Ameliorations in the Biomarker Indices of Dyslipidemia and Atherosclerotic Plaque by the Inhibition of HMG – CoA Reductase and Antioxidant Potential of Phytoconstituents of an Aqueous Seed Extract of *Acacia Senegal* (L.) Willd in Rabbits

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Research

Keywords: HMG-CoA reductase, Biomarker indices, Atherosclerotic plaque, molecular dynamics, Acacia senegal, Dyslipidemia

Posted Date: March 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-314959/v1

Version of Record: A version of this preprint was published at PLOS ONE on March 3rd, 2022. See the published version at https://doi.org/10.1371/journal.pone.0264646.

Abstract

Background: The HMG-CoA inhibitor are used to control adverse cardiovascular event caused by Hypercholesterolemia and dyslipidaemia. The current study was aimed to evaluate the ability of phytoconstituents of an aqueous seed extract of *Acacia senegal* (L.) Willd to inhibit HMG-CoA reductase and regress the formation of atherosclerotic plaque.

Methods: The chemical fingerprinting of the test extract was assessed by LC-MS. Consequently, the assessments of *in-vitro, in-vivo,* and *in-silico* were performed by following the standard methods.

Results: The *in-vitro* assessment of the test extract revealed 74.1 % inhibition potential of HMG-CoA reductase. *In-vivo* evaluations of the test extract indicated that treated hypercholesterolemic rabbits exhibited a significant ($P \leq 0.001$) ameliorations in the biomarker indices of the dyslipidaemia, such as the atherogenic index, Castelli risk index (I&II), atherogenic coefficient along with lipid profile. Concomitantly, significant reductions were observed in the atherosclerotic plaque area and antioxidants. The *in-silico* study of molecular docking shown interactions capabilities of key phytoconstituents of the test extract with target protein of HMG-CoA reductase which further validated by the molecular dynamics through potentail energy, NPT, NVT, RSMD and others. Subsequently, the ADMET analysis shown ideal druggability.

Conclusion: The results indicate that phytoconstituents of an aqueous seed extract of *Acacia senegal* (L.) Willd. could inhibit HMG-CoA reductase and improve the levels of antioxidants activity that may reduce symptoms associated with hypercholesterolemia.

Introduction

Cardiovascular disease is one of the major reasons of mortality across the developed and developing countries. Dyslipidaemia is the process that leads to increased lipid deposition on arterial wall that promote atherosclerosis[1]. The existing therapeutics of dyslipidemia involve cholesterol lowering drugs specifically statin and fibrates. The mechanism of statins involves inhibition HMG-CoA enzyme[2]. Although, there are several adverse effects associated with these synthetic drugs. In view of this, the present study explores natural plant product to have HMG–CoA reductase and antioxidant potential. Plant products are not only used in traditional medicine but are also in demand globally as potential sources for the development of new drugs [3]. The Indigenous traditional herbal remedies contain unique formulations of local herbs and herbal extracts that have been developed based on conventional knowledge and local wisdom [4–6]. The ability of several traditional medicines to treat and resolve cardiovascular problems and linked metabolic disorders have been well documented [7]. In this regard, polyherbal formulation of five local herbs (Panchkuta), such as *Prosopis cineraria* pod (Sangari), seed of *Acacia senegal (L.) Willd.* (Kumbat or Kumatiya), fruit of *Capparis decidua* (Ker), pulp of *Casio melo* (Kachar), and pulp of *Mangifera indica* (Amchoor) that are endemic to the Western Rajasthan region (Thar desert) of India, have been historically used to treat cardiovascular problems in rural communities

[8, 9]. *Acacia senegal* (L.) Willd. seed is one of the key ingredients in this herbal medicine (panchkuta) of which several medicinal properties have been demonstrated in our previous studies [10–12]. Exudates of *Acacia senegal* (L.) Willd., which is commonly known as gum Arabic, have also been reported to exhibit hypocholesterolemic activity in animals as well as Sudanese human subjects [13, 14]. Extracts of seeds of *Acacia senegal* (L) also have the ability to inhibit serine proteinase activity [15]. Several reports have provided on information on the ethnopharmacological applications of foods and herbal medicines indigenous to the arid regions of African countries and the Indian subcontinent[15–17]. *Acacia senegal* (L.) Willd. is typically known by its common name, white gum tree, and is a member of the Leguminosae-Mimosoideae[18], while seed extracts of *Acacia senegal* (L.) Willd. is locally known as kumbat or kumatiya in Rajasthan [19, 20]. The present study also identified the major phytoconstituents present in the seed extracts of *Acacia senegal* and assess its anti-atherosclerotic properties in hypercholesterolemic rabbits using a combination of *in-vitro*, *in-silico*, and *in-vivo* methodology.

Material And Methods

Plant material and extraction

Dried seeds of *Acacia senegal* (L.) Willd. were purchased from a local store in Jodhpur (Rajasthan), India. Taxonomic confirmation of the seeds was based on a comparison with an herbarium accession by a botanical expert in the Department of Botany, Jai Narain Vyas University, Jodhpur. Seed extract was obtained using a standard Soxhlet procedure.

Identification of the phytoconstituents

Identification of predominant phytoconstituents present in the seed extracts was based on LC-MS (Liquid chromatography and Mass spectroscopy) [21,22]. The LC-MS data were subsequently analysed using Masshunter software developed by Agilent. Peaks generated in both positive and negative modes of ionization, with ≥3500 ionization counts, were considered using a peak spacing tolerance of 0.0090m/z for reasonable resolution of the chromatogram. Chromatogram peaks were assigned masses based upon MS-MS fragmentation patterns specific for the identified phytocompound. The metabolite profile was confirmed using mass Bank workstation software along with public database information. The samples (SAIF 436) were analysed by the SAIF (Sophisticated Analytic Instrumental Facility), CDRI, Lucknow, UP, India.

Doses of standard statin drug and seed extract dosage

A supply of 20 mg tablets of Atorlip (atorvastatin) was obtained from a local pharmacy in Jodhpur and administered doses were calculated based on body weight of the test rabbits. The seed extract was administered orally at a dose of 400 mg/kg body weight per day for 45 days based on an LD_{50} assessment and previously published studies [23,24].

In-vitro inhibition of HMG -CoA reductase activity

The HMG-CoA reductase inhibition assay was performed *in-vitro* using a kit (Sigma Aldrich) according to the manufacturer's instructions and previous reports in the literature [25,26]. The inhibitory activity of increasing concentrations (0.32mg/ml 0.62 mg/ml 1.25 mg/ml, and 5mg/ml) of the seed and a standard statin drug (Pravastatin) provided with the kit were determined by measuring absorbance at 340 nm. The IC_{50} was calculated based on the obtained inhibition curve for HMGR of the seed extract and the standard drug. The assay is based on the decrease in absorbance resulting from the tested compound and measures the oxidation of NADPH by the catalytic subunit of HMGR in the presence of the substrate HMG-CoA.

Groups of experimental animals

New Zealand white male adult rabbits weighing approximately 1.5±0.1 kg were used in the experiments. Four groups (two control groups and two treated groups) of rabbits were established with six rabbits in each group. Animals were acclimatized for 10 days prior to the onset of the experiment and were maintained in cages in a controlled environment (26 ± 3°C and 12 h of light and dark cycles). The animals were fed a balanced diet supplemented with micronutrients and vitamins. The experimental protocol for use of the animals was recommended by the Institutional Animal Ethics Committee (IAEC) based on the standard norms of the CPCSEA (Reg. No.1646/GO/a/12/CPCSEA valid up to 27.03.23).

Experimental groups were assigned as follows:

Group I: Vehicle control

Group II: Hypercholesterolemic control

Group III: Group administered seed extracts of *Acacia senegal* (L.) Willd.

Group IV: Group administered standard statin drug (Atorvastatin).

The duration of the experiment was 60 days inclusive of the time needed to induce hypercholesterolemia (15days) and administer the treatments (45days)

Induction of hypercholesterolemia

Hypercholesterolemia was induced in the test rabbits by feeding them a high fat diet and a cholesterol powder supplement for 15 days. The cholesterol powder supplement was formulated at 500mg cholesterol powder/kg body weight per day mixed with 5ml coconut oil [27,28]. The induction of hypercholesterolemia was confirmed by weekly biochemical assessments of the blood lipid profile and calculation of the atherogenic index using standard methods.

