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Antidiabetic and Antihyperlipidemic activity of Chrysoeriol in diabetic rats, role of HMG CoA reductase, LCAT and LPL: *In vivo and in silico* approaches

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ABSTRACT

Objective: Diabetes mellitus is associated with dyslipidemia, which is a significant risk factor for cardiovascular complications. The present study was designed to examine the antihyperlipidemic effect of Chrysoeriol (CS) in streptozotocin (STZ) induced diabetic rats. **Methods:** Diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (40 mg/kg b.w.). **Results**: Diabetic rats showed increased plasma glucose, Glycosylated Hb, total cholesterol, triglycerides, free fatty acids, phospholipids, low density lipoprotein, very low density liprotein, atherogenic index and decreased insulin and high density lipoprotein in diabetic rats. The activity of 3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA) reductase significantly increased and decreased levels of lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase (LCAT). In addition, the molecular docking of Chrysoeriol against HMG CoA reductase, LPL and LCAT involved in cholesterol biosynthesis using the Argus software. Diabetic rats were treated with Chrysoeriol shifted all these parameters towards normality. Chrysoeriol has shown the best ligand binding energy -7.59, -7.82 and -6.94 Kcal/mol. **Conclusions**: In conclusion, this study indicates that Chrysoeriol showed an antihyperlipidemic effect in addition to its antidiabetic effect in diabetic rats.

KEYWORDS: Chrysoeriol, Streptozotocin, Antihyperlipidemic, Diabetes mellitus, 3-hydroxy 3-methylglutaryl coenzyme A reductase, Lecithin cholesterol acyltransferase

1. INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycaemia, hypercholesterolemia and hypertriglyceridemia resulting from deserts in insulin secretion followed by dysfunction and failure of organs, especially the eyes, kidneys, nerves, heart and arteries[1]. It is estimated that approximately 5% of the global population are diabetic, with 85–95% of this being assigned to non-insulin dependent DM or type 2 diabetes[2].

In addition to hyperglycaemia, type 2 diabetic individuals suffer from a constellation of metabolic CV risk factors, most importantly the modified lipid profile characterized by elevated levels of circulating FFAs and TGs, a reduction in HDL-C along with excess fat deposition in various tissues including the liver. An abnormal aggregation of fat in the liver and muscle elicits insulin resistance that culminate in beta cell reduction in type 2 diabetes[3]. The currently available drug regimens for the treatment of type 2 diabetes are known to cause some

adverse drug reactions[4]. Hence, the effective management of DM relies in optimizing glucose control along with lipid homeostasis that could predispose patients to cardiovascular complications. Phytochemicals are promoted for the prevention and treatment of numerous health conditions, including cancer, heart disease, diabetes, and high blood pressure. Some phytochemicals have either antioxidant or insulinotrophic activity on the existing ß-cells[5].

CH belongs to the family Sapindaceae. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snakebite its roots for nervous diseases, as a diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic and sudorific[6], its leaves and stalks are used in the treatment of diarrhea, dysentery and headache and as a poultice for swellings[7]. The whole plant CH contains saponins, traces of alkaloids, flavanoids, proanthocyadin, apigenin, and phytosterols (e.g. stigmasterol).

Chrysoeriol (5,7-dihydroxy-2-(4-hydroxy-3- methoxyphenyl) chromen-4-one) and diosmetin were possessed many biological effects similar to luteolin, such as antioxidant, anti-inflammatory, and anti-osteoporotic effects[8, 9]. Furthermore, CS were natural pro drugs in cancer prevention[10, 11] and We also viewed the free radical scavenging activity[12]. The structure of CS is depicted as below (Fig.1).

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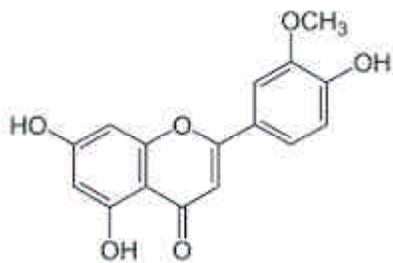└─────────────────────────────────────┘

Fig.1. Structure of Chrysoeriol

In this view, the aim of the present study was to investigate the antihyperlipidemic effects of CS on plasma, tissue lipid profiles and lipid metabolizing enzymes in STZ- induced diabetic rats.

