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Pharmacological screening of newly synthesized compounds, CGH-1 and CGH-2 for hypolipidemic activity in rats

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Abstract: In the present study, we have investigated the effect of two newly synthesized compounds, CGH-1 and CGH-2 for their potential as hypolipidemic agents against high cholesterol diet (HCD)-induced atherosclerosis in rats. CGH-1 & CGH-2 were synthesized as ring open derivatives of the parent compound, LM-1554. Hyperlipidemia was induced by administration of high cholesterol diet in rats. Test compounds, CGH-1 & CGH-2 were administered at dose of 40 & 100 mg/kg along with high cholesterol diet. Blood samples were collected after 40 days administration of high cholesterol diet for the estimation of serum lipid profile (TC, TGs, HDL-C& LDL-C). The animals were sacrificed on 41st day and thoracic aorta was isolated from each group for histopathological studies (H & E staining). Compound CGH-1 was found to be more effective as compared to CGH-2 in treated groups in reducing the cholesterol level in serum. The compounds are supposed to act through cholesterol absorption in GIT, specifically *via* ACAT and/or CETP inhibition. It could provide the rationale for their therapeutic potential in treating hyperlipidemia.

Keywords: Hyperlipidemia, Atherosclerosis, Animal model, CETP, ACAT

Introduction

Cardiovascular diseases are the major cause of morbidity and mortality worldwide and responsible for 30-35% of death in industrialized countries [1]. Hypercholesterolemia is considered as one of the most important risk factors and consequently a primary therapeutic target [2]. Measurement of serum lipid concentration helps in identification of the subject with cardio metabolic abnormalities or risk of cardiovascular diseases. Atherosclerosis is considered as the major cause of more than half of all deaths in the western worldwhereas; hyperlipidemia is the known cause of coronary heart disease and ischemic heart disease [1].

Although, the benefits of lowering cholesterol level has been widely known for the prevention of heart diseases [3-4]. Alternative modes of treatment of CVS diseases are being investigated. Due to various limitations in the available therapies, there is extreme need to explore some new targets against hyperlipidemia. Acetyl-Coenzyme A: cholesterol acyltransferase (ACAT) and cholesteryl ester transfer protein (CETP) are the new targets which are directly or indirectly involved in hyperlipidemia. ACAT supposed to converts the free cholesterol into cholesterylesters whereas CETP mediates the net transfer of cholesteryl esters from atheroprotective high density lipoproteins to atherogenic low density lipoproteins or very low density lipoproteins [5-6]. Hence, ACAT and CETP can be entitled as "entwined twins" and simultaneous targeting of both the enzymes can yield best therapeutic results.

Compound LM-1554 **1** is considered as the potent anti-hyperlipidemic compound and has poor bioavailability and low volume of distribution [7]. Beside this, Jain and co-workers [8] have further modified this basic scaffold to improve the biological profile of LM-1554. They have reported condensed 2-chloromethyl-4-chloro/hydroxyl-5,6-disubstitutedthieno(2,3-*d*) pyrimidine**2**. Inspired from these studies, we have designed new series of compounds for the evaluation of their anti-hyperlipidemic activity. CGH-1 **3a** and CGH-2 **3b** are considered as the ring open derivatives of LM-1554**1**, and are expected to show improved hypolipidemic activity (Fig. 1).

Material and methods

a)Drugs and chemicals used: Ezetimibe, CGH-1 and CGH-2 were used in the experimental protocol. Ezetimibe was received ex-gratia from Indswift Pharmaceuticals Ltd., Baddi (HP), Indiawhereas CGH-1 and CGH-2 has been synthesized. Total Cholesterol kit, Triglycerides kit and HDL-C kit (Coral Clinical System Pvt. Ltd, Goa, India) were used for the estimation of serum lipid profile.

b) Experimental animals: Wistar rats weighing 180-200 gm of either sex were used. All animals were housed at ambient temperature (25±3°C) and relative humidity (55±5%) with fixed 12h light/dark cycle. Animals had free access to

