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EVALUATION OF ANTIDYSLIPIDEMIC EFFECT OF MANGIFERIN AND AMAROGENTIN FROM *SWERTIA CHIRAYITA* EXTRACT IN HFD INDUCED CHARLES FOSTER RAT MODEL AND *IN-VITRO* ANTIOXIDANT ACTIVITY AND THEIR DOCKING STUDIES

Rakesh Kumar Asthana [*1], Rasna Gupta [1], Nidhi Agrawal [2], Atul Srivastava [3], Upma Chaturvedi [3], Sanjeev Kanojiya [2], Ashok Kumar Khanna [3], Gitika Bhatia [3] and Vishnu Lal Sharma [1]

Medicinal and Process Chemistry Division [1], SAIF Division [2], Biochemistry Division [3], CSIR-Central Drug Research Institute, Lucknow - 226031, Uttar Pradesh, India.

Keywords:

Swertia chirayita, cardiovascular diseases, Mangiferin, Amarogentin, Antidyslipidemic, Antioxidant

Correspondence to Author:
Rakesh Kumar Asthana

Principal Technical Officer, Medicinal and Process Chemistry Division CSIR-Central Drug Research Institute, Sector 10 Jankipuram Extension, Lucknow - 226031, Uttar Pradesh, India.

E-mail: asthanacdri@gmail.com

ABSTRACT: *Swertia chirayita* (SC) has been traditionally used for the treatment of several metabolic disorders related to health problems. We have investigated the antidyslipidemic and antioxidant potential of *Swertia chirayita* (SC) plant, a potent antidiabetic, and its isolated markers. The LC-ESI-MS standardized hot water extracts of different batches from the whole plant of *Swertia chirayita*, were tested *in-vivo* for their antidyslipidemic activity in Triton (WR-1339) and high-fat diet (HFD) induced dyslipidemic Charles Foster rats and *in vitro* for their antioxidant activity. Further solvent fractionation and isolation resulted in the identification of antidyslipidemic Mangiferin (MG) and Amarogentin (AM). Treatment of hyperlipidemic rats with MG and AM at 100 and 200mg/kg doses caused reduction in plasma levels of Low density lipoprotein (LDL) 24, 26% and very low - density lipoprotein (VLDL) 19, 25% as well as increase in levels of High density lipoprotein (HDL) 18, 22% respectively. In rats with HFD, MG and AM significantly reduced levels of VLDL lipids (26% and 27%) as well as Low-density lipoprotein-Total cholesterol (LDL-TC) 27% and 30% in hyperlipidemic rats at 100 and 200 mg/kg doses. This is the first report of AM demonstrated as an antidyslipidemic agent. MG and AM also showed inhibition of superoxide anion, hydroxyl free radicals and microsomal lipid peroxidation by 24 & 28%, 25 & 31% and 27 & 25%, respectively. Also, both MG and AM also demonstrated more effective inhibition at 5 µM and 10 µM of HMGR (HMG-CoA reductase) in comparison to Pravastatin, which was further confirmed by docking studies.

INTRODUCTION: Cardiovascular disease (CVD) remains the leading cause of death in the developed countries and is major cause of mortality and disease in the Indian subcontinent.

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The World Health Organization (WHO) estimates that CVD is today the largest single contributor to global mortality and will continue to dominate mortality trends in the future [1]. Increase in low-density lipoprotein (LDL), triglyceride (TG) and total cholesterol (TC) levels with decrease of high-density lipoprotein (HDL) levels have been established as one of the risk factors for CVD[2].

Clinical and epidemiological studies have consistently shown that low plasma levels of high-density lipoproteins cholesterol (HDL-C) are

strongly and independently associated with an elevated risk of CVD[3].

Reactive oxygen species (ROS) and oxidative stress are also important contributors to CVD[4]. Oxidative stress followed by elevated blood lipid levels, especially of LDL and TG, is recognized as a primary cause in the initiation and progression of CVD [5]. Enhanced lipid peroxidation acts as a potential contributor to increased cardiovascular risk in low HDL phenotype [6]. Normal functional HDL has high antioxidant potential [7]. Therefore, agents that increase HDL cholesterol concentration in the blood and thereby the ratio of HDL-C to TC (HDL-C/TC) and reduce oxidative stress would have promising therapeutic utility as anti-dyslipidemic and antioxidant agents.

In continuation of our research programs on bio-evaluation of medicinal plants in India, we have investigated the antidyslipidemic and antioxidant potential of *Swertia chirayita* (Roxb. ex Flem., Family: Gentianaceae). It is an indigenous medicinal plant of temperate Himalaya. The decoction of the plant has been used traditionally for the treatment of liver disease, febrifuge, anthelmintic, laxative and malarial fever [8, 9]. Studies on the biological activities of different extracts of SC revealed that the plant possesses antioxidant [10], antihyperglycemic [11, 12] anti-carcinogenic [13], analgesic [14], antimicrobial [15] properties.