2. MATERIALS AND METHODS

Plant Material
The plant CH leaves were collected in March-April 2012 from the Department of Agriculture Annamalai University and authenticated by Department of Botany and a voucher specimen was deposited at the herbarium of botany further reference.

Isolation of Chrysoeriol from *Cardiospermum halicacabum* leaves
Chrysoeriol was isolated from the leaves *Cardiospermum halicacabum* and tested its antioxidant potency *in vitro*[12]. The isolation of CS was followed by the method[13].

Chemicals
Streptozotocin was purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Commercial diagnostic kits were obtained from Qualigens Diagnostics, Mumbai, India for determination of cholesterol, HDL-C and TGs. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Animals
Adult Male albino Wistar rats (9 weeks old; 180–200 g) were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in air-conditioned room ($25 \pm 10\,^0C$) with a 12 h light/dark cycle. Feed and water were provided ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital, Annamalai University (Reg. No. 160/1999/CPCSEA, Proposal No: 539).

Induction of experimental diabetes
Experimental diabetes was induced in 12 h fasted rats by single (i.p.) injection of STZ (40 mg/kg B.W.) dissolved in cold citrate buffer (0.1 M, pH 4.5). STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemia. STZ-injected animals exhibited hyperglycemia within a few days. Diabetic rats were confirmed by measuring the elevated plasma glucose (by the glucose oxidase method) 72 h after injection with STZ. The animals with glucose above 235 mg/dl were selected for the experiment.

Experimental design
The experimental animals were divided into five groups, each group consists of a minimum of six rats detailed as given below. CS and glibenclamide were administered orally for 45 days.

Group I	:	Normal control (0.5% DMSO)
Group II	:	Control + Chrysoeriol (20 mg/kg BW)
Group III	:	Diabetic control (0.5% DMSO)
Group IV	:	Diabetic + Chrysoeriol (20 mg/kg BW)
Group V	:	Diabetic + glibenclamide (600 µg/kg BW)

Anesthetized between 8:00 a.m. and 9:00 a.m., using ketamine (24 mg/kg b.w) and sacrificed by cervical decapitation. Blood samples were collected in tubes containing EDTA. The plasma was obtained after centrifugation ($2000 \times g$ for 20 min at 4^0C) and used for various biochemical measurements. The liver, kidney and heart were excised immediately, washed with ice cold isotonic saline and stored at-80 ^0C until analyzed.

Biochemical estimations
Plasma glucose levels were estimated using a commercial kit (Sigma Diagnostics Private Limited, Baroda, India) by the method[14]. HbA1c was estimated by diagnostic kit (Agappe Diagnostic Private Limited, India)[15], Plasma insulin was assayed by ELISA kit (Boeheringer–Manneheim Kit, Mannheim, Germany) respectively.

Extraction of lipids
Total lipids were extracted from plasma and tissues according to the method[16]. The total cholesterol was estimated by the method[17]. The TG was estimated by the method[18]. FFAs were estimated by the method[19]. PLs level was estimated by the method[20].

Measurement of plasma lipid profile levels
Plasma total cholesterol (TC), TGs, HDL-C, LDL-C and HDL-C were determined using Diagnostic Kit Qualigens Diagnostics, Mumbai, India. VLDL-C and LDL-C were calculated as VLDL-C = TG/5 and LDL-C = total cholesterol - (HDLC + VLDL-C) [21]respectively.

Estimation of lipid marker enzymes
The activity of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase in the liver was assayed by the method[22]. Plasma lipoprotein lipase (LPL) activity was assayed by the method[23]. Plasma lecithin cholesterol acyl transferase (LCAT) activity was assayed by the method[24].

Docking study

The 3-D crystal structure of the targeted diabetic protein Chrysoeriol (PDB: ID: 5280666) was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb). Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and PyMOL (Python molecular visualization software, 2006). The chemical name CS was screened according to Lipinski's rule of five, a compound having not more than 3 hydrogen bond donors (OH and NH groups), not more than 6 hydrogen bond acceptors (notably N and O), molecular weight under 300 g/mol, partition coefficient log P of less than 1.7 and rotatable bonds of less than 2 was taken as drug molecule and docking procedure was carried out against the HMG CoA, LPL and LCAT[25].