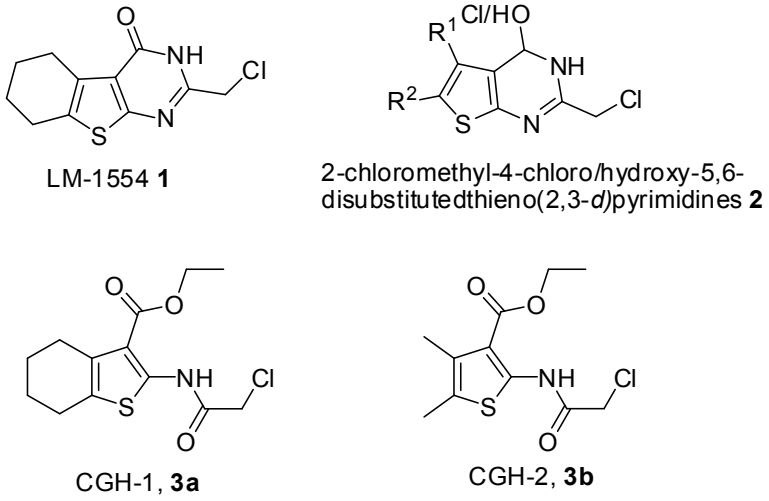


Fig. 1 Some of the potent anhyperlipidemic compounds.

Fig. 1 Basic scheme for synthesis of 2-chloroacetamido thiophenes (CGH-1 & CGH-2)

standard pellet diet and water given *adlibitum*. The experimental protocol (Ref. No. ISFCP/IAEC/CPCSEA/M9/2014/171) was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity study

Doses of CGH-1 and 2 were selected from acute toxicity studies performed according to OECD (Organization for Economic Co-operation and Development) guidelines; Section 423. On the basis of the toxicity study, 40 and 100 mg/kg doses were taken for *in-vivo* studies.

High cholesterol diet (HCD) –Induced model for atherosclerosis in rats

a) Experimental grouping

Hyperlipidemia in rats was induced by administration of HCD (4% cholesterol, 1% cholic acid and groundnut oil, q.s.) for 40 days in standard rat chow diet [11]. The rats were divided into seven groups (n=6).

Group 1, served as normal control (saline), Group 2, received HCD, Group 3, received HCD + Ezetimibe (7.5 mg/kg), Group 4 & 5 received HCD + CGH-2 (40 & 100 mg/kg), Group 6 & 7, received CGH-1 (40& 100 mg/kg). Food intake and weight gain in rats of each group were observed for 40 days.

b) Estimation of serum lipid profile

On 41st day, blood samples were collected from the tail vein after 8 h fasting and allowed to clot for 30 minutes at room temperature, then centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimation of lipid profile. On 41st day, serum lipid profile (HDL-C, TC, TGs, LDL-C) was then estimated using respective Coral kits. The serum total cholesterol (TC) was estimated by cholesterol oxidase peroxidase (CHOD/PAP) method [12], serum TGs was estimated by glycerophosphate oxidase peroxidase (GOD/PAP) method [13] and HDL was estimated by PEG (Polyethylene Glycol) precipitation method [12].

LDL-C level was estimated using the following formula;

(Freidewald's Formula)

LDL cholesterol (mg/dl) = (Total Cholesterol) – (Triglycerides/5) – (HDL Cholesterol)

c) Assessment of histological parameters Hematoxylin& Eosin (H & E) staining

The animals were sacrificed on 41st day for serum lipid estimation and the thoracic aorta was isolated for histopathological studies. Each tissue was fixed overnight in 10% formaldehyde at 4°C. Tissues were embedded in paraffin and cut into 4 µm cross-sections. Microscopic examination was performed with hematoxylin-eosin stained sections to assess fatty changes in thoracic aorta.

Statistical analysis

All the results obtained were expressed as mean ± S.E.M. The data obtained for behavioral parameters were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test. Whereas, data obtained for all biochemical parameters were analyzed using one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons post-hoc test. $P<0.05$ was considered statistically significant.