The liquid chromatography-electron spray ionization-mass spectroscopy (LC-ESI-MS) standardized hot water extract its, solvent fractions and two glycosides, of SC, a potent antidiabetic[16], were studied in Triton (WR-1339) and HFD induced hyperlipidemic rats model. Their effect on cholesterol biosynthesis and antioxidant activity was also studied in the *in-vitro* system, which was confirmed by molecular docking.

MATERIALS AND METHODS:

Chemical and Reagents: Triton (WR-1339), standard drug gemfibrozil, and test kit for HMGR along with other chemicals were procured from Sigma Chemical Company St Luis, MO, (USA).

Plant Material and Preparation of Extract: Whole plant materials of SC were collected during Sep-Oct 2002 (Botany Reg. no. 8359, Batch no. 46 and 47 and Sep-Oct 2007 Botany Reg. No. 8563, Batch No. 48) from Simana Darjeeling (West Bengal) India and authenticated by Botany Division of CDRI, Lucknow, where the specimens are preserved. The process comprises the steps of hot extraction of powdered whole plant material (1.0 Kg) of SC for 2 h in deionized boiling water (8L x 4). The combined extract was filtered with fine cloth, and the filtrate was concentrated under reduced pressure at 45 ºC. These hot water extracts (*i.e.*, 46/A1, 47/A1, and 48/A1) were used for the biological evaluation and separation of fractions as well as isolation of compounds.

Isolation of Fractions and Compounds: The extract was fractionated with hot methanol (500 mL × 4) and combined fractions were concentrated to complete removal of solvent (F2). The residue (F3) was then fractionated with 10% methanol in water (200 mL × 4) which on concentration to dryness gave (F4) and the remaining residue (F5) which was dried in an oven at 50 ºC for 4-5 h and then used for the experiment. F2 was dissolved in methanol and concentrated up to half of its volume and kept in a conical flask at 10ºC for 24 h, a yellowish amorphous compound settled at the bottom of the flask which was filtered and passed through Sephadex LH-20 column in Water (H_2O), H_2O-methanol (8:2, 6:4, 5:5, 4:6, 2:8) and methanol. Eluted fractions were monitored on reverse phase-18 (RP-18) TLC plate in H_2O: methanol and H_2O: acetonitrile (6:4 and 5:5) and TLC plates were sprayed with 5% ceric sulfate and spots were developed by heating at 110 ºC. MG **Fig. 1** was isolated after repeated crystallization from methanol in a 0.4% yield. The methanol fraction was subjected to repeat column chromatography over silica gel in 5-10% methanol-chloroform mixture gave AM **Fig. 1** in 0.2% yield.

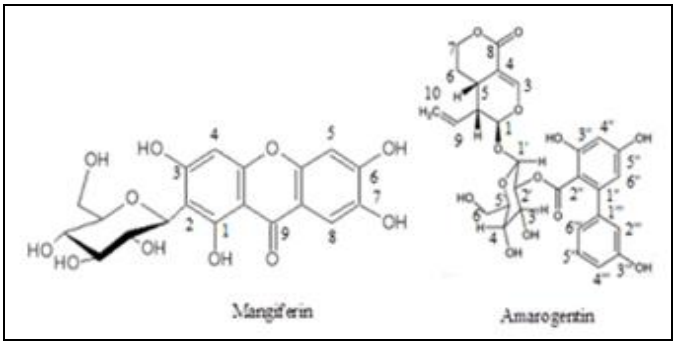

FIG. 1: STRUCTURE OF MANGIFERIN AND AMAROGENTIN

Structure of these compounds was carried out by spectroscopic techniques and confirmed by comparison of its experimental data with that of reported in literature [17].

Sample Analysis LC-ESI-MS and MS/MS Analysis: The LC-ESI-MS and MS/MS analysis of hot water extract (46-A1) of the plant were performed on Waters TQD triple quadruple mass spectrometer (USA) equipped with Waters H-Class Acquity UPLC system and electron spray ionization source. Mobile phase (A) acetonitrile and (B) 5 mM ammonium acetate buffer were used and linear gradient elution performed at the flow rate of 0.350 mL min^{-1} (as 90-80% B in 0-4 min; 80-50% B in 4-6 min; 50-20% B in 6-8 min.). Waters BEH C-18 100 × 2.1 mm, the 1.7µm column was used and ESI source potentials were capillary voltage 3.5 kV; cone potential at 30 V for LC-ESI-MS experiments. Nitrogen was used as the nebulizing and drying gas at flow rates of 50 and 650 L h^{-1} respectively. Online collision-induced dissociation (CID) experiments were performed at 25 eV and argon used as the collision gas. Data acquisition and processing were carried out using Mass Lynx V4.1 SCN 714 software. The spectra were accumulated from the top of Extracted ion chromatogram (EIC) peak **Fig. 2**.

