL, as well as their byproducts, are reflected in high triglyceride levels. A key role in the onset and development of atherosclerosis and related cardiovascular disorders is played by low density lipoproteins, which can undergo oxidative alterations as a result of increased oxidative stress and increased oxygen free radical generation. Inhibiting oxidant chain reactions at low concentrations, antioxidants are substances that operate as oxidation process inhibitors, removing the risk of harmful processes [10]. Methanolic extract of *Gossypium herbaceum* shown better IC₅₀ value in comparison to standard ascorbic acid.

Propylthiouracil is used to treat hyperthyroidism that is also accompanied with hypercholesterolemia, which is a rise in triglycerides, LDL cholesterol, and total cholesterol in blood serum caused by disruption of cholesterol metabolism. Triton X-100, a non-ionic detergent provokes acute hyperlipidaemia, by increasing hepatic cholesterol biosynthesis [11].

In the present study, METP was investigated for antihyperlipidemic effect by Triton X-100 and propylthiouracil induced models. The results of this study, which examined the effects of MEGH (100 and 200 mg/kg bd.wt) in hyperlipidemic rats, showed a significant reduction in plasma and hepatic lipid profiles as well as an increase in plasma HDL in MEGH-treated as opposed to hyperlipidemic rats. This suggests that MEGH is effective in preventing the elevation seen in various lipid profile components under experimentally induced hyperlipidemia caused by PTU and Triton X 100. Higher plasma HDL concentrations have been linked to a lower risk of coronary artery disease, according to epidemiological research. In hypercholesteremic rats, flavonoids in MEGH have been shown to raise HDL levels while lowering LDL and VLDL levels. Through the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, statins lower LDL-C and total cholesterol. Statins can reduce LDL-C levels, which can reduce mortality and disease progression in patients with clinical ASCVD, according to numerous studies [12]. In comparison to the disease control group, atorvastatin decreased TG, TC, LDL, and VLDL while increasing HDL.

Hence the antihyperlipidemic effect of MEGH *i.e* decrease in TG, TCL, LDL, VLDL and elevation in HDL facilitates the transport of triglyceride or cholesterol from serum to liver where it is catabolized and excreted out of the body and produce an antioxidant effect. *In silico* analysis by molecular docking revealed plant constituents showed response to LCAT activator, Inhibited HMG-CoA reductase and antioxidant activity. One of the key regulators

of plasma high-density lipoprotein cholesterol (HDL-C) and a key player in the reverse cholesterol transport (RCT) mechanism is lecithin cholesterol acyltransferase (LCAT). LCAT is a plasma enzyme that produces the cholesterol esters found in human plasma and is primarily found in circulation with high density lipoproteins (HDL). The amount of unesterified cholesterol in plasma is likewise decreased by cholesterol esterification caused by LCAT [13]. Flavone 3-ol, Gossypol, Cyanidin and catechin showed good docking score compared to other compounds. Statins remain the most prescribed drugs for the treatment of hyperlipidaemia. They inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase through competitive inhibition. Deleterious effect of postprandial hyperlipidemia is mediated via induction of oxidative stress [14]. Ramachandran plot showed the presence of amino acid in most favourable region is greater than 86%. In the present study the superposition of Flavone 3-ol, Gossypol, Cyanidin, catechin and other compounds docking found with LCAT activator (PDB ID: 6MVD), HMG-CoA reductase inhibitor (PDB ID: 3CCZ) and Antioxidant (PDB: 1URM) have validated the accuracy of our docking study and Ramachandran plot. The main class of phenolic chemicals contained in MEGH are flavonoids, which may help prevent hyperlipidemia through their antioxidant action.

5. CONCLUSION

In PTU-induced and Triton X-100-induced hyperlipidemia models, methanolic extract of *Gossypium herbaceum* demonstrated significant antioxidant activity against hydrogen peroxide radical assay and significantly decreased triglycerides, total cholesterol levels, LDL and VLDL levels while increasing HDL. The histopathology study of *Gossypium herbaceum* showed recovery of the damaged liver cells in Propylthiouracil treated group. The cells of the intoxicated liver were reformed. The degree of vascularization was also reduced as compared to hyperlipidemic group that may due to presence of cyanidin, delphinidin, gossypol, catechin and flavonoids. The phytoconstituents present in MEGH showed good docking score compared to standard atorvastatin. Ramachandran plot showed the presence of aminogroups in favourable region is >86%. Hence it can be concluded that Methanolic extract of *Gossypium herbaceum* has showed significant antihyperlipidemic activity.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

Ethical Approval:

All the pharmacological experimental protocols were approved by the Institutional animal ethics committee (IAEC)

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