Synthesis of the CGH-1 (3a)and CHG-2 (3b). a) Synthesis of 2-amino-3-carbethoxy-4, 5, 6,

7-tetrahydrobenzo(*b*)thiophene (4)

Cyclohexanone (12.0 g, 0.122 mol), sulfur (3.92 g, 0.122 mol), ethyl cyanoacetate (13.82 g, 0.122 mol) and methanol (25 ml) were mixed in a 250 ml round bottom flask and stirred together at 60 ºC. To this well stirred mixture, diethylamine (11.55 g, 0.152 mol) was added dropwise in 0.5 h and stirring was continued for 3 h at ambient temperature. The reaction mixture was allowed to attain RT and thereafter kept in refrigerator overnight. The solid separated was filtered and washed with 20 ml chilled 50% aq. methanol. The product having m.p. 110-112°C was characterized as **4**. Purity of the product was checked up by TLC using ethyl acetate:hexane (7:3) as mobile phase.

Mol. Formula: $C_{11}H_{15}NO_2S$; MW 225.31; $R_f$value: 0.69; Colour: yellow;IR (KBr) cm^{-1}: 3404.97, 3299.51 (γ_{N-H}), 3167.37, 3076.70, 2986.47 (γ_{C-H}), 1647 (γ_{COOEt}); ^1H NMR (300MHz, $CDCl_3$, TMS =0)δ: 1.38 (*t*, 3H, *J*= 5.1 Hz), 1.78 (*s*,4H), 2.63(*s*,2H),2.75(*s*,2H), 4.35-4.28(*m*,2H),11.25(*s*,2H).

b) Preparation of CGH-1 (ethyl-2-(2-chloroacetamido)-4,5,6,7-tetrahydrobenzo (*b*)thiophene-3-carboxylate) (3a)

Chloroacetylchloride**6** (3.5 ml; 0.044 mol) in toluene (18 ml) was added dropwise to a stirring solution of **4**(10 g; 0.044 mol) in a solvent mixture of toluene (35 ml) and pyridine (dry) (7.17 ml; 0.088 mol). The product **3a** separated out from the reaction mixture within 2-3 h and solid was filtered and washed with water (250 ml). The filtrate was then acidified using dil. HCl (to remove excess pyridine), followed by addition of water (to remove acid) and extracted with ethyl acetate. The organic layer was washed 3 times with water and was dried over $Na_2SO_4$to remove any traces of water. The final dried product was obtained by evaporating the ethyl acetate at low temperature. The product with m.p. 111°-116°C was characterized as **3a**.

Mol. Formula: $C_{13}H_{16}ClNO_3S$; MW 301.05; R_f value: 0.65; Colour: brown; IR (KBr) cm^{-1}: 3184.68 (γ_{N-H}), 2939.01 (γ_{C-H} st. aliphatic), 1663.30 (γ_{CONH}), 785.89 (γ_{C-Cl}); ^1H NMR (300MHz, $CDCl_3$, TMS =0)δ: 1.39(*t*,3H, *J*=6.9Hz),1.80(*s*,4H), 2.66(*s*,2H), 2.78(*s*,2H), 4.25(*s*,2H), 4.50-4.33(*m*,2H), 12.17(*s*,1H).

c) Synthesis of 2-amino-3-carbethoxy-4,5-dimethylthiophene (5)

Ethylmethylketone (2-butanone) (7.0 g, 0.097mol), sulfur (3.11 g, 0.097 mol) and ethylcyanoacetate (10.9 g, 0.097 mol) were reacted in methanol (20 ml) in presence of diethylamine (8.86 g, 0.121 mol) as per the procedure described for compound **4**. The product having m.p. 92°-93°C was characterized as **5**.