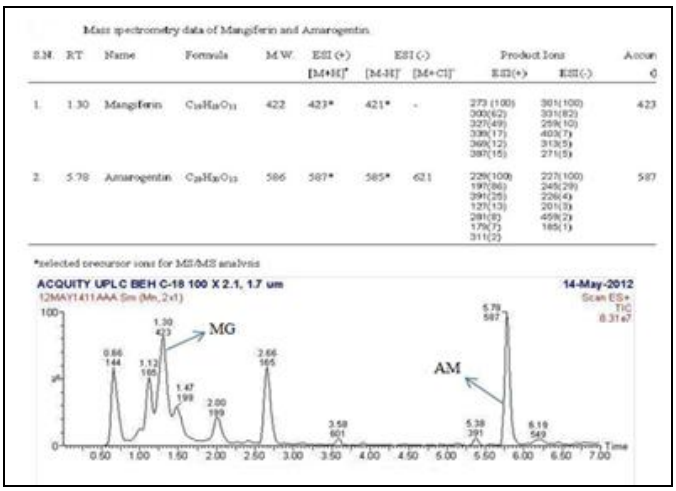

FIG. 2: LC-ESI-MS AND MS/MS ANALYSIS

Experimental Animals: Male adult rats of Charles Foster (CF) strain (100-150g) bred in the animal house of the institute were used after approval of the Animal Ethics Committee (IAEC/2010/149). The animals were kept in controlled conditions of temperature (25-26 °C), relative humidity (60-80%) and 12/12h light/ dark cycle (light from 8:00 am to 8:00 pm) and provided with standard pellet diet (Lipton India Ltd) and water *ad libitum*.

Experiment Design for Antidyslipidemic Activity: In the first set of experiment, animals were divided into 14 groups *i.e.* 1.Control, 2. Triton treated, 3. Triton + 46/A1, 4. Triton + 46/F2, 5. Triton + 46/F3, 6. Triton + 46/F4, 7. Triton + 46/F5, 8. Triton + 47/A1, 9. Triton + 47/F2, 10. Triton + 47/F3, 11.Triton + 48/A1, 12.Triton + 48/F2, 13. Triton + 48/F3 and 14. Triton + gemfibrozil. In, another set of experiment, the animals were divided into seven groups *i.e.* 1. Control, 2. Triton treated, 3. Triton + MG 100mg/ kg b. w. (MG-1), 4. Triton + MG 200mg/kg b.w. (MG-2), 5. Triton + AM, 100mg/kg b.w. (AM-1), 6. Triton + AM 200mg/kg b. w. (AM-2) and 7. Triton + gemfibrozil.

Triton and HFD Induced Hyperlipidemia: The rats were divided into control, triton, and triton plus drug-treated groups of six rats in each. In the acute experiment, triton was administered (400mg/kg body-wt.) by intraperitoneal injection for 18 hours. These test material and gemfibrozil (Cipla Ltd, Bombay, India) were macerated with 0.2% aqueous gum acacia suspension and fed orally (200mg/kg body-wt.) simultaneously with triton. In the chronic experiment, hyperlipidemia was produced by feeding with HFD once a day for 30 days. Drugs were administered (100 mg/kg body-wt.) orally simultaneously with HFD in drug-treated groups. Control animals received the same amount of natural saline or groundnut oil. At the end of experiments, rats fasted overnight, and blood was withdrawn. The animals were killed, and the liver was excised immediately. The plasma was analyzed for TC, TG, PL (phospholipids) and lecithin cholesterol acyltransferase activity (LCAT) and post heparin lipolytic activity (PHLA) activity.

Biochemical Analysis of Plasma/Serum: LCAT and PHLA activity was assayed by reported procedure [18, 19]. Serum was fractionated into VLDL, LDL, and HDL by polyanionic precipitation methods [20]. Serums, as well as lipoproteins, were analyzed for their TC, PL, and TG by standard procedures reported earlier[21].

Biochemical Analysis of Liver: Liver was homogenized (10% w/v) in cold 100mM phosphate

buffer pH 7.2 and used for the assay of total lipolytic activity [19]. The lipid extract of each homogenate was used for estimation of TC, PL, TG, and TP (total protein).

Faecal Bile Acids: The rat faeces were collected from all groups throughout 30 days and processed for estimation of cholic and deoxycholic acid[22].

Cholesterol Biosynthesis: The effect of MG and AM on cholesterol biosynthesis was estimated as their effect on HMGR, in an *in-vitro* experiment using protocol and reagents provided with a standard test kit from Sigma Chemical Company.

Antioxidant Activity: The effect of test substances on the generation of superoxide anions and hydroxyl free radical were measured in the *in-vitro* enzymatic systems as described earlier [23].

Statistical Analysis: Data were analyzed using student't' test.

Hyperlipidemic groups were compared with control, hyperlipidemic, and drug-treated with hyperlipidemic group P=<0.05 was considered as significant.

***In-vitro* HMGR Inhibitory Activity:** The HMGR assay was performed using the HMGR assay kit from Sigma-Aldrich. HMG-CoA (substrate), NADPH, assay buffer, and enzyme HMGR were applied with the supplied assay kit.

Molecular Modeling: Three-dimensional coordinates of MG and AM were extracted from PubChem compound database (Pub chem compound Id. 5464041 and 115149 respectively) for docking purposes against Human HMGR (pdb id: 1Hw9) using Flex X implemented in Sybyl 7.1 [24]. Flex X applies incremental building method for flexible docking of small molecules. Gasteiger-Huckel charges were assigned to all the ligands before docking calculation. All the values were kept in the default for docking studies. Docking parameters were validated through the redocking of crystallized Simvastatin (6c).