Collection of serum samples for biochemical analysis and histopathology

Twenty-four-hour fasted animals were autopsied under mild anaesthesia at the completion of the experiment and blood samples were obtained from direct cardiac and hepatic vein puncture. The

collected blood was kept in EDTA-coated vials and serum was separated by centrifugation for 15 min at 3000 rpm.

Serum lipid profile and atherogenic index

Total cholesterol[29], HDL-cholesterol[30], and triglyceride (TG)[31] were determined using standard methods and the lipid profile was constructed following Friedewald's formula (Kumar, 2014). The following indices were calculated using the indicated formulas:

LDL-cholesterol = Total cholesterol - HDL-cholesterol - VLDL-cholesterol

Where VLDL= triglyceride/5

The Castelli risk index – I (Total cholesterol/HDL), Castelli risk index – II (LDL/HDL)[33] and the Atherogenic index = Log (Triglyceride / HDL-cholesterol) [34].

Antioxidants and peroxidation assays of serum

Serum antioxidant levels were determined for catalase[35], superoxide dismutase (SOD)[36], GSH (reduced glutathione)[37], and FRAP (Ferric reducing antioxidant potential) [38] using standard protocols based on redox reaction end products measured as absorbance at an appropriate wavelength. The degree of lipid peroxidation (LPO) in serum was determined by assessing thiobarbituric acid reactive substances (TBARS) and is represented as malondialdehyde (MDA) content, following the modified method of Ohkawa [39].

Histology and planimetric (morphometry) study of aorta

A 2-3 cm length of the ascending aorta of autopsied animals was removed and fixed in 10% formalin. The aortic tissues were subsequently dehydrated in a graded ethanol series and eventually embedded in paraffin wax. The paraffin-embedded samples of aorta were sectioned at a thickness of 5 microns and processed for staining and histopathological analysis [10,40]. The morphometric measurements and planimetric assessments of the sectioned samples of aorta were performed using a Camera Lucida [27,40].

Molecular Docking

Molecular interactions of identified compounds with HMG-CoA reductase was analyzed using Autodock 4.2[41,42]. The catalytic portion of human HMG-CoA reductase (1HW8) was downloaded from a protein data bank and processed using PyMol to extract the co-crystallised ligand inhibitor atorvastatin, remove unwanted water molecules, and correct for chain integration. Three-dimensional structures of the compounds identified in the seed extract and the known inhibitors (pravastatin, atorvastatin) were downloaded from Pubchem Database. Ligand processing was performed using PyMol and hydrogen was added to the structures. The developed docking protocol was validated by performing re-docking with prepared co-crystalized ligand and prepared receptor protein and maps were generated. Post-validation of

the docking protocol of the test compounds was performed by independently docking them with target receptor proteins. The parameters of molecular interactions were obtained through ligand conformations, binding energies, and linked assessments.

Molecular dynamics

Molecular dynamics (MD) simulation studies were performed using GROMACS to understand conformational dynamics of docked complexes (Atorvastatin, Eicosonoid, Flavan-3-ol, Linoleic acid and Pravastatin) with 1HW8. All atoms simulation method was used to gain the insight by solving newton's equation of motion. MD simulations of Atorvastatin_1HW8(HMG-CoA reductase), Eicosonoid_1HW8, Flavan-3-ol_1HW8, Linoleic acid_1HW8 and Pravastatin_1HW8 complexes were performed with the GROMACS 2020.2 package using CHARMM36 force field[43]. The topology of 1HW8 was generated using pdb2gmx modules of GROMACS In addition, PRODRG 2.5 an automated server was used to generate the topology of ligand separately[44]. For solvation of protein, dodecahedron box was used, and protein was placed at least 1.0 nm from the edge of the box. Energy minimization was performed after adding required charges to the system. In one phase potential energy was minimized at maximum force of 1000.0 KJ/mol/nm using 50,000 energy minimization steps cut-off. The temperature coupling was performed by considering the protein structure and ligand as one at a temperature of 310K for 100ps and coordinates of the complex was saved after every 10ps. Pressure equilibrium was also attained using Parrinello-Rahman pressure coupling. The LINCS algorithm was used for constraining all the bonds. Finally, the systems were submitted to molecular dynamics simulation for 1ns to observe stability of Atorvastatin_1HW8, Eicosonoid_1HW8, Flavan-3-ol_1HW8, Linoleic acid_1HW8 and Pravastatin_1HW8 complexes. Structural analysis (RMSD, RMSF and Radius of Gyration) was performed using rmsd, rmsf and gyrate modules of GROMACS and their graphs were generated with xm grace (Graphing, Advanced Computation and Exploration program).

Pharmacokinetic Analysis

ADMET analysis was performed using Drulito software with the standard protocol used to determine the ideal pharmacokinetic profile of the test compounds considered for drug development [45,46]. The test compounds were curtained by two filters: the Lipinski rule and the blood brain barrier (BBB) requirement. The Lipinski rule indicates that an ideal drug molecule should weigh below 500g/mol, the number of hydrogen bond donors should be less than or equal to 5 and the number of hydrogen bond acceptor should be $\leq$ 10, with a partition coefficient $\leq$ 5. The test compound should pass the BBB if the number of hydrogen bonds present is approximately 8-10 and no acidic groups should be present in the molecule. TPSA (total polar surface area) represents the bioavailability of the drug molecule according to Veber's rule which indicates that a TPSA less than or identical to 140Å will have good oral bioavailability.

Statistical Analysis

The data on the biochemical parameters are expressed as a mean ± SEM (standard error of the mean). A one-way analysis of variance (ANOVA) was conducted followed by Tukey's multiple comparison tests

using GraphPad Prism 7.0 software. Graphical representations of the data were constructed using MS Excel 2018.

Results

LCMS analysis of the seed extract

The monoisotopic mass obtained for phytoconstituents was calculated as M + H or M-H ions in QTOF mass hunter software and verified by MS/MS and identified using the data METLINE software and published literature. Results indicated that the seed extract contained nine major phytoconstituents (Table 1and Fig. 1).

In-vitro **inhibition of HMG-CoA reductase activity**

The seed extract and the standard statin drug, pravastatin, exhibited a maximum 74.1 % and 91.4% inhibition of HMG-CoA reductase activity, respectively. Increasing gradient of concentrations of the seed extract were assessed. Enzyme activity was calculated based on the product rate per minute. The IC_{50} of the seed extract, calculated from the inhibition curve, was 0.064µg/ml (Fig. 2A &2B).

Atherogenic index, Castelli risk indexes (I &II), and the lipid profile

Biomarker indices of dyslipidemia, such as the atherogenic index, Catelli risk index – I (Total cholesterol/HDL), Castelli risk index – II (LDL/HDL), and the lipid profile significantly ($P \leq 0.001$) increased up to ten-fold, relative to the vehicle group, in rabbits that were fed the high fat diet supplemented with cholesterol powder. Treatment with the seed extract or atorvastatin resulted in a significant reduction in the atherogenic index, LDL/HDL ratio, and lipid profile that were near normal relative to the untreated rabbits (Figs. 3 & 4).

Effect On Peroxidation And Antioxidants Levels

The levels of peroxidation and antioxidants (SOD, CAT and GSH) were abnormal in hypercholesterolemic rabbits. In contrast, however, administration of the seed extract or atorvastatin resulted in significant reduction ($P \leq 0.001$) in MDA in hypercholesterolemic rabbits, relative to the untreated, hypercholesterolemic rabbits. Moreover, the levels of catalase, SOD and GSH were significantly elevated in hypercholesterolemic rabbits treated with the seed extract. Increased levels of total antioxidants were observed in the rabbits treated with the seed extract, as determined by using a FRAP assay (Fig. 5).

Histology And Morphometric (planimetric) Analysis Of The Aorta

The aortal wall of the vehicle control group (non-hypercholesterolemic) of rabbits was composed of three distinct layers (intima, media and adventitia) and exhibited a compact wall area and enlarged lumen (Fig. 6A). In contrast, the aortal wall of hypercholesterolemic rabbits exhibited an abnormal wall area with the presence of bulging structures of atherogenic plaque and a reduced lumen volume (Fig. 6B). Treatment of the hypercholesterolemic rabbits with the seed extract resulted in a significant ($P \leq 0.001$) reduction in the aortal total wall area and plaque along with an enlargement in lumen volume relative to the untreated, hypercholesterolemic rabbits. The effect was even greater than the reduction exhibited in response to treatment with the standard statin drug (Fig. 6C and 6D; Fig. 7).