Mol. formula: $C_9H_{13}NO_2S$; MW 199.07; R_f value: 0.66; Colour: yellow; IR (KBr) cm^{-1}: 3424.47, 3310.78 (γ_{N-H}), 2932.13 (γ_{C-H}), 1655.47 (γ_{COOEt}); ^1H NMR (300MHz, $CDCl_3$, TMS =0)δ: 1.34(*t*,3H, *J*=5.4Hz), 2.56(*s*,3H), 2.45 (*s*,3H), 4.48-4.34(*m*,2H), 11.51(*s*,2H).

d)Preparation of CGH-2 (ethyl-2-(2-chloroacetamido)-4,5-dimethylthiophene-3-carboxylate) (3b)

Chloroacetylchloride**6** (2.18 ml, 0.027 mol) in toluene (12 ml) was added dropwise with stirring of **5** (5.5 g, 0.027 mol) in a solvent mixture of toluene (25 ml) and pyridine (4.45 ml, 0.055 mol). The product **3b** separated out from the reaction mixture and was filtered, washed with water (250 ml) following the same procedure as for **3a**. The product having m.p. 102°-107°C was characterized as **3b**.

Mol. formula: $C_{11}H_{14}ClNO_3S$; MW 275.04; $R_f$value: 0.70; Colour: light brown; IR (KBr)

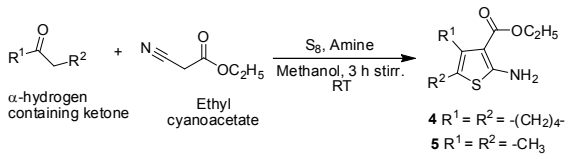
cm^{-1}: 3430 (γ_{N-H}), 2920 (γ_{C-H} aliphatic), 1680 (γ_{CONH}), 790 (γ_{C-Cl}); ^1H NMR (300MHz, $CDCl_3$, TMS =0)δ: 1.36(*t*,3H,*J*=5.8Hz),2.50(*s*,3H), 2.44(*s*,3H), 4.20 (*s*,2H), 4.48-4.36(*m*,2H), 12.12 (*s*,1H).

Results: Chemistry

Synthesis of 2-amino-3-carboxylate thiophenes(Gewald synthesis)

Gewald reaction is an organic reaction involving the Knoevenegaelcondensation of a ketone (or an aldehyde when R^2 = H) with a α-cyanoester in the presence of elemental sulfur and base to give a poly-substituted 2-amino-thiophene **4**and **5**[9-10]. Generally, the reaction is useful for the synthesis of 2-amino thiophene-3-carboxylic esters, carbonitriles and carboxamides.

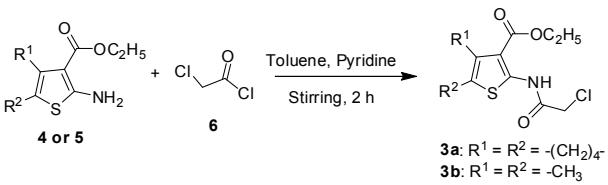
General scheme for synthesis of 2-amino-3-carbethoxyethyl ester thiophene**4**and **5**is given in Scheme-1.



Scheme-1 Synthesis of 2-amino-3-carboxylate thiophenes.

Synthesis of 2-chloroacetamido thiophenes

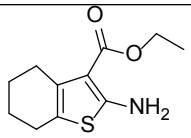
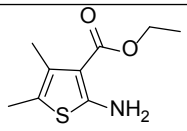
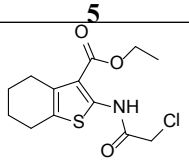
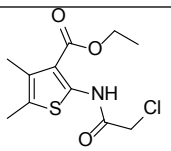
Synthesis of 2-chloroacetamido thiophenes**3** has been carried out by nucleophilic attack of 2-amino thiophene**4**on carbonyl carbon of chloroacetylchloride**6** in the presence of organic base, pyridine (Scheme-2).



Scheme-2 Synthesis of 2-chloroacetamido thiophenes.