RESULTS AND DISCUSSION:

Effect of SC Extract on Triton-Induced Hyperlipidemia: The effect of triton was found to be reversed by the hot water extracts and fractions of SC **Fig. 3**.

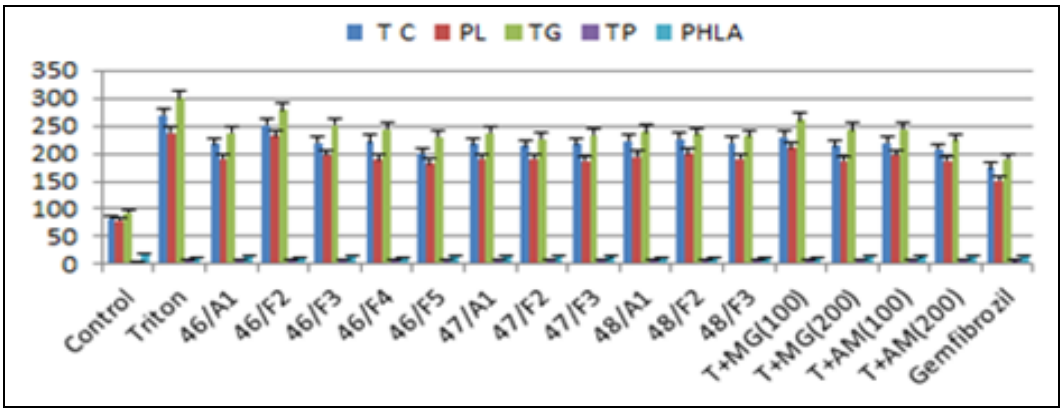

FIG. 3: TRITON TREATED RATS COMPARED WITH CONTROL, TRITON LUS DRUG TREATED COMPARED WITH TRITON. (LIPID LOWERING ACTIVITY OF SWERITA CHIRAYITA A1, F2-F5, MG AND AM IN TRITON TREATED HYPERLIPIDEMIC RATS

Treatment of hyperlipidemic animals with MG and AM caused reduction in plasma levels of TC (20, 23%), TG (19, 25%), PL (21, 21%), TP (23, 20%), LDL (24, 26%), VLDL (19, 25%) and atherogenic index (35, 39%) as well as increase in levels of HDL (18, 22%), HDL/LDL ratio at 100 and 200 mg/kg doses respectively **Fig. 4a, b**. MG and AM have demonstrated the better effect on HMGR inhibition at 5µM and at the 10µM concentration in comparison to pravastatin, a potent inhibitor of HMGR **Fig. 4c** and activated LCAT (20, 24%) and PHLA (22, 21%), (Fig. 4d).

Effect of SC Extract on High Fat Diet-Induced Dyslipidemia: Further studies on MG and AM were carried out using HFD - induced hyperlipidemic rats. The HFD induced Hyperlipidemic rats model is more suitable for study because the rats consume the diet and water *ad libitum*, similar to human eating patterns, rather

than being force-fed. The results of the feeding of HFD in rats suggested an increase in their serum levels of TC, PL, and TG by 131%, 91% and 89% respectively **Table A1**.