Molecular Docking

HMG-CoA has a catalytic groove comprising amino acid residues from 426 to 888. The catalytic portion is composed of Cys688, Thr689, Asp690 and Lys691. The side chain of Lys691 is positioned in the middle of the active site. The flap, primarily composed of Glu559 and Asp767, is in the front of the active site. Among the identified phytoconstituents, eicosanoic acid, linoleic acid, digallic acid, and flavan-3-ol displayed polar interactions with the catalytic residues of the receptor protein (Table 2, Fig. 8A-8E). In contrast, gallocatechin, taxifolin, and myricetin did not exhibit any interaction with the HMG-CoA molecule.

Table 1
Identified masses from UPLC-QTOF mass spectroscopy constituents in an aqueous extract of *Acacia. senegal* (L.) Willd. seed in negative and positive electron ionization modes

S.No.	Identified compound Name	Formula	Monoisotopic mass (g/mol)	Retention time (min)	m-z/ m + z values
1.	Fisetinidol	$C_{15}H_{14}O_5$	274.1	1.05 min	273.1
2.	Linoleic acid	$C_{18}H_{32}O_2$	280.4	2.31 min	279.4
3.	Eicosonoic acid	$C_{20}H_{40}O_2$	312.02	3.84 min	311.09
4.	Lupenone	$C_{30}H_{48}O$	424.5	23.00 min	423.5
5.	Flavan-3-ol	$C_{15}H_{14}O_2$	226.04	4.47 min	249.0
6.	Myricetin	$C_{15}H_{10}O_8$	318.3	4.58 min	341.3
7.	Digallic acid	$C_{14}H_{10}O_9$	322.2	13.10 min	323.2
8.	Taxifolin	$C_{15}H_{12}O_7$	304.3	16.23 min	327.3
9.	Gallocatechin	$C_{15}H_{14}O_7$	306.3	16.23 min	307.3

Table 2
Molecular docking investigations of identified phytocompounds of aqueous extract of *Acacia senegal* (L.) Willd. seed with target enzyme of HMG-CoA reductase

S.No.	Ligand	Binding Energy (Kcal/mol)	No. of H-bonds	Bond length (Å)	Interacting residues
Identified Phytoconstituents					
1.	Fisetinidol	0.8			
2.	Linoleic acid	-3.4	3	2.7, 2.5, 2.1	Asp767, Asp690, Lys692
3.	Eicosonoic acid	-5.0	3	3.3, 2.4, 2.4	Asp690, Lys691, Glu559
4.	Lupenone	NA			
5.	Flavan-3-ol	-3.4	1	2.4	Arg590
6.	Myricetin	7	NA	NA	NA
7.	Digallic acid	-3.7	9	2.7, 2.3, (2.8, 1.8, 2.2), (1.8, 2.6), 2.2, 2.3	Lys692, Ala751, Lys691, Asn7555, Ser684, Arg590
8.	Taxifolin	0.9	0		
9.	Gallocatechin	7.5	NA	NA	NA
Positive control					
1.	Pravastatin	-7.0	2	1.8, 2.1	Asp690, Lys691
2.	Atorvastatin	-7.8	1	2.2	Asp690

Admet Analysis Of Pharmacokinetics

ADMET studies of the identified phytoconstituents indicated that, among the identified phytoconstituents in the seed extract, only the flavonoid, flavan-3-ol, conforms to the Lipinski rule of five along with the potential to cross the BBB. Although eicosanoic acid and linoleic acid both displayed a molecular interaction with HMG-CoA in the docking analysis, they did not conform with the Lipinski rule of five for an ideal drug molecule. Fisetinidol and taxifolin exhibited ideal drug profiles but lack the ability to cross the BBB and did not interact with the target protein in the docking analysis (Table 3).

Table 3
Pharmacokinetics ADMET prediction by Drulito against Lipinski rule of five and blood-brain-barrier filter of phytocompounds of aqueous extract of *Acacia. senegal* (L.) Willd. seed

Compound	MW	logP	AlogP	HBA	HBD	TPSA	nHB	nAcidic group	Filter L/B
Fisetinidol	274.08	0.933	-0.373	5	4	90.15	9	0	L
Linoleic acid	280.24	7.865	-0.948	2	1	37.3	3	1	
Eicosonoic acid	312.3	9.846	-5.05	2	1	37.3	3	1	
Lupenone	424.37	11.294	3.801	1	0	17.07	1	0	
Flavan-3-ol	226.1	1.591	1.316	2	1	29.46	3	0	L/B
Myricetin	318.04	2.182	-1.807	8	6	147.68	14	0	
Digallic acid	322.02	1.77	-1.178	9	6	164.75	15	1	
Taxifolin	304.06	0.803	-1.369	7	5	127.45	12	0	L
Gallocatechin	306.07	1.2	-1.499	7	6	130.61	13	0	

MW = molecular weight; logP = partition coefficient; AlogP = octanol–water partition coefficient; HBA = hydrogen bond acceptor; HBD = hydrogen bond donor; TPSA = total polar surface area; nHB = number of hydrogen bond; nAcidic group = number of acidic group; Filter L = Lipinski rule of five and B = blood brain barrier

Discussion

The prevailing strategy for the management of hypercholesterolemia is the use of HMG-CoA reductase inhibitors which work by inhibiting cholesterol synthesis by HMG-CoA reductase in the liver and removal of excess cholesterol level in peripheral circulation by several mechanisms of reverse cholesterol transport [47, 48]. Excess cholesterol in the circulatory system is indicated by biomarker indices of dyslipidaemia and abnormal lipoproteins ratios, which can be regulated by proper fractional esterification of cholesterol and reverse cholesterol transport (RCT) [49, 50]. Cholesterol present in the intestine is first absorbed in the form of chylomicron (triglyceride rich complex) and is then modified and packaged as high-density lipoprotein (HDL) cholesterol. Therefore, the ratio of triglyceride to HDL is indicative of the levels of peripheral cholesterol in circulation. Abnormal cholesterol esterification rates in apoB-lipoprotein-depleted plasma (fractional esterification) and lipoprotein particle size result in dyslipidaemia [49, 51]. In animal model, specifically hypercholesteraemic rabbits, exhibit elevated levels of the biomarker indices of dyslipidaemia, such as the logarithm of the TG/HDL ratio, total cholesterol/ HDL (Castelli risk index -I (CRI-I)) and LDL-cholesterol/HDL-cholesterol (Castelli risk index-II (CRI-II)). In the present study, the treatment of hypercholesterolemic rabbits with an aqueous seed extract of *Acacia senegal* (L.) Willd. caused a significant reduction in the atherogenic index and CRI – I&II, indicating improved fractional esterification of cholesterol and reverse cholesterol transport. These results are similar to a previously

reported study [33]. The lipid profile i.e. total cholesterol, triglyceride, VLDL-cholesterol, and LDL-cholesterol were significantly improved by treatment with the aqueous seed extract of *Acacia senegal* (L.) Willd. The seed extract appears to significantly inhibit cholesterol biosynthesis in hepatic tissues, as demonstrated in the *in-vitro* HMG-CoA reductase inhibition assay, as well as the *in vivo* studies in hypercholesterolemic rabbits. A variety of phytocompounds have been reported to have capacity to inhibit HMG-CoA reductase, a key enzyme in cholesterol biosynthesis, by inducing the activation of sterol regulatory element binding protein-2 (SERBP-2) and modifications in LDL receptors that lead to reduced cholesterol production and other parameters of the lipid profile [48, 52].