Amino acid binding sites

The Pubchem database was used for retrieving the CS molecules. The selected chemical (specification) by using the Marvin Sketch Software. The predicted binding site, based on the binding energy, and amino acids make up of the binding cavity. The predicted ligand binding site residues are LPL (PRO62, PHE52, ALA51,CYS63, LYS73, LEU67) HMG-CoA(TYR533, PRO513, ARG515, PRO926, TYR517, PRO903, PRO813) LCAT (SER33, LEU31, CYS56, ILE53). The molecular docking was performed using Argus lab, widely distributed public domain molecular docking software. The inhibitor and target protein were geometrically optimized and docked using docking engine Argus lab.

Statistical analysis

The experimental results are expressed as the means ± SD and subjected to One-Way Analysis of Variance (ANOVA), using a computer software package (SPSS version 16.0, SPSS Inc., Cary, NC) and the comparisons of significant groups were performed using the Duncan Multiple Range Test (DMRT), at p < 0.05.

3. RESULTS

Table 1 shows the levels of plasma glucose, HbA1c and insulin in control and diabetic rats. There was a significantly increased levels of plasma glucose, HbA1c and a decreased in plasma insulin in diabetic rats compared with control rats. Oral administration of Chrysoeriol to diabetic rats significantly decreased the levels of glucose, HbA1c and increased the levels of insulin compared with diabetic control rats.

Table 2 depicts the levels of plasma TC, TGs, PLs and FFAs in control and diabetic rats. The levels of plasma TC, TGs, PLs, FFAs, LDL-C, VLDL-C and atherogenic index increased, whereas levels of HDL-C

was decreased in diabetic rats compared with the control rats. Oral treatment with Chrysoeriol of diabetic rats were significantly reversed all these changes to near normal levels.

Table 1. Effect of CS on, plasma glucose, insulin and glycosylated haemoglobin in STZ- induced diabetic rats

Groups	Glucose (mg/dl)	Glycosylated Hb (mg/g of Hb)	Insulin (μU/mL)
Normal control	90.36±8.27[a]	4.70±0.32[a]	17.68±1.36[a]
Normal+ CS (20 mg/kg BW)	88.17±6.96[a]	4.26±0.30[a]	18.88±1.51[a]
Diabetic control	282.16±22.56[b]	13.02±1.26[b]	6.23±0.49[b]
Diabetic + CS (20 mg/kg BW)	133.27±10.42[c]	6.52±0.52[c]	15.87±1.49[c]
Diabetic + Glibenclamide (600 μg/kg BW)	125.40±8.91[c]	5.82±0.43[c]	16.39±1.22[c]

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly value *(a,b,c) at P< 0.05 (DMRT).*

Table 2. Effect of CS on TC, TGs, HDL-C, VLDL-C, LDL-C, FFAs, PLs and atherogenic index in the plasma of control and experimental rats.

Groups	Normal control	Control + CS (20 mg/kg b.w.)	Diabetic control
TC (mg/dL)	76.84±4.27[a]	72.84±3.94[a]	153.63±9.56[b]
TGs (mg/dL)	57.43±3.22[a]	55.77±2.09[a]	164.39±8.37[b]
HDL-C (mg/dL)	48.21±2.78[a]	47.73±4.83[a]	23.84±1.96[b]
VLDL-C (mg/dL)	11.48±0.86[a]	11.15±0.72[a]	32.87±1.87[b]
LDL-C (mg/dL)	16.51±1.24[a]	13.96±1.01[a]	96.92±6.79[b]
FFAs (mg/dL)	63.24±2.64[a]	61.17±3.19[a]	109.39±8.02[b]
PLs (mg/dL)	78.48±3.87[a]	76.72±4.50[a]	145.06±11.27[b]
Atherogenic index	1.49±0.03[a]	1.52±0.05[a]	6.44±0.14[b]

Groups	Diabetic + CS (20 mg/kg b.w.)	Diabetic + glibenclamide (600 μg/kg b.w.)
TC (mg/dL)	90.26±6.02[c]	82.08±5.83[d]
TGs (mg/dL)	86.25±4.76[c]	79.42±6.19[d]
HDL-C (mg/dL)	41.86±3.02[c]	43.91±3.57[ac]
VLDL-C (mg/dL)	17.25±1.64[c]	15.88±1.12[cd]
LDL-C (mg/dL)	31.14±2.64[c]	22.29±1.58[d]
FFAs (mg/dL)	74.86±5.77[c]	65.51±4.86[a]
PLs (mg/dL)	105.84±8.46[c]	99.09±6.04[d]
Atherogenic index	2.15±0.06[c]	1.86±0.02[d]

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at P< 0.05 (DMRT).