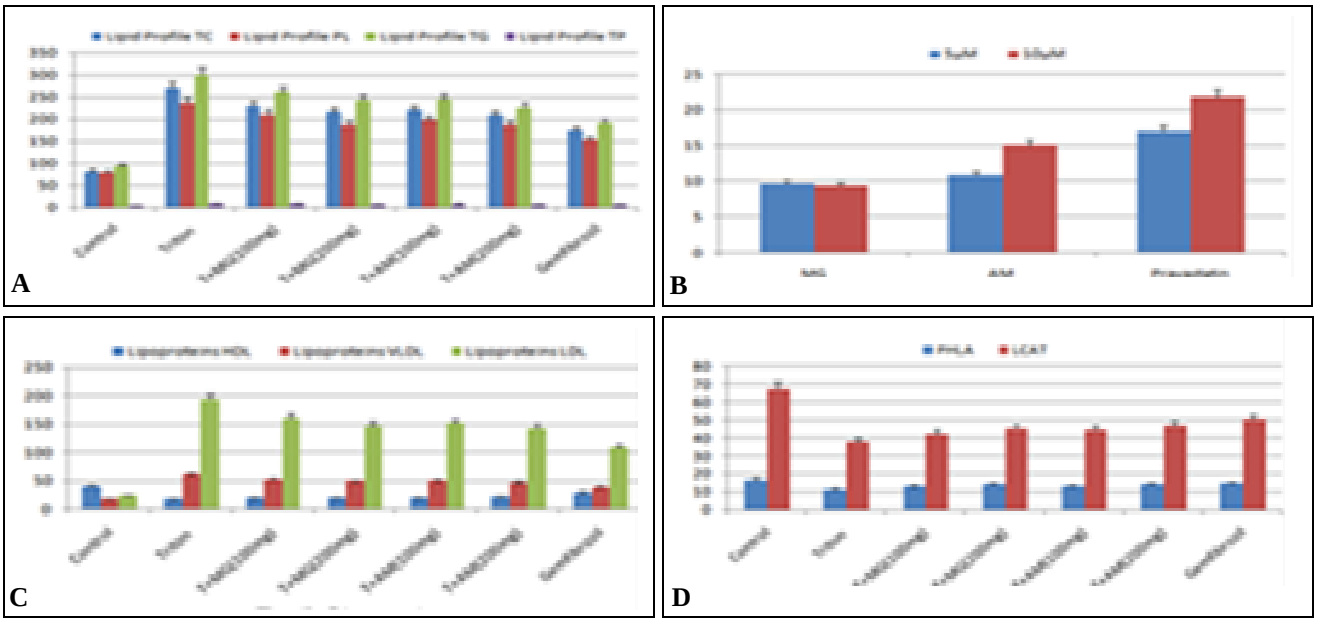

FIG. 4: A. EFFECT OF MG AND AM ON LIPID, B. EFFECT OF MG AND AM ON LIPO-PROTEIN LEVEL OF HYPERLIPIDEMIC RATS, C. EFFECT OF MG AND AM ON HMG-CoA REDUCTASE, D. EFFECT OF MG AND AM ON LIPOLYTIC ENZYME OF PLASMA

TABLE A1: EFFECT OF MG AND AM ON LIPID COMPOSITION IN SERUM LIPOPROTEINS IN HFD INDUCED HYPERLIPIDEMIC RATS

Parameters	Control	HFD treated	HFD+MG1	HFD+MG2	HFD+AM1	HFD+AM2	HFD + Gemfibrozil
(A1) Serum							
TG[a]	86.66 ±5.10	200.58±15.12 ***(+2.31F)	160.00 ±13.00**(-20)	169.00 ±11.82*(18)	150.00 ±15.00***(-25)	138.00 ±10.00(-31)	195.20 ±13.48***(-35)
PL[a]	86.00 ±7.70	168.80±12.30 ***(+1.91F)	130.00 ±8.90**(-22)	135.00 ±11.00**(20)	125.00 ±12.00***(-25)	120.00 ±10.82(-29)	142.22 ±11.00***(-33)
TC[a]	106.88 ±9.0	201.93±14.44 ***(+1.88F)	145.20±12.11 ***(-28)	158.00 ±14.00**(21)	143.00 ±14.00***(-29)	140.00 ±10.77(-31)	152.60 ±11.66***(-34)
Protein[b]	6.05 ±0.17	10.80±0.33** *(+1.79F)	8.60 ±0.17**(-21)	8.70 ±0.74**(-20)	8.40 ±0.89***(-23)	7.60 ±0.40(-30)	8.48 ±0.32***(-30)
(A2) VLDL							
TC[a]	6.30 ±0.38	10.87±1.00** *(+1.72F)	9.49 ±0.10*(-13)	9.43 ±0.35*(-13)	9.50 ±0.41*(-14)	9.10 ±0.27*(-16)	8.70 ±0.18*(-20)
PL[a]	10.00 ±0.70	18.14±1.03** *(+1.81F)	14.97 ±1.30*(-17)	14.84 ±1.00*(-17)	14.62 ±0.88*(-19)	14.50 ±0.92**(-20)	14.20 ±0.78**(-22)
TG[a]	45.60 ±4.00	90.30±7.37** *(+1.98F)	73.37 ±5.12*(-19)	70.76 ±6.00**(-21)	70.29 ±5.00**	69.61 ±4.12***(18)	67. 81 ±2.84***(-25)
AP[b]	6.30 ±0.50	12.12±1.80** *(+1.92F)	9.56 ±0.60**(21)	9.30 ±0.70(-23)	9.15 ±0.25***(-26)	9.00 ±0.38***(-26)	8.80 ±0.31***(27)
(A3) LDL							
TC[a]	8.23 ±0.60	17.23±1.48** *(+2.09F)	13.28 ±1.00**(-22)	13.00 ±0.79***(-24)	12.80 ±1.10***(-26)	12.50 ±1.18***(-27)	12.00 ±1.00***(-30)
PL[a]	8.14 ±0.47	15.73±1.77** *(+1.93F)	13.20 ±1.18*(-16)	13.00 ±1.00*(-17)	12.83 ±0.79*(-18)	12.63 ±1.08**(-20)	12.50 ±0.69***(-21)
TG[a]	18.47 ±0.56	30.23±2.12** *(+1.63F)	25.60 ±2.00*(-15)	25.40 ±1.87*(-16)	25.10 ±1.66*(-17)	24.85 ±1.99*(-17)	24.40 ±1.64***(-19)
AP[b]	17.56 ±1.07	28.62±2.24** *(+1.62F)	24.00 ±2.00*(-16)	23.37 ±1.90*(-18)	23.10 ±1.79*(-17)	22.80 ±9.88**(-25)	22.20 ±1.43*(-22)
(A4) HDL							
TC[a]	45.38 ±4.00	38.14±2.80*(-16)	44.00 ±3.87*(+13)	39.88 ±3.00[NS](+4)	40.10 ±3.33[NS](+5)	40.33 ±3.00[NS](+5)	41.60 ±3.60[NS](+8)
PL[a]	37.40 ±3.14	28.60±2.00** *(-24)	33.17 ±2.60*(+14)	34.10 ±2.70*(+16)	35.21 ±2.81*(+19)	35.28 ±2.17*(+19)	36.00 ±2.81**(+21)
TG[a]	15.10 ±1.00	12.10±0.17*(-20)	15.10 ±1.10*(+20)	15.00 ±1.15*(+19)	15.27 ±1.00**(+21)	15.30 ±1.08**(+21)	15.68 ±1.30***(+23)