Following the protocol, we have synthesized

Table 1: Physical characterization of thiophenes and 2-chloroacetamido thiophenes

S.No.	Compound	Mol. formula	Mol. wt. (calculated)	m.p.	% age yield
1.	 **4**	$C_{11}H_{15}NO_2S$	225.31	110°-112°C (uncorrected)	52%
2.	 **5**	$C_9H_{13}NO_2S$	199.07	92°-93°C (uncorrected)	49%
3.	 **3a**	$C_{13}H_{16}ClNO_3S$	301.05	111°-116°C (uncorrected)	33%
4.	 **3b**	$C_{11}H_{14}ClNO_3S$	275.04	102°-107°C (uncorrected)	51%

intermediate 2-aminothiophenes and 2-chloroacetamido thiophenes and physical characterization is given in Table 1.

In-Vivo Results

a) Effect of newly synthesized compounds, CGH-1 & CGH-2 on body weight in high cholesterol diet (HCD)-induced atherosclerosis in rats

HCD (4% cholesterol, 1% cholic acid and groundnut oil, q.s.) was given to the rats for 40 days to induce hyperlipidemia. Further, ezetimibe (7.5 mg/kg), CGH-1 (40 & 100 mg/kg) & CGH-2 (40 & 100 mg/kg) were administered daily orally to the rats for 40 days along with HCD. There was significant increase in body weights of HCD-fed rats as compared to saline group. Treatment with ezetimibe (7.5 mg/kg), CGH-1 (40 & 100 mg/kg) & CGH-2 (40 & 100 mg/kg) showed significant and dose dependent decrease in body weight as compared to high cholesterol diet fed rats on 20th and 40th day (Fig. 2).

atherosclerosis in rats. All values are expressed as a mean± SEM (n=6). $^{*}p<0.05$ versus saline, $^{\#}p<0.05$ versus HCD treated group, $^{@}p<0.05$ versus CGH-2 (40), $^{\$}p<0.05$ versus CGH-1 (40), $^{+}p<0.05$ versus CGH-2 (100) on 20th and 40th day.

b) Effect of CGH-1 & CGH-2 on the levels of total cholesterol (TC) and triglycerides (TGs) in HCD-induced atherosclerosis in rats

HCD was given to the rats for 40 days to induce the hyperlipidemia. Treatment protocol had been started with ezetimibe (7.5 mg/kg) i.e. standard drug, CGH-1 (40 & 100 mg/kg) and CGH-2 (40 & 100 mg/kg) orally for 40 days. TC and TGs levels were significantly increased in HCD fed rats as compared to saline group but ezetimibe (7.5 mg/kg), CGH-1 (40 & 100 mg/kg) & CGH-2 (40 & 100 mg/kg) treated groups showed significant decrease in the TC and TGs levels in the dose dependent manner as compared to HCD group. Also, CGH-1 was found to be better hypolipidemic drug as compared to CGH-2 (Fig. 3& 4).