Table 3 shows the levels of TC, TGs, FFAs and PLs in liver, kidney and heart of control and diabetic rats. Diabetic rats showed elevated levels of TC, TGs, FFAs and PLs when compared with normal rats.

Rats treated with Chrysoeriol there was a significant reduction in the content of TC, TGs, FFAs and PLs in both the tissues, when compared with diabetic control rats.

Table 3. Effect of CS on TC, TGs, FFAs and PLs in the liver, kidney and heart of control and experimental rats.

Groups	Normal Control	Control + CS (20 mg/kg b.w.)	Diabetic control
TC (mg/g wet tissue)			
Liver	4.18 ± 0.27[a]	3.54 ± 0.18[a]	6.40 ± 0.41[b]
Kidney	4.53 ± 0.22[a]	4.32 ± 0.29[a]	6.14 ± 0.43[b]
Heart	2.56 ± 0.12[a]	2.28 ± 0.09[a]	5.14 ± 0.25[b]
TGs (mg/g wet tissue)			
Liver	3.49 ± 0.30[a]	3.32 ± 0.25[a]	7.52 ± 0.47[b]
Kidney	3.69 ± 0.35[a]	3.34 ± 0.23[a]	7.04 ± 0.41[b]
Heart	4.09 ± 0.25[a]	3.98 ± 0.22[a]	6.12 ± 0.32[b]
FFAs (mg/g wet tissue)			
Liver	7.72 ± 0.42[a]	7.58 ± 0.55[a]	12.33 ± 0.74[b]
Kidney	4.43 ± 0.35[a]	4.24 ± 0.32[a]	9.68 ± 0.65[b]
Heart	5.22 ± 0.32[a]	5.17 ± 0.22[a]	14.14 ± 1.23[b]
PLs (mg/g wet tissue)			
Liver	22.02 ± 1.28[a]	22.44 ± 1.42[a]	45.27 ± 3.16[b]
Kidney	17.02 ± 1.29[a]	17.07 ± 1.39[a]	32.32 ± 2.73[b]
Heart	12.02 ± 1.12[a]	12.34 ± 0.72[a]	24.17 ± 1.67[b]

Groups	Diabetic + CS (20 mg/kg b.w.)	Diabetic + glibenclamide (600 µg/kg b.w.)
TC (mg/g wet tissue)		
Liver	4.87 ± 0.35[c]	4.36 ± 0.31[a]
Kidney	5.43 ± 0.27[c]	4.34 ± 0.41[d]
Heart	2.77 ± 0.17[c]	2.34 ± 0.13[ad]
TGs (mg/g wet tissue)		
Liver	4.43 ± 0.44[c]	4.12 ± 0.43[d]
Kidney	4.44 ± 0.33[c]	4.14 ± 0.25[c]
Heart	4.54 ± 0.20[c]	4.12 ± 0.31[ad]
FFAs (mg/g wet tissue)		
Liver	9.24 ± 0.63[c]	8.42 ± 0.54[d]
Kidney	6.06 ± 0.35[c]	5.32 ± 0.40[d]
Heart	8.25 ± 0.21[c]	7.09 ± 0.42[ad]
PLs (mg/g wet tissue)		
Liver	27.32 ± 1.70[c]	25.73 ± 1.19[ac]
Kidney	20.34 ± 1.67[c]	19.23 ± 1.52[c]
Heart	15.02 ± 1.14[c]	14.23 ± 1.04[d]

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at P< 0.05 (DMRT).