AP[b]	168.20 ±12.20	120.30±13.00 ***(-28)	140.37 ±13.00*(+14)	143.88 ±13.70*(+16)	144.17 ±12.14*(+17)	144.20 ±12.44*(+1)	145.00 ±14.00*(+17)
(A5) Plasma							
LCAT[c] Activity	68.00 ±6.00	38.81±3.00** *(-42)	48.40 ±4.00*(+20)	49.16 ±3.87**(+21)	49.66 ±3.18**(+22)	50.00 ±3.97**(+22)	52.88 ±4.39***(+26)
PHLA[d]	17.80 ±1.00	10.00±0.81** *(-44)	13.85 ±0.87***(+28)	14.00 ±1.00***(+29)	14.17 ±0.79***(+29)	15.00 ±1.48***(+3)	15.79 ±1.30***(+37)

Units-(a) mg/dl serum, (b) g/dl, (c) n mol cholesterol released/h/L plasma. (d) n mol free fatty acid formed/hours/ml plasmid value are mean± SD of six ratio P<0.005; P<0.01; P<0.05; HFD compound with control, cholesterol and drug treated with cholesterol. MG 1(100mg/kg), MG 2 (200mg/kg), AM1 (100mg/kg), AM2 (200mg/kg)

Feeding with these extracts, fractions, MG, AM, and gemfibrozil reversed the level of these serum lipids (18-31%) in HFD and drug-treated animals. The analysis of hyperlipidemic serum showed a marked increase in the levels of lipids and Apoproteins constituting β-lipoproteins and those effects were pronounced for VLDL-TG (198%) and LDL-TC (209%) and increase in level. Treatment with these test samples and gemfibrozil significantly reduced these levels of VLDL lipids (17-26% and 20-27%) as well as LDL-TC (22-27% and 30%), PL (16-20% and 27%), TG (15-17% and 19%) and Apo-LDL (16-20% and 22%) respectively (Table A2, A3), and at the same time level of HDL and Apo-HDL in hyperlipidemic rats. HFD feeding caused the inhibition of plasma LCAT (44%), PHLA (41%) (Table A5), total lipolytic activity (45%) in the liver and caused a significant decrease in faecal excretion of cholic acid (42%) and deoxycholic acid (56%). Treatment with MG and AM lowered the level of TC, PL, TG and protein in liver (52%, 55%, 52% and 45%) and simultaneously also showed to recovered the level of cholic and deoxycholic acid by MG and AM (21-23% and 33-38%) and gemfibrozil (26 and 41%) in HFD and drug fed animals.

Antioxidant Activity of SC Extract: The extracts (A1) and fractions (F2, F3, F4, and F5) of plant inhibited *in-vitro* generation of superoxide anion and hydroxyl free radicals by 5.0-31.6% and 7.3-47.6%, respectively. The fraction 47/F2 showed maximum inhibition of superoxide anion generation at 200µg/mL concentration, whereas the generation of hydroxyl free radicals was maximally inhibited by the extract 46/A1 at 200µg/mL concentration. The microsomal lipid peroxidation was found to be inhibited by 3.9–28.7% in the presence of test substances, which was maximum by the fraction 47/F2 at 200µg/mL concentration. MG and AM showed inhibition of superoxide anion, hydroxyl free radicals and microsomal peroxidation by 24 & 28%, 25 & 31% and 27 & 25%, respectively **Fig. 5**.