Excessive amounts of peripheral LDL-cholesterol induce the generation of an excessive level of free radicals resulting in oxidative stress. This causes endothelial dysfunction and leads to the further progression of atherosclerotic plaque and reduced lumen volume in the aorta. Similar observations have been noted in hypercholesterolemic animals accompanied by an excess level of cholesterol in the peripheral circulatory system, as well as the progression of atherosclerosis. In the present study, hypercholesterolemic rabbits treated with the seed extract exhibited lower levels of free radicals and elevated levels of catalase, SOD and GSH, which are responsible for scavenging and degrading free radicals. In addition, treatment with the seed extract also resulted in a significant regression in atherosclerotic plaque which would have reversed the progress of atherosclerosis. Previous studies have indicated that hypercholesterolemia promotes atherosclerosis by generating oxidative stress which causes an imbalance between host antioxidant capability and the level of oxidative stress-inducing molecules including reactive oxygen (ROS), nitrogen (RNS), and halogen species, non-radical as well as free radical species. Oxidative stress leads to peroxidation of cellular proteins, lipids, and DNA, resulting in cell injury or cell death, which activates cell death signalling pathways that are responsible for accelerating atherogenesis [53]. In the present study, treatment of hypercholesterolemic rabbits with the seed extract elevated the levels of catalase, SOD and GSH and thus the free radical scavenging capacity of the cell. This effect reduced the atherogenic plaque area and increased the lumen volume. Natural and synthetic antioxidants have been reported to play a crucial role in the prevention and treatment of atherosclerosis through different mechanisms, including inhibition of LDL oxidation [54], decreasing the generation of ROS [55], inhibition of cytokine discharge, the regression of atherosclerotic plaque formation [56] and platelet accumulation [57], the prevention of mononuclear cell infiltration, improvement in endothelial dysfunction [53] and vasodilation, increasing nitric oxide (NO) bioavailability [58], modulating the expression of adhesion molecules, and reducing foam cell formation [57]. The phytochemical analysis of the seed extract identified several predominant phytoconstituents, including fisetinidol, linoleic acid, eicosanoic acid, lupenone, flavan-3-ol, myricetin, digallic acid, taxifolin, and gallocatechin. The *in silico* molecular docking analysis indicated that eicosanoic acid, linoleic acid, and flavan-3-ol are capable of binding to the target enzyme, HMG-CoA reductase [41]. Accordingly, the molecular dynamics simulation validates the stability of the complex system in polar solution was observed using the parameters of RMSD (root mean square deviation), RMSFs (root means square fluctuations), and radius of gyration[59]. The system pressure and temperature are encouraging the structural deviations distress the density, viscosity, thermodynamic properties, and chemical kinetics

within melts as indicated by respective graphs[60, 61]. Compassionately, the ADMET profile of the major phytoconstituents present in the seed extract indicated that the compounds have ideal pharmacokinetic properties conforming to the Lipinski rule, have good bioavailability, and are capable of crossing the blood brain barrier [45].

Conclusion

In conclusion, an aqueous seed extract of *Acacia senegal* (L.) Willd. inhibits HMG-CoA reductase and demonstrate significant antioxidant properties *in vitro.* These properties might be responsible to regress atherosclerosis and reduce hypercholesterolemia as evident by the improvements in biomarker indices of dyslipidaemia observed *in vivo* in hypercholesterolemic rabbits. The major phytoconstituents identified in the seed extract by LCMS are eicosanoic acid, linoleic acid, and flavan-3-ol, could interact with the target enzyme, HMG-CoA reductase as demonstrated by molecular docking studies. Conclusively, the present result indicates that the phytoconstituents present in an aqueous extract of *Acacia senegal* (L.) Willd. seeds have the potential ability to reduce hypercholesterolemia by inhibiting HMG-CoA reductase and slowing the progression of atherosclerotic plaque formation in hypercholesterolemic rabbits.

Abbreviations

ADMET-Absorption, Distribution, Metabolism, Excretion, and Toxicity

FRAP- Ferric reducing antioxidant potential

FTIR- Fourier-transform infrared spectroscopy

GCMS- Gas chromatography–mass spectrometry

GSH- Glutathione Sulfhydryl (Reduced Glutathione)

HDL-High Density Lipoprotein

HMG-CoA Reductase- 3-Hydroxy-3-Methyl-Glutaryl-*CoA reductase*

IC_{50}- Inhibition Concentration$_{50}$

LCMS- *Liquid chromatography*–mass spectrometry

LDL – Low Density Lipoprotein

LPO-Lipid Peroxidation

QTOF MS- Quadrupole Time-of-flight Mass Spectrometry

SERBP-2- Sterol Regulatory Element Binding Protein-2

SGOT- Serum glutamic oxaloacetic transaminase

SGPT- Serum glutamic pyruvic transaminase

SOD-*Superoxide Dismutase*

TBARS- thiobarbituric acid reactive substances

VLDL-Very Low-Density Lipoprotein

Declarations

Acknowledgement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors would like to extend their sincere appreciations to the Researchers Supporting Project Number (RSP-2020/134), King Saud University, Riyadh, Saudi Arabia as well as CSIR, New Delhi (JRF 09/098(0137)/2019-EMR-I).

Authors Contributions

HR & JKC-Experimental protocol design and creating a draft of the manuscript, JKC-*in-vitro* study of HMG-CoA reductase, AP& SA-Review of the results and the manuscript, PR & PK-*In-vivo* Study, SK & PK, AK and AP -Phytochemistry and *in-silico* study, writing and editing of manuscript, GS & BPS – data curation and editing, AAA, AH, AFA, MFSA & EFA-Editing and funding.

Funding

This study was not conducted by specific research grant.

Availability of data and materials

All data used in this study has been included in this article and supplementary data can be provided upon reasonable request.

Ethics approval and consent to participate.

The experimental protocol was approved by IAEC (Institutional Animal Ethical Committee) Department of Zoology, JNVU, Jodhpur which is registered under CPCSEA (Reg. No.**1646/GO/a/12/CPCSEA valid up to 27.03.23**). All the participants agreed to publish this work.

Consent for publication

All the participants approved the final version for the publication and provided their consent.

Competing interests

It is declared that there are no conflicts of interest for the authors participating in this study.

Funding

This study was not conducted by specific research grant.

Conflicts of interest/Competing interests

It is declared that there are no conflicts of interest for the authors participating in this study.