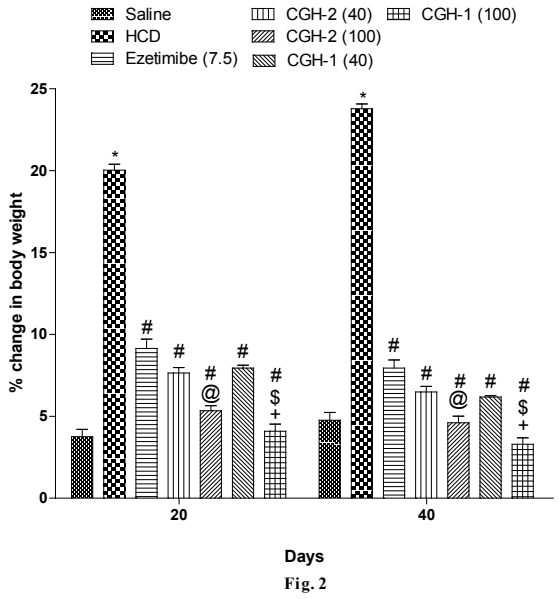


Fig. 2

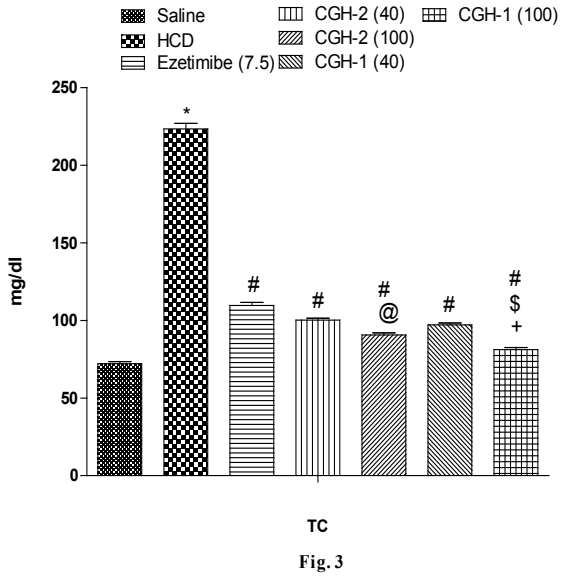


Fig. 3

Fig.2 Effect of CGH-1 & CGH-2 on body weight in high cholesterol diet (HCD)-induced

Fig.3 Effect of CGH-1 & CGH-2 on total cholesterol (TC) levels in HCD-induced

atherosclerosis in rats. All values are expressed as a mean± SEM (n=6). *p<0.05 versus saline, #p<0.05 versus HCD treated group, @p<0.05 versus CGH-2 (40), $p<0.05 versus CGH-1 (40), +p<0.05 versus CGH-2 (100) on 41st day.

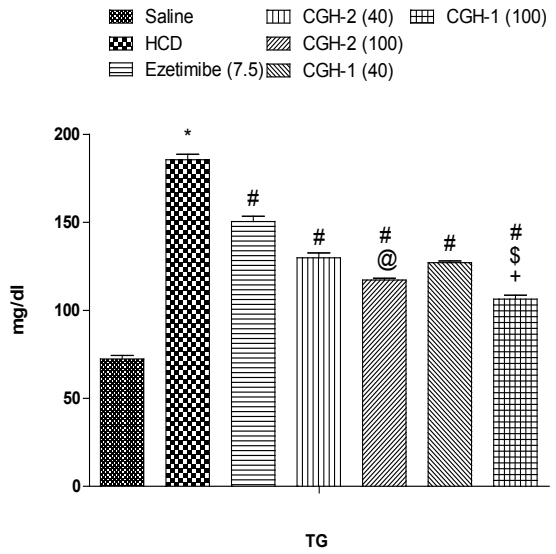


Fig. 4

Fig.4 Effect of CGH-1 & CGH-2 on triglycerides (TGs) levels in HCD-induced atherosclerosis in rats. All values are expressed as a mean± SEM (n=6*p<0.05 versus saline, #p<0.05 versus HCD treated group, @p<0.05 versus CGH-2 (40), $p<0.05 versus CGH-1 (40), +p<0.05 versus CGH-2 (100) on 41st day.

c) Effect of CGH-1 & CGH-2 on the levels of HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C) in HCD-induced atherosclerosis in rats

4% cholesterol, 1% cholic acid suspended in groundnut oil was mixed in normal chow diet and given to the rats for 40 days. It produced hyperlipidemia in rats. Ezetimibe (7.5 mg/kg), CGH-1 (40 & 100 mg/kg) and CGH-2 (40 & 100 mg/kg) were administered orally to the rats along with HCD. HCD group appeared to show significant decrease in HDL-C levels and increase in LDL-C levels when compared to saline group but ezetimibe (7.5 mg/kg), CGH-1(40 & 100 mg/kg) and CGH-2 (40 & 100 mg/kg) increased the levels of HDL-C and decreased the levels of LDL-C (Fig. 5 & 6).