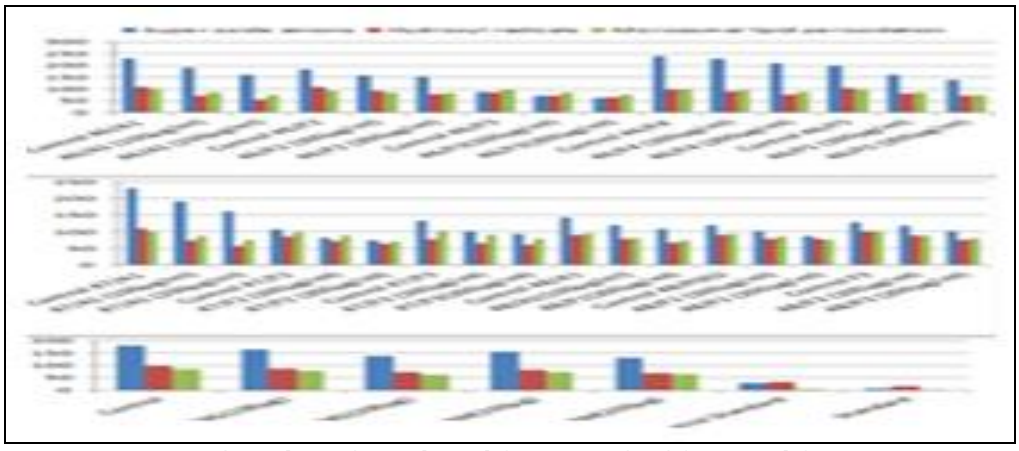

FIG. 5: ANTIOXIDANT ACTIVITY OF SC EXTRACT

Docking Studies: Docking propose numerous conserved interactions between MG and AM and receptor. Both the MG and AM bind in the same active site with several polar as well as hydrophobic interactions with the active site residues that are also consistent with the binding of co-crystallized ligand simvastatin. Polar side chains of Glu559, Arg590, Asp690, and Asn658 are steadily involved in polar interactions with both MG and AM molecules while major hydrophobic interactions are contributed by Met657, Leu853, Ala751, Ala856 **Fig. 6a**.

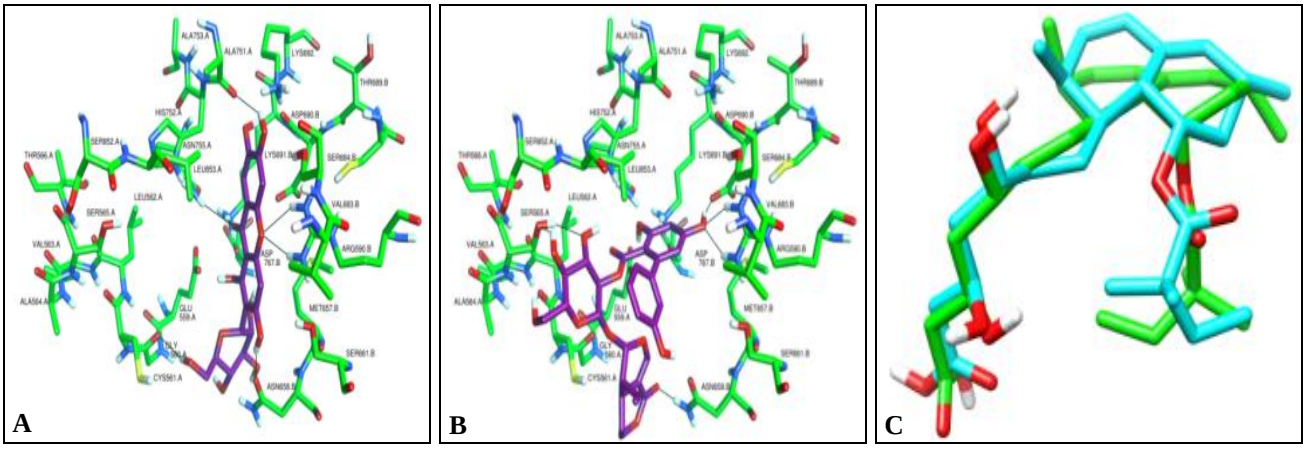

FIG. 6: INTERMOLECULAR INTERACTIONS OF ACTIVE SITE RESIDUES OF HMG- COA REDUCTASE (PDB ID 1HW9) WITH DOCKED CONFORMATION OF (A) MANGIFERIN AND(B) AMAROGENTIN (C) SUPERIMPOSITION OF DOCKED SIMVASTATIN (IN CYAN) OVER CO-CRYSTALIZED SIMVASTATIN (IN GREEN) WITH HMG- COA REDUCTASE (1HW9)

Several polar interactions are found with residues Asp690, Lys691and Ser684, which belongs to cis-loop, which is essential for the formation of HMG-binding site **Fig. 6b**. Flex X docking score (Energy) was found to be -30.589 and -15.708 Kcal/mol for MG and AM, respectively.