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Figures

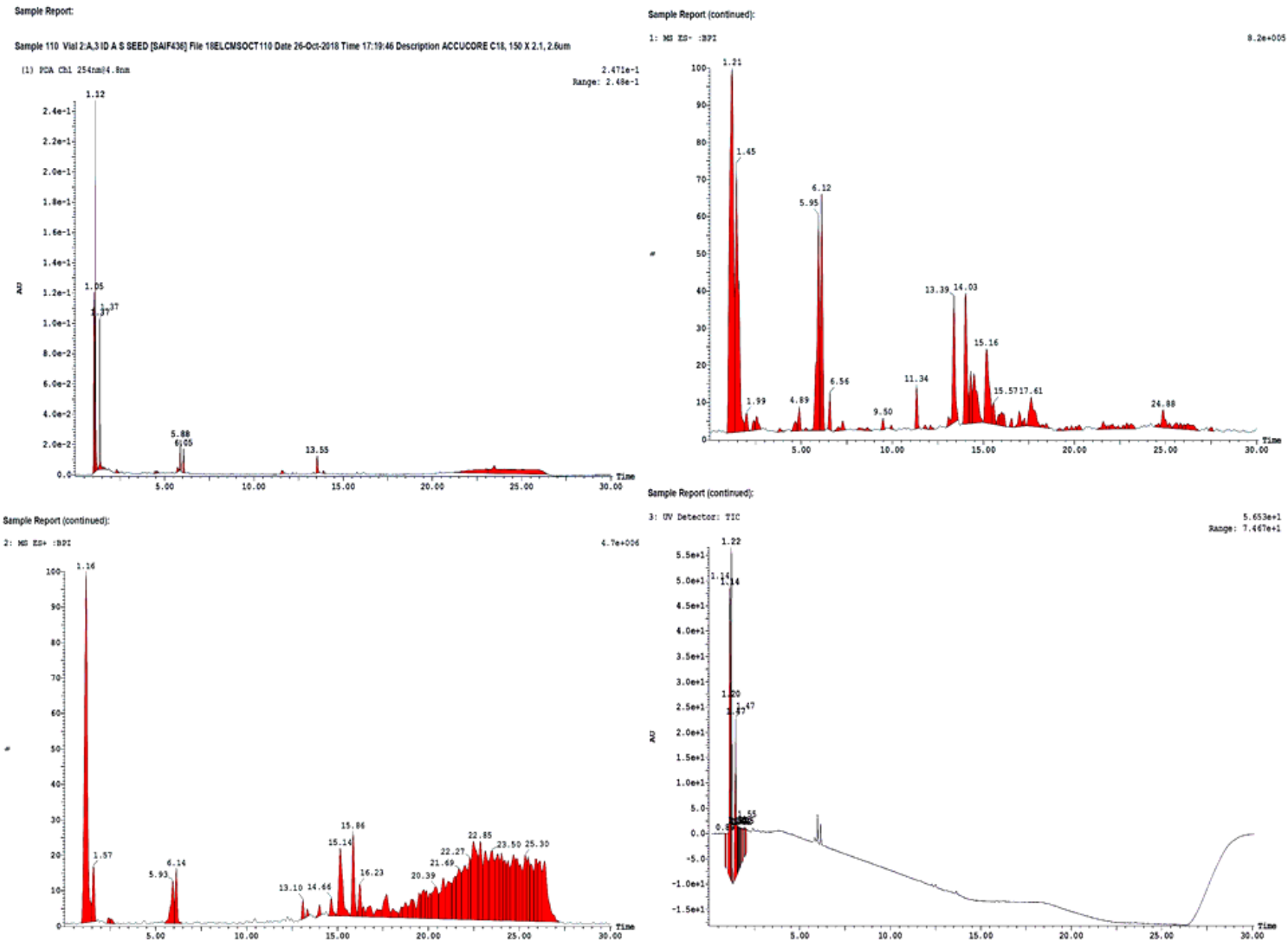

Figure 1

QTOF analyses of aqueous extract of Acacia. senegal (L.) Willd. seed extract

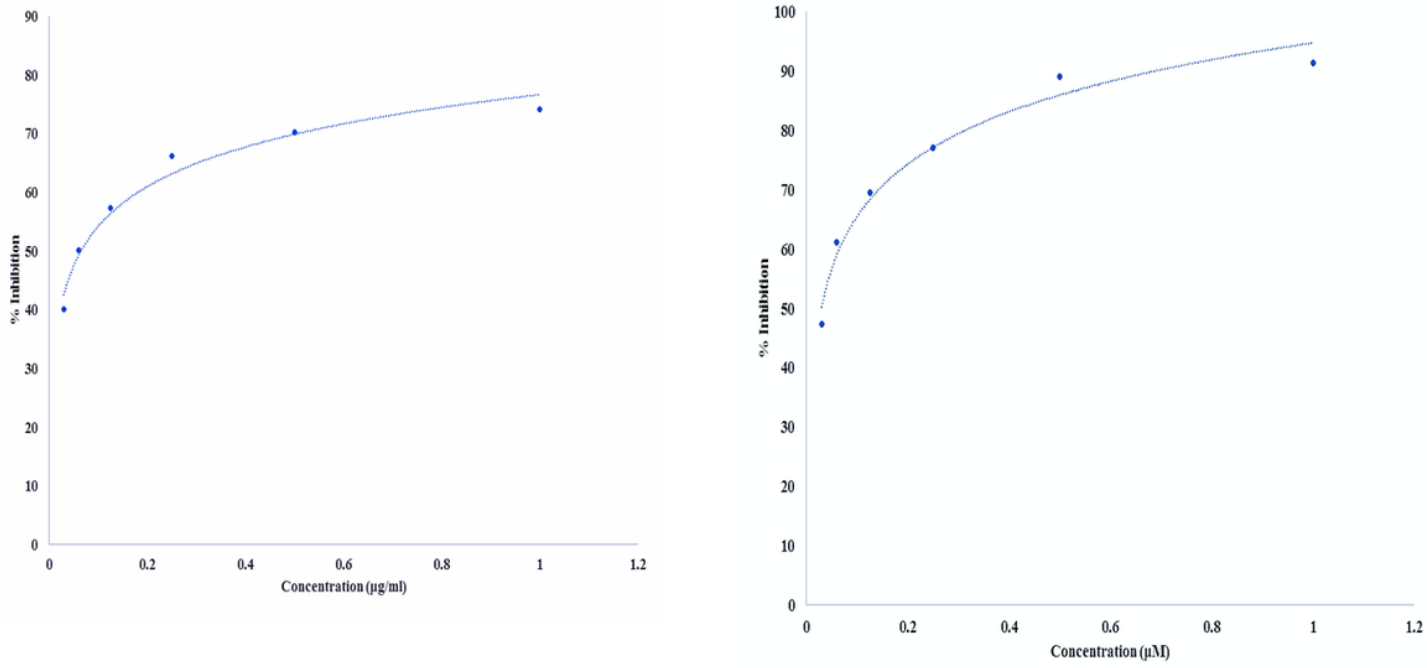

Figure 2

A: HMG-CoA reductase inhibition against ascending concentration gradient of the aqueous extract of Acacia. senegal (L.) Willd. seed (Equation- y = 9.7365ln(x) + 76.671, R^2 = 0.9725, IC50=0.064µg/ml) B: HMG-CoA reductase inhibition against ascending concentration gradient of the standard drug (Pravastatin) (Equation-y = 12.686ln(x) + 94.755, R^2 = 0.9749, IC50=0.029µM)

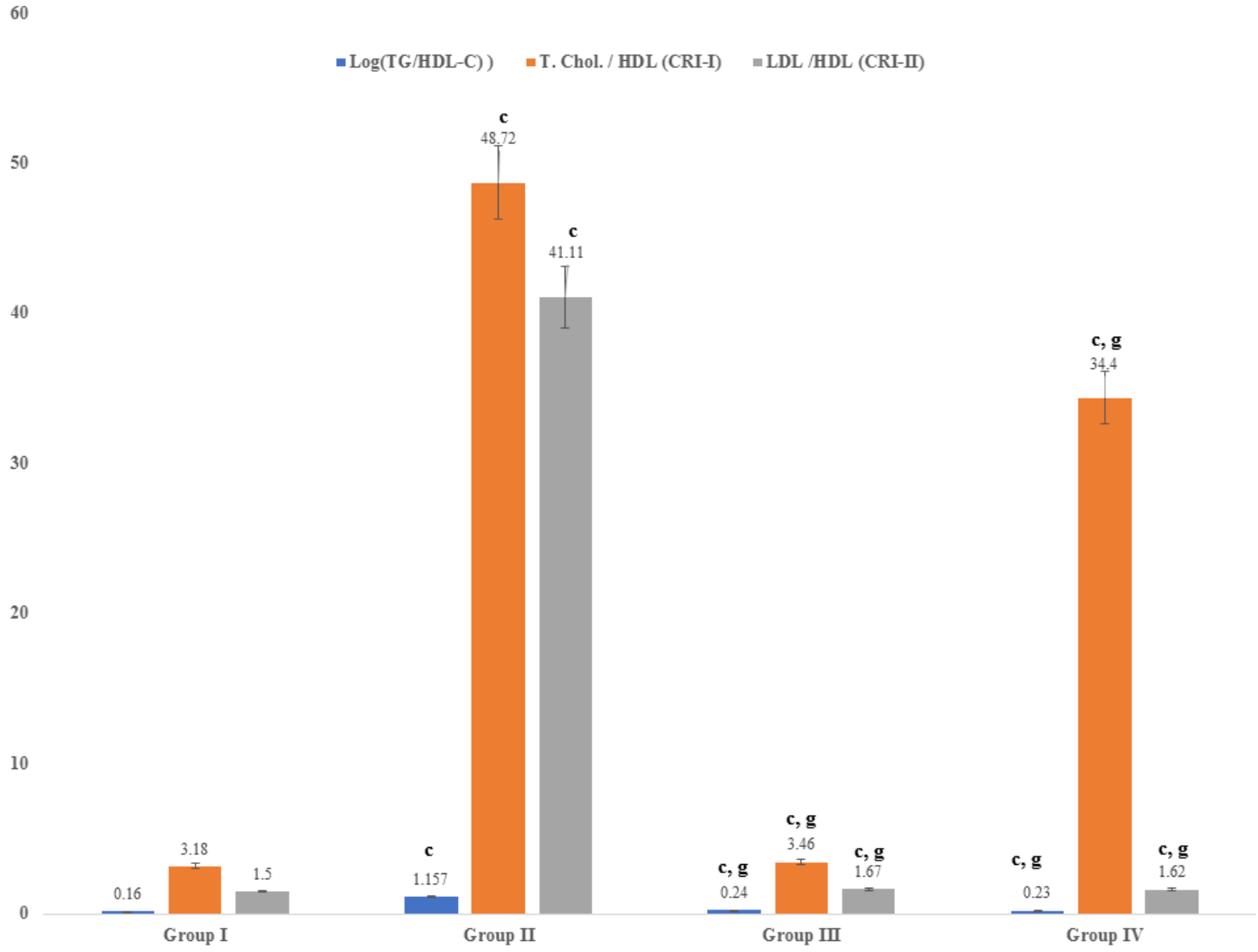

Figure 3

Effect on biomarker indices of dyslipidemia i.e. Castelli Risk Factors (I & II) and atherogenic index (AI) of phytochemicals of an aqueous extract of Acacia. senegal (L.) Willd. seed