The activities of LCAT, LPL HMG-CoA reductase of normal and diabetic rats are shown in Table 4. Decreased activities of LCAT, LPL and increased activity of HMG-CoA reductase were observed in plasma of diabetic rats as compared to normal rats. Administration of

Chrysoeriol to the diabetic rats showed significantly increased in the activities of LCAT, LPL and decrease the activity of HMG-CoA reductase, when compared to diabetic control rats.
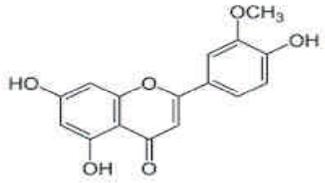
Molecular modelling (docking) study was carried out for series of CS for HMG CoA reductase, LCAT and LPL. Thus, the protein was targeted against pocket. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 5 and 6. Given the three-dimensional structure of a target receptor molecule, usually a protein; Chrysoeriol having potential affinity towards site is designed rationally, with the aid of computational methods (Fig. 2,3 and 4).

Table 4. Effect of CS on the activities of plasma LPL, LCAT and liver HMG-CoA in normal and diabetic rats.

Groups	LPL (µmoles of glycerol liberated/h/l)	LCAT (µmoles of cholesterol esterified/h/l)	HMGCoA/ mevalonate ratio
Normal control	7.38±0.47[a]	72.05±5.27[a]	1.62±0.09[a]
Normal+ CS (20 mg/kg BW)	7.41±0.49[a]	72.12±5.34[a]	1.58±0.08[a]
Diabetic control	5.09±0.32[b]	58.22±4.72[b]	0.84±0.04[b]
Diabetic + CS (20 mg/kg BW)	6.40±0.50[c]	65.43±5.62[c]	1.35±0.06[c]
Diabetic + glibenclamide (600 µg/kg BW)	6.75±0.62[c]	66.27±5.78[c]	1.42±0.07[c]

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly value *(a,b,c)*
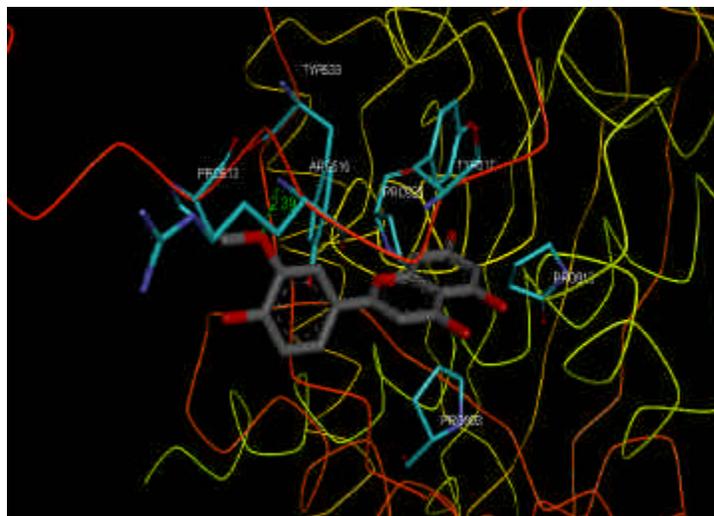*at P< 0.05 (DMRT).*

Table 5. Docking results of CS

Compound name	Pubchem ID	Structure
Chrysoeriol	5280666	

Molecular weight (g/mol)	Molecular Formula	ClogP	Hydrogen bond acceptors	Hydrogen bond donors.	Rotatable bonds.
300.26288	$C_{16}H_{12}O_6$	1.7	6	3	2

Table 6. Docking results of CS against LPL, LCAT and HMG-COA protein.

S.No	Target proteins [PDB ID]	No of H-bonds	H-bonds distance	Docked Protein residues	Binding energy [kcal/mol]
1	lipoprotein lipase (LPL) (ILPA)	4	3.16,2.87,2.55,2.38	PRO62, PHE52, ALA51,CYS63, LYS73, LEU67	-7.82
2	3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA) reductase (2Q11)	1	2.39	TYR533, PRO513, ARG515, PRO926, TYR517, PRO903, PRO813	-7.59
3	lecithin cholesterol acyltransferase (LCAT) (4X90)	1	3.00	SER33, LEU31, CYS56, ILE53	-6.94

(a)

(b)