CONCLUSION: In conclusion, it was observed that standardized hot water extracts from a different collection and two major markers (MG) and (AM) of SC were effective in *in-vivo* antidyslipidemic activity in both Triton and HFD-induced hyperlipidemic rat model and also demonstrated *in-vitro* antioxidant activity. Treatment of hyperlipidemic rats with MG and AM caused a reduction in plasma levels of LDL and VLDL, as well as increase in levels of HDL, was also observed. In rats with HFD, MG and AM significantly reduced levels of VLDL lipids as well as LDL-TC in hyperlipidemic rats at 100 and 200 mg/kg doses. The antidyslipidemic activity of Amarogentin has been reported for the first time. MG and AM also showed significant inhibition of superoxide anion, hydroxyl free radicals and microsomal lipid peroxidation. In comparison to Pravastatin, MG and AM are found to be potential cholesterol-lowering agents as they demonstrated much better inhibition of HMGR. The docking results suggested that modifications in MG and AM structure might improve its HMGR activity, which will be useful in structure-based design of new chemical entities. Thus, our results support that *Swertia chirayita* hot water extract has therapeutic potential for the treatment of cardiovascular disease and its related disorders.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. WHO. World health statistics. Geneva: World Health Organization; 2009e.
2. Milionis HJ, Kakafika AI, Tsouli SG, Athyros VG, Bairaktari ET, Seferiadis KI and Elisaf MS: Effect of statin treatment on uric acid homeostasis in patients with primary hyperlipidemia. Am Heart J 2004; 148: 635-40.
3. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG and Danesh J: Major lipids, apolipoproteins, and risk of vascular disease: JAMA 2009; 302: 1993-00.
4. Sugamura K and John FK: Free Radic Biol Med 2011; 51(5): 978-92.
5. Drechsler M, Megens RT, Van Zandvoort M, Weber C and Soehnlein O: Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis, Circulation 2010; 122: 1837-45.
6. Vazzana N, Ganci A, Cefalù AB, Lattanzio S, Noto D, Santoro N, Saggini R, Puccetti L, Averna M and Davì G: Enhanced lipid peroxidation and platelet activation as potential contributors to increased cardiovascular risk in the low HDL phenotype: Am Heart Assoc 2013; doi: 10.1161/JAHA.113.000063
7. Podrez EA: Antioxidant properties of high-density lipoprotein and atherosclerosis: Clin Exp Pharmacol Physiol 2010; 37: 719-25.
8. Chatterjee A and Pakrashi SC: In: The Treatise on Indian Medicinal Plants Vol. 1, P.I.D., C.S.I.R, New Delhi 1991; 94-96.

9. Stehouwer CDA, Lambert J, Donkar AJM and van Hinsbergh VWM: Endothelial dysfunction and pathogenesis of diabetic angiopathy. Cardiovascular Research 1997; 34: 55-58.
10. Chen Y, Huang B, Jingsheng H, Han L, Zhan Y and Wang Y: *In-vitro* and *in-vivo* antioxidant effects of the ethanolic extract of *Swertia chirayita*. Journal of Ethnopharmacology 2011; 136: 309-15.
11. Verma VK, Sarva KK and Zaman MK: Antihyperglycemic activity of *Swertia Chirayita* and *Andrographis Paniculata* plant extracts in streptozotocin-induced diabetic rats. International Journal of pharmacy and pharmaceutical sciences 2013; 5: 305-11.
12. Suryawanshi S, Asthana RK and Gupta RC: Assessment of systemic interaction between S. chirata extract and its bioactive constituents in rabbits. Phytotherapy Res 2009; 23: 1036-38.
13. Saha P and Das S: Highlighting the anti-carcinogenic potential of an ayurvedic medicinal plant, *Swertia chirata*. Asian Pac J Cancer Prev 2010; 11(6): 1445-49.
14. Sharma N, Aggarwal SG, Kala RP and Kumar A: Analgesic activity of *Swertia chirayita*. World journal of pharmacy and pharmaceutical sciences. 2013; 2: 3667-75.
15. Ahirwal L, Singh S and Mehta A: Antimicrobial screening of methanol and aqueous extracts of *Swertia chirata*. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3: 142-46.
16. Suryawanshi S, Mehrotra N, Asthana RK and Gupta RC: Simultaneous estimation of Mangiferin & four secoiridoid glycosides in rat plasma using liquid chromatography tandem mass spectrometry & its application to pharmacokinetic study of herbal preparatiom. J Chromatography B 2007; 858(1-2): 211-19.
17. Chakravarty AK, Mukhopadhyay S, Moitra SK and Das B: Syringaresinol, A hepatoprotective agent and other constituents from Swertia chirata. Indian J Chem 1994; 338: 405-08.
18. Nagasaki TA and Kanuma YA: New colorimetric method for determination of plasma lecithin: cholesterol acyltransferase activity. Clin Chem Acta. 1977; 75: 371-75.
19. Wing DR and Robinson DS: Clearing factor lipase in adepose tissue. Biochem J 1968; 109: 841-49.
20. Borstein M and Legmann P: Monograph on atherosclerosis in Lipoprotein precipitation, ed by T. B. Claakson. S. Sagar, London. 1982; 2: 76-83.
21. Chandar R, Singh C and Kapoor NK: Effect of chromic ethanol administration on serum lipoprotein lipid profile in rats. Biochem. Life Sci Adv 1988; 7: 25-27.
22. Mosbach EH, Klenisky HJ, Hai P and Kendall FE: Determination of deoxycholic and cholic acid in bile. Arch. Biochem. Biophys 1954; 51: 402-09.
23. Bhatia G, Puri A, Maurya R, Yadav PP, Khanna AK and Saxena JK: Anti-dyslipidemic and Antioxidant activities of different fractions of *Pongamia pinnata* (lin.) fruits. Medicinal Chemistry Research 2008; 17: 281-89.
24. Sybyl, version 7.1, Tripos, Inc., St.Leuis, MO 2005.

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