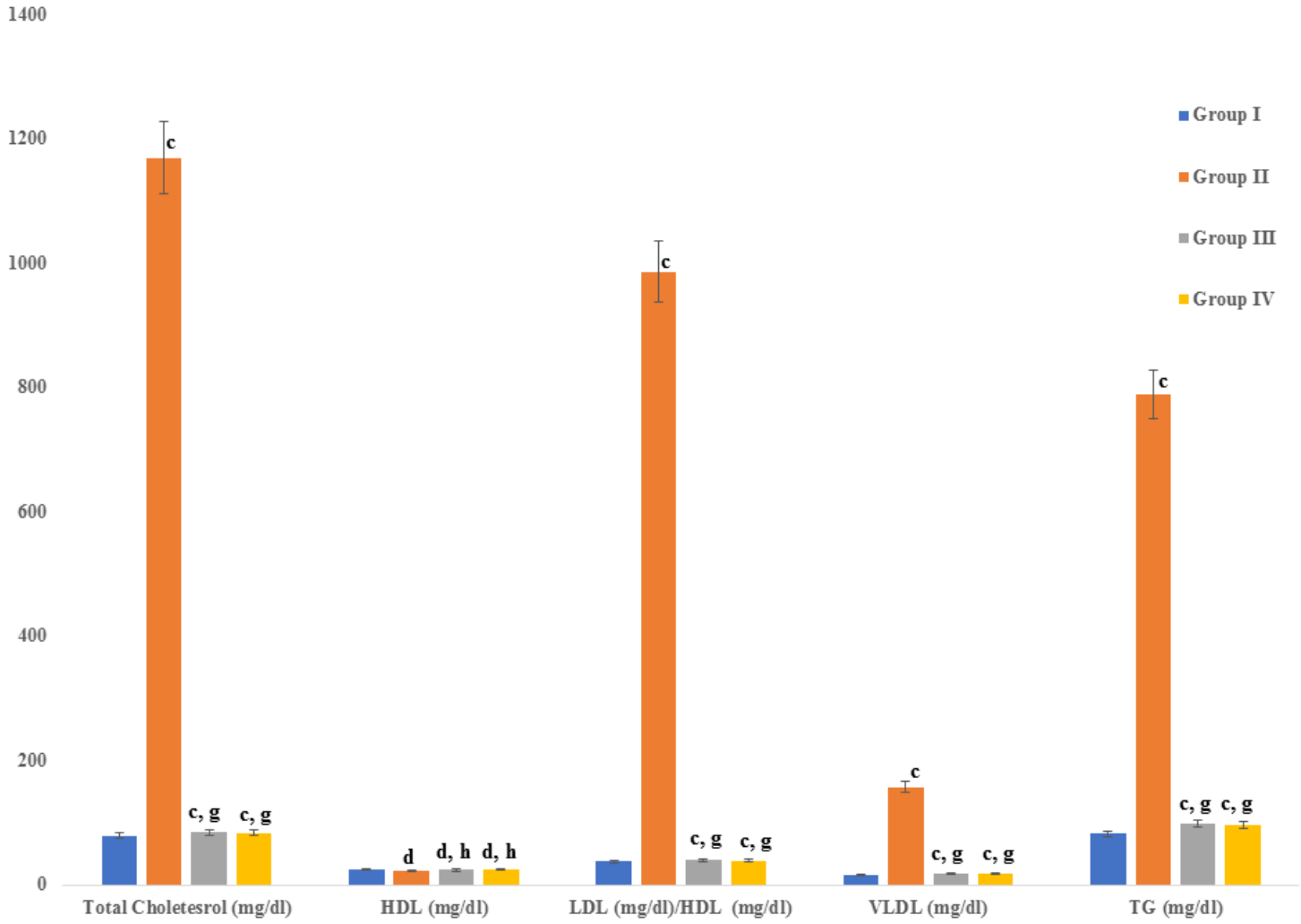

Figure 4

Effect of an aqueous extract of Acacia. senegal (L.) Willd. seed treatment on lipid profile

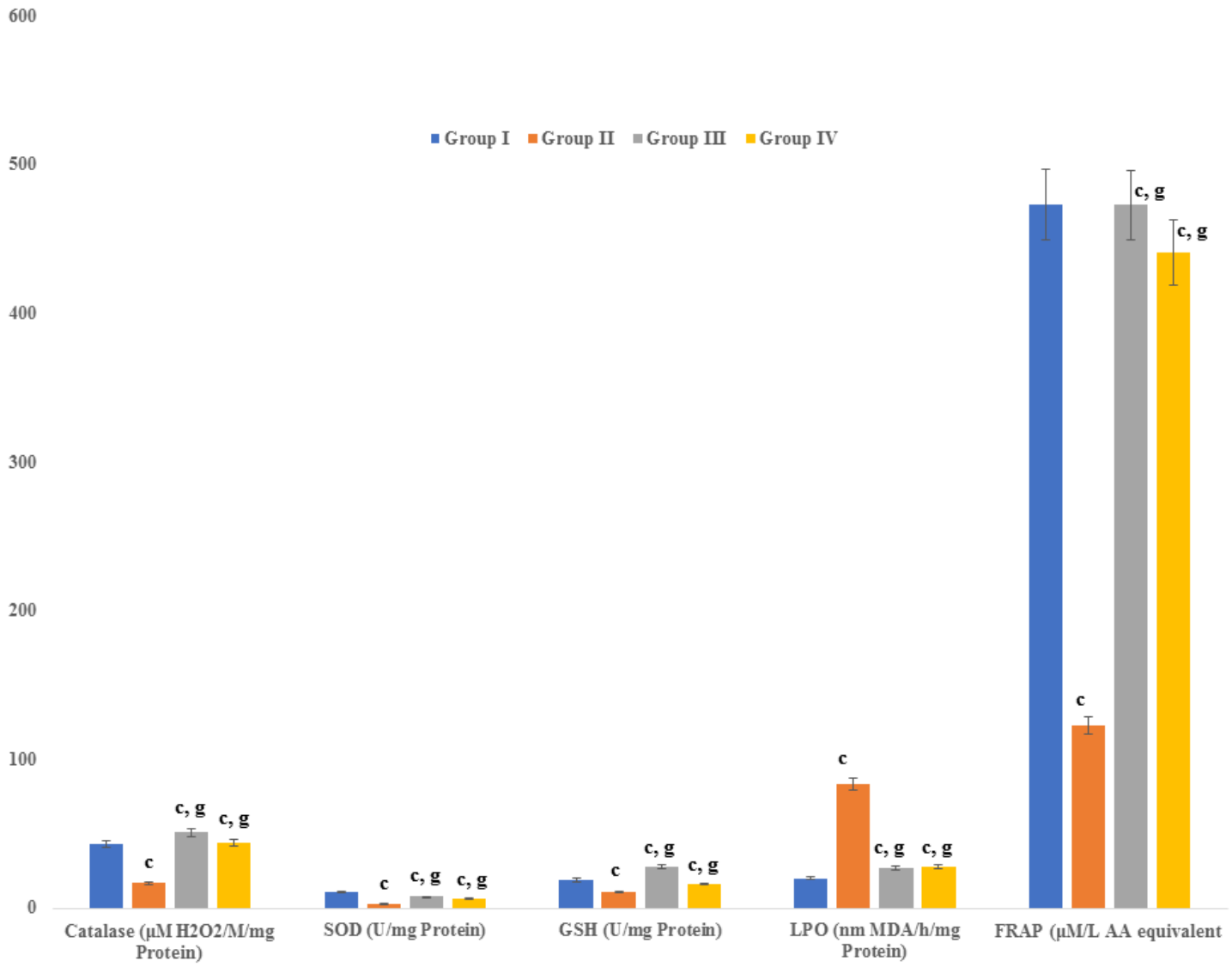

Figure 5

Effect of an aqueous extract of Acacia. senegal (L.) Willd. seed on antioxidant levels in treatment groups