Fig. 2. Docking study (a) protein lipoprotein lipase (LPL)– ligand (CS) docking structure, (b) neighbour residues of ligand molecules.
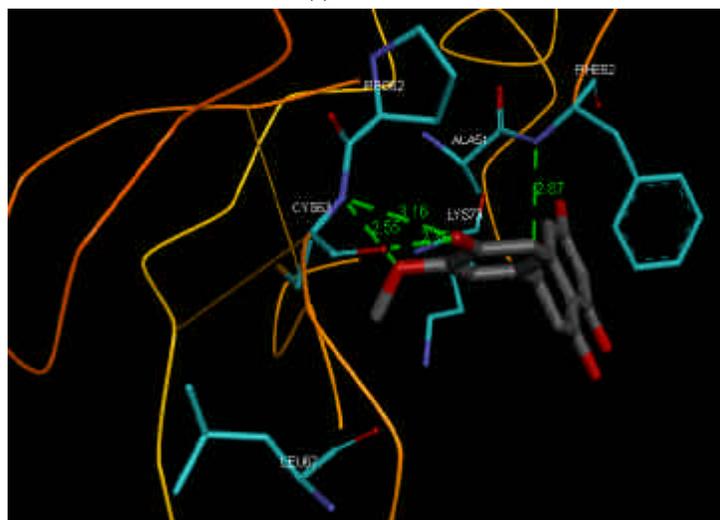

(a)
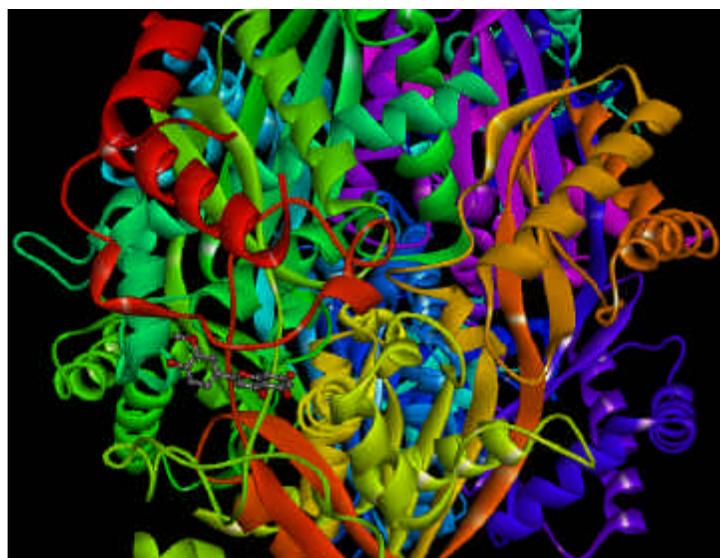

(b)

Fig. 3. Docking study (a) protein (HMG CoA reductase) – ligand (CS) docking structure, (b) neighbour residues of ligand molecules.
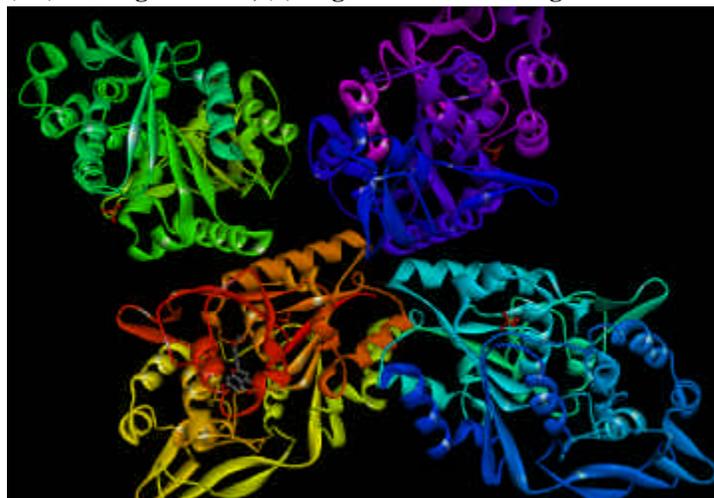

(a)

(b)

Fig. 4. Docking study (a) protein lecithin cholesterol acyltransferase (LCAT). ligand (CS) docking structure, (b) neighbour residues of ligand molecules.