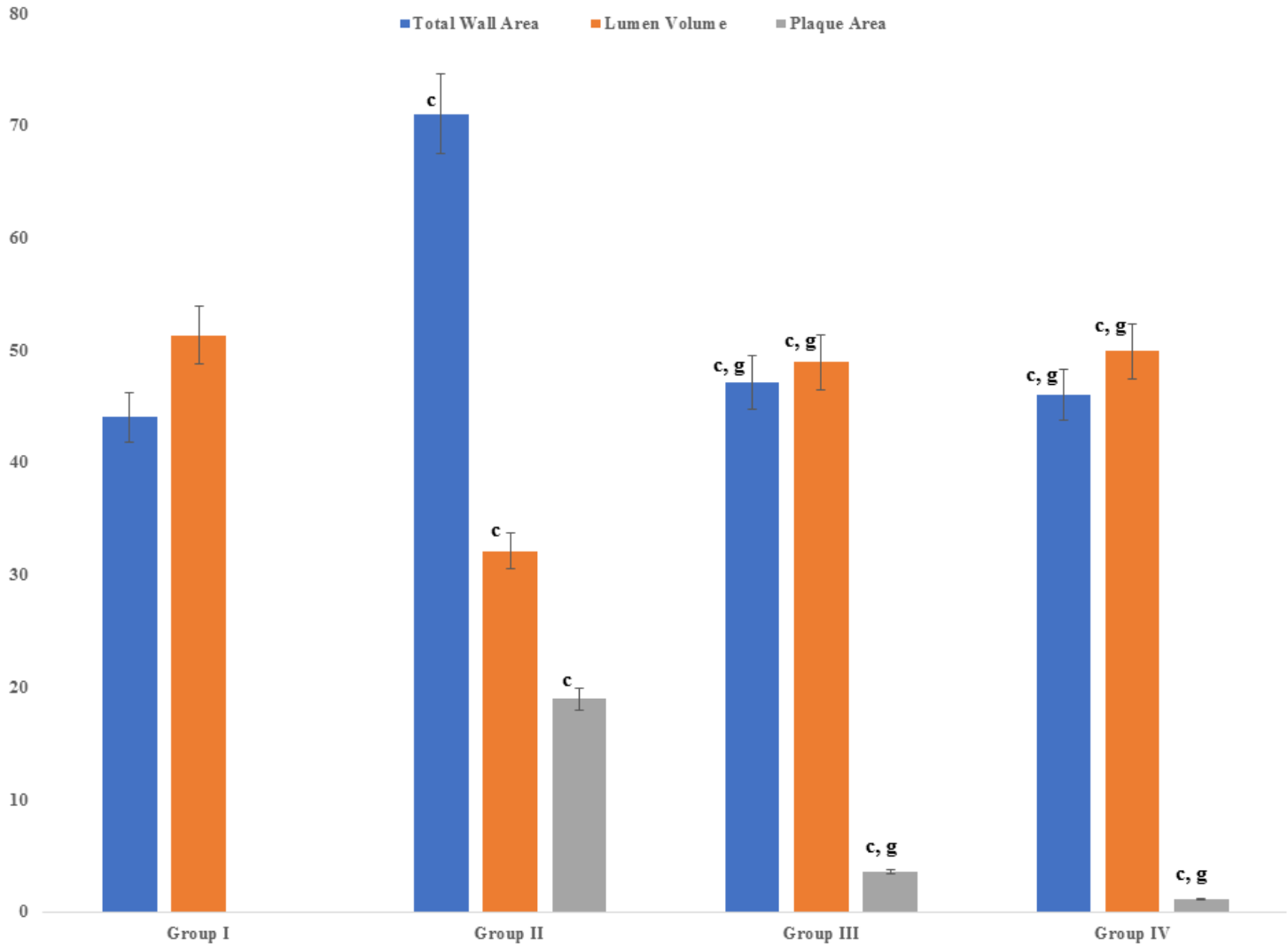

Figure 6

Effect of an aqueous extract of Acacia. senegal (L.) Willd. seed on planimetry of aorta

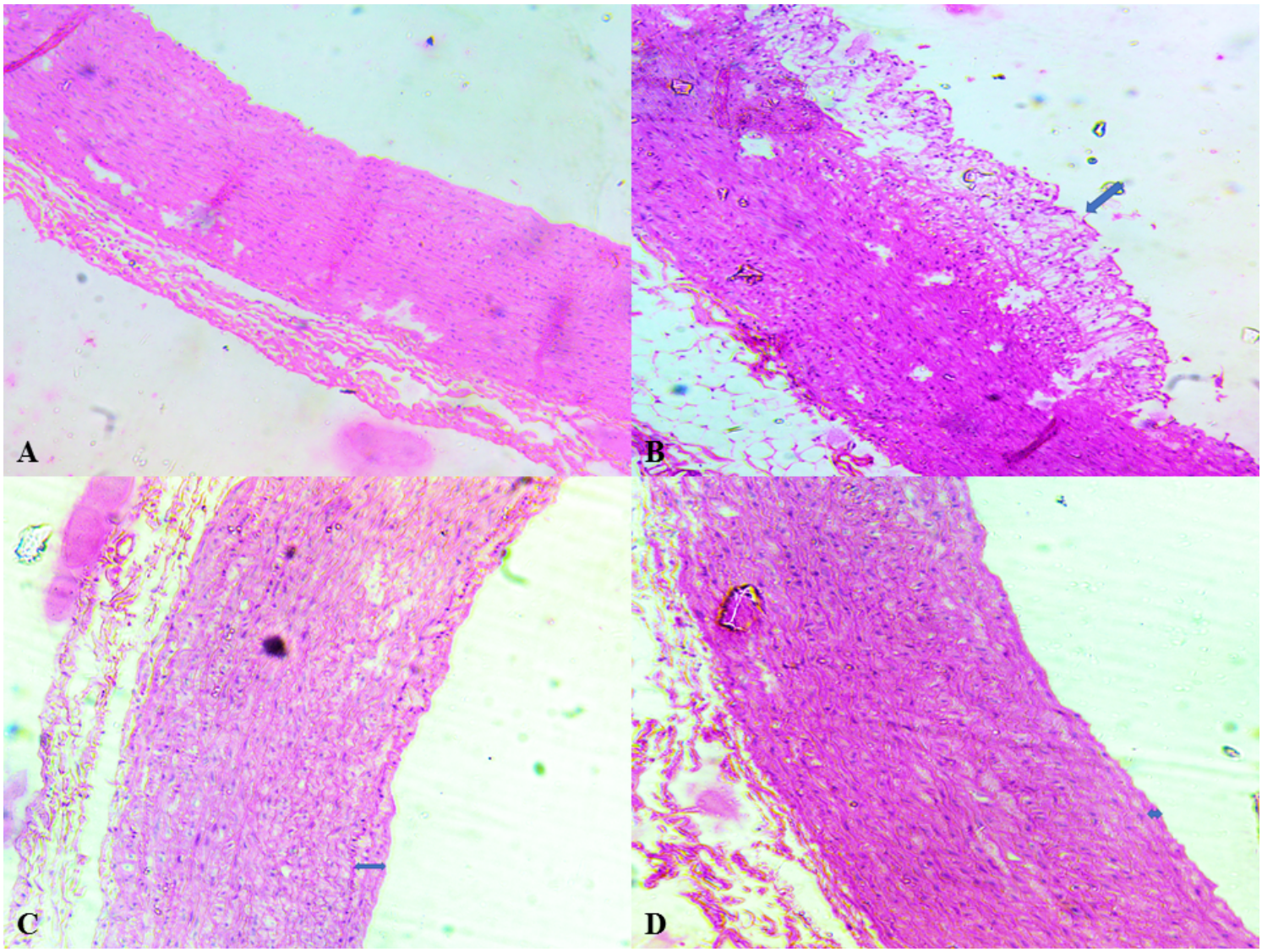

Figure 7

Effect of an aqueous extract of Acacia. senegal (L.) Willd. seed on histopathology of aortas of treatments groups (400X, H& E), A – Histoarchitecture of vehicle control aorta: Exhibiting normal structure with composition three layers i.e. intima, media and adventitia, B – Histoarchitecture of hypercholesterolemia aorta: The arrow indicating the presence of atherosclerotic plaque, C- Histoarchitecture of Acacia. senegal (L.) Willd. seed extract (aqueous) treated aorta: The arrow indicating the reduced area of atherogenic aorta, D-Histoarchitecture of atorvastatin treated aorta: Histoarchitectural restorations by treatment of atorvastatin by indicating the arrow.

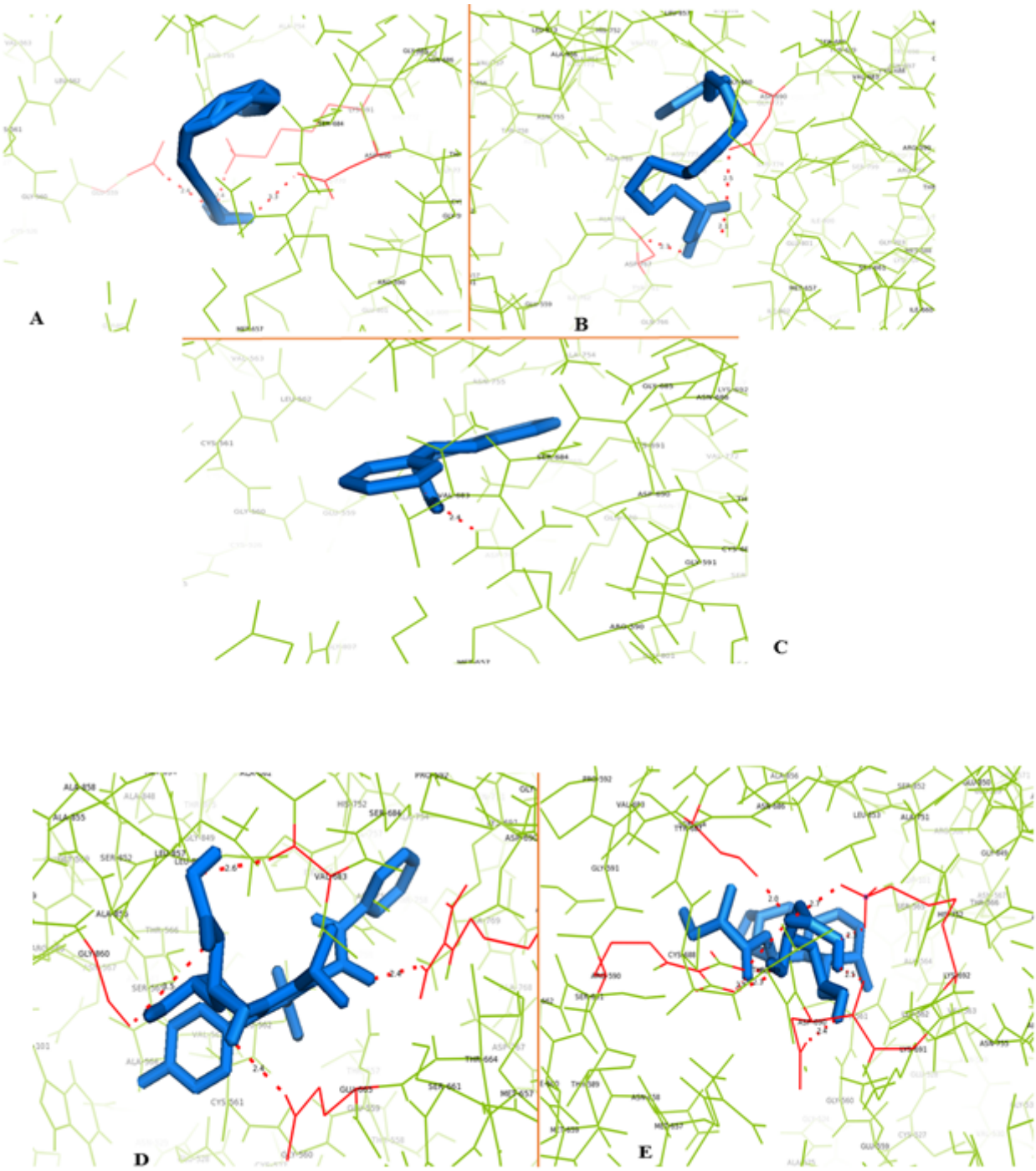

Figure 8

Molecular interactions of identified compounds studied using docking analysis; (A)- HMG-CoA interaction with eicosonoic acid; (B)- HMG-CoA interaction with linoleic acid; (C)- HMG-CoA interaction with flavan-3-ol; (D)- HMG-CoA interaction with atorvastatin; (E)- HMG-CoA interaction with pravastatin.

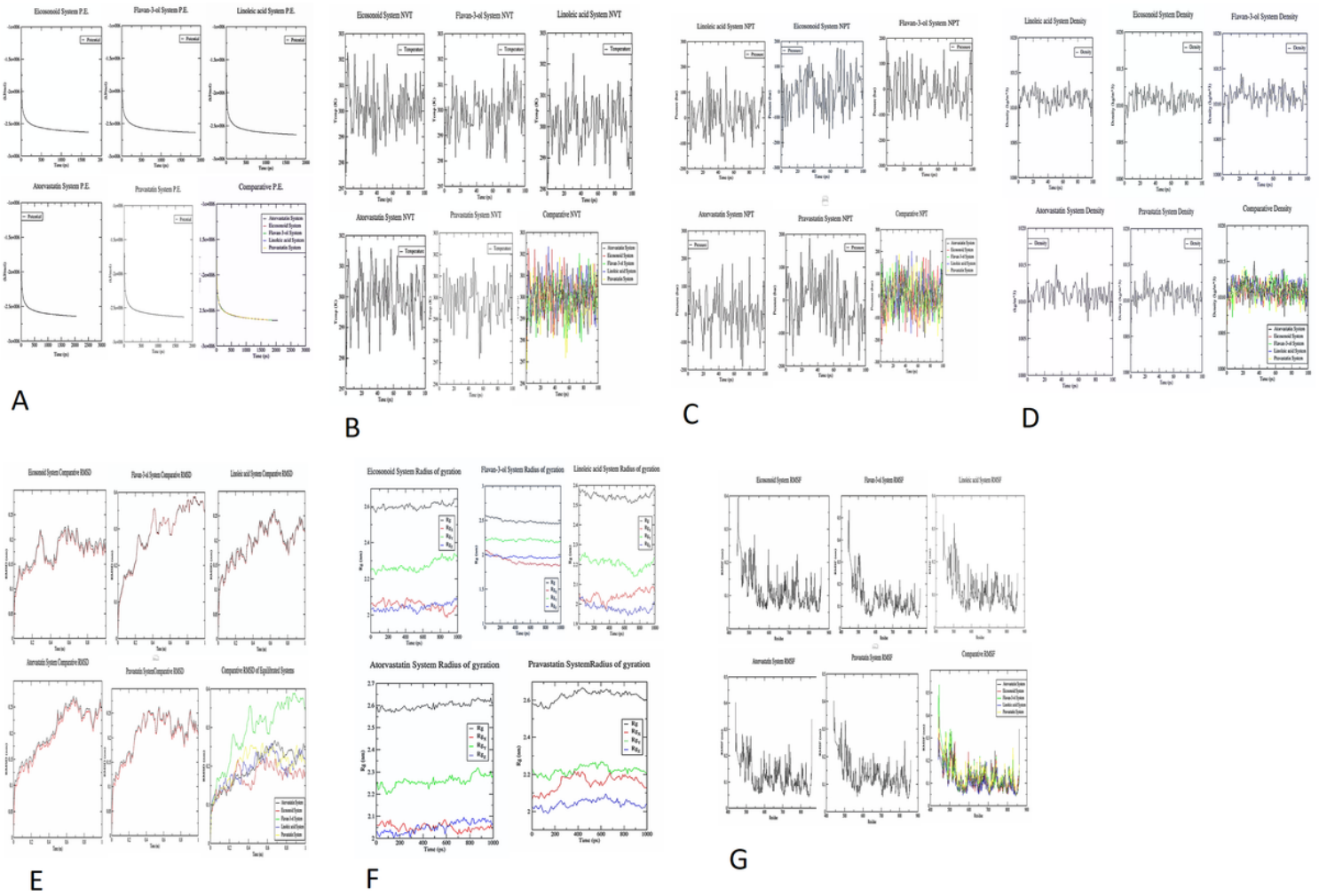

Figure 9

A: Potential Energy Minimization of Eicosonoid System achieved at 1681 P.E steps, Flavan-3-ol System achieved at 1873 P.E steps, Flavan-3-ol System achieved at 1873 P.E steps, atorvastatin system achieved at 2060 P.E. steps, Pravastatin System achieved at 1754 P.E steps and Comparative Potential Energy Minimization all five Systems. B: System Temperature graphs of Eicosonoid, flavan-3-ol, linoleic acid, atorvastatin and pravastatin after temperature minimization for 100 picoseconds C: System Pressure graphs of Eicosonoid, flavan-3-ol, atorvastatin, pravastatin and comparative view accounted after NPT Equilibration for 100ps D: System Density graphs of linoleic acid, Eicosonoid, flavan-3-ol, atorvastatin, pravastatin and comparative view accounted after Equilibration for 100ps E: System RMSD of Eicosonoid, Flavan-3-ol, linoleic acid, Pravastatin and atorvastatin. Crystal Backbone (black) Equilibrated Structure Backbone (red) F: Radius of gyration for Eicosonoid, Flavan-3-ol, linoleic acid, atorvastatin and pravastatin system accounted after 1000 ps. G: System RMSF accounted during the 1ns of MD simulations run of Eicosonoid, flavan-3-ol, linoleic acid, atorvastatin, pravastatin and comparative.