4. DISCUSSION

STZ-induced hyperglycaemia has been depicted as a useful experimental model to study the activity of hypoglycemic agents. Previous literature exposed that the antihyperglycemic potential of Diosmetin by assessing its modulatory effects on the activities of carbohydrate metabolizing enzymes in the hepatic tissues of STZ–induced diabetic rats[26]. In our results, the exalted levels of blood glucose and HbA1c in diabetic rats were returned back to near normal levels treated with CS showing its antihyperglycemic potential in diabetic rats. In the present investigating, we attempted to assess the putative antihyperlipidemic effect of CS in diabetic rats. A number of phytochemicals have been described to possess hypoglycemic effects and the possible mechanism proposed for such hypoglycemic action could be through an increased insulin secretion from ß-cells of islets of Langerhans[27] or its release of bound insulin or such hypoglycemic effects of flavonoid and isoflavone could also be because of their insulin-like actions[26, 28]. Similar mechanism may be creditworthy for the hypoglycemic action shown by CS in diabetic rats.

In our study, a marked increase in the lipid content of plasma, liver, kidney and heart was found in STZ- induced diabetic rats that are mainly due to increased militarisation of FFAs from peripheral depots. It is well known that insulin inhibits the hormone sensitive lipase[29]. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. Therefore, hyperlipidemia that qualifies the diabetic state may be regarded as a consequence of uninhibited actions of lipolytic hormones on the fat depots[30]. The higher concentrations of cholesterol can increase the risk of oxidative disease treat due to susceptibility of cholesterol to oxidation while it is in circulation. A deficiency of insulin is associated with increased in cholesterol levels due to the raised mobilization of lipids from the adipose tissue to the plasma. The increased concentration of FFAs in the liver, kidney and heart may be due to lipid break up, which leads to increased generation of NADPH and activation of NADPH- dependent microsomal lipid peroxidation[31]. Additionally, PLs are vital components of biomembranes and play an crucial role in the transport of TGs[32]. In diabetic rats, the elevated level of PLs may be due to the elevated levels of FFAs, TG and total chlolesterol[33], which can promote the synthesis of PLs[34]. Moreover, hypertriglyceridemia is a common finding in patients with diabetes mellitus related with vascular complication[35]. Braun and Severson[36] have reported that a deficiency of LPL activity may contribute significantly to the elevation of TGs in diabetes. Lopez-Virella[37], reported that treatment of diabetes with insulin served to lower plasma triglyceride levels by returning LPL activity to normal. In this context, a fall in TGs level following administration of CS might be due to increased insulin secretion, which in turn increases LPL activity. In addition, engulfmentt of insulin secretion also inhibits hormone sensitive lipase and increases the utilization of glucose and thereby decreasing the mobilization of FFAs from the fat depots. The decreased level of FFAs is also associated with decreased actions of lipolytic hormones.

Lipoproteins are an independent risk factor for the development of atherosclerotic disease. In our study an increase in plasma LDL, VLDL and atherogenic index along with a decrease in HDL were observed in diabetic rats. This increased LDL concentration in the plasma of diabetic rats might be due to the defect in insulin secretion. In particular, many studies have found LDL to be the most dangerous among the plasma lipids, and the oxidation of LDL leads to its increased incursion of arterial walls[38]. When there is excess LDL in the blood and also accumulate in the extracellular sub endothelial space of arteries and are highly atherogenic and toxic to vascular cells, thereby extending to atherosclerosis, hypertension, obesity, functional depression in some organs, etc[39]. Type 2 diabetes is often associated with an increased LDL fraction that is most of hepatic origin. The observed increase in plasma VLDL in the untreated group may be due to the increased re-uptake of VLDL from the liver or due to decreased affinity to LPL[40]. HDL carries cholesterol and cholesterol ester from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids[41]. This pathway plays a very important role in bringing down cholesterol levels in the blood and peripheral tissues, and in inhibiting atherosclerotic plaque formation in the aorta. The Lower level of plasma HDL has been observed in adults with type 2 diabetes mellitus[42]. The decreased plasma HDL level in diabetic rats may be due to the decreased production of HDL by the intestine and liver and or due to the glycation of HDL or its apoproteins by high glucose rendering it more pro-atherogenic. Our results showed that administration of CS experts potential antihyperlipidemic effects in diabetic rats as shown by the further decrease in levels of LDL, VLDL, atherogenic index and increase in the level of HDL. CS thus has the potential to prevent the formation of atherosclerosis and CHD, which are the secondary complications of diabetes mellitus. Studies have shown that atherogenic index is a good predictor of cardiovascular disease risk as well as efficient monitoring of the effectiveness of lipid-lowering therapies since the LDL/HDL ratio has been conceived more prognostic than LDL or HDL alone[43].

LCAT is the key enzyme in mediating the esterification of cholesterol on circulating lipoproteins. It is critically important in facilitating HDL particle stability and HDL maturation. Chronic hyperglycemia leads to non-enzymatic glycation of proteins, including Apo A-I. Glycation of HDL on serine residues in its most abundant protein moiety (Apo A-I) causes conformational changes eventually resulting in reduced LCAT activity[44,45]. In addition, LCAT is possibly glycated and inacti-

vated due to high concentrations of glucose in plasma. The decreased LCAT activity was observed in the plasma of diabetic rats in this study. The decreased LCAT activity markedly decreased the levels of mature HDL particles, which may finally lead to severe impairment of the HDL-C synthesis as well as TGs metabolism in diabetic rats. We observed a significant increase in plasma LCAT activity of CS administered diabetic rats when compared with diabetic rats. This hypolipidemic effect of CS may be due to an increase insulin secretion and decrease plasma glucose that ultimately led to increase plasma LCAT activity. This could be revelatory of an enhanced capacity for HDL maturation and the shift towards improved HDL level seen with CS treatment. This finding is ordered with other studies reporting that diosmin, a flavonoid treatment increase LCAT activity[46].

LPL is a key enzyme in TGs catabolism which supplies the body with fatty acids by hydrolysing TG[47]. It plays a key role in hydrolyzing TGs in chylomicrons and VLDL at the first step in their metabolism. LPL is one of the enzymes regulated by insulin and its plasma activity excogitates insulin sensitivity[48]. The decreased LPL activity seen in the untreated rats may be due to its glycation[49]. Deficiency of lipoprotein lipase activity may conduce significantly to the elevation of TGs in diabetes and those levels were lowered upon treatment with insulin by increasing the activity of lipoprotein lipase[50]. In this context, the hypolipidemic effect of CS can be explained as a result of direct reduction in the blood glucose concentration via raised secretion of insulin which in turn increases lipoprotein lipase activity. In addition, enhancement of insulin secretion also inhibits hormone sensitive lipase and increases the utilization of glucose and thereby decreasing the mobilization of FFAs from the fat depots.

HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis. Some inhibitors of HMG-CoA reductase can inhibit cholesterol synthesis and lower cholesterol levels. Decreased HMG-CoA/mevalonate ratio indicates increased activity of the enzyme. The previous study reported that organic phosphates may phosphorylate and inhibit the HMG-CoA reductase, the key enzyme in cholesterol synthesis[51,52]. This pathway plays an important role in reducing cholesterol levels in the blood and peripheral tissues, and inhibiting atherosclerotic plaque formation in the aorta[53]. Rising evidence indicates that the activity of HMG-CoA reductase is increased in diabetic rats [54, 55] and deficiency of insulin is associated with increased HMG-CoA reductase activity[56]. In the present study, we found that treatment with Chrysoeriol in diabetic rats had a positive effect on the marker enzymes of lipid metabolism. The increased insulin output in diabetic animals caused by Chrysoeriol could activate LPL thereby increasing the HDL fraction in the animals, lowering the intestinal absorption of cholesterol and enhancing the excretion of consumed cholesterol and inhibition of HMG-CoA reductase.

Bioinformatics analysis offers a convenient methodology for efficient *in silico* preliminary analysis of possible function of new drugs. The target protein and inhibitors were geometrically optimized. Chrysoeriol as an inhibitor was docked against the active site of the target protein using Argus lab. Chrysoeriol has a binding energy of -7.59,-7.82 and -6.94 kcal/mol.

5.CONCLUSION

In conclusion, from the present findings, it is well documented that the Chrysoeriol plays a part in the management of diabetes and the prevention of its vascular complications in diabetic rats and it may be useful in the treatment of hyperlipidaemia in diabetic patients.

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