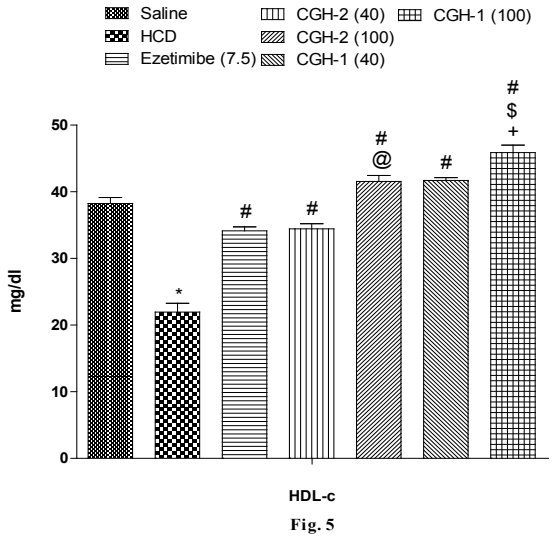


Fig. 5

Fig.5 Effect of CGH-1 & CGH-2 on HDL cholesterol (HDL-C) levels in HCD-induced atherosclerosis in rats. All values are expressed as a mean± SEM (n=6). *p<0.05 versus saline, #p<0.05 versus HCD treated group, @p<0.05 versus CGH-2 (40), $p<0.05 versus CGH-1 (40), +p<0.05 versus CGH-2 (100) on 41st day.

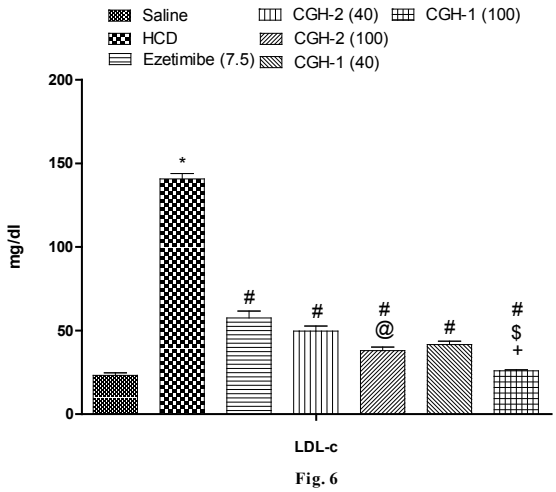


Fig. 6

Fig.6 Effect of CGH-1 & CGH-2 on LDL

cholesterol (LDL-C) levels in HCD-induced atherosclerosis in rats. All values are expressed as a mean± SEM (n=6). $^{*}p<0.05$ versus saline, $^{\#}p<0.05$ versus HCD treated group, $^{@}p<0.05$ versus CGH-2 (40), $^{\$}p<0.05$ versus CGH-1 (40), $^{+}p<0.05$ versus CGH-2 (100) on 41st day.

Histopathological parameters

In animals fed with normal diet, the aorta did not show accumulation of lipids whereas in cholesterol-fed rats, the aorta showed deposition of cholesterol as shown by arrows in Fig. 7 to 13. CGH-1 (40 & 100 mg/kg) showed decrease in the accumulation of cholesterol as compared to CGH-2 (40 & 100 mg/kg) and ezetimibe (7.5 mg/kg). Also, among the two doses of CGH-1, 100 mg/kg proved to be better hypolipidemic drug as shown in Fig. 13.

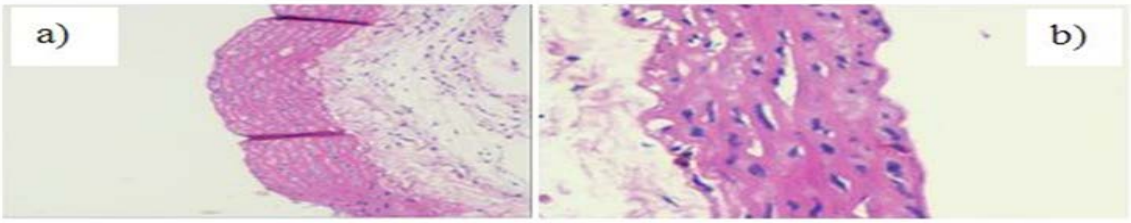


Fig. 7

Fig. 7 Saline treated rats showing normal thoracic aorta; a) imaging at 40x b) imaging at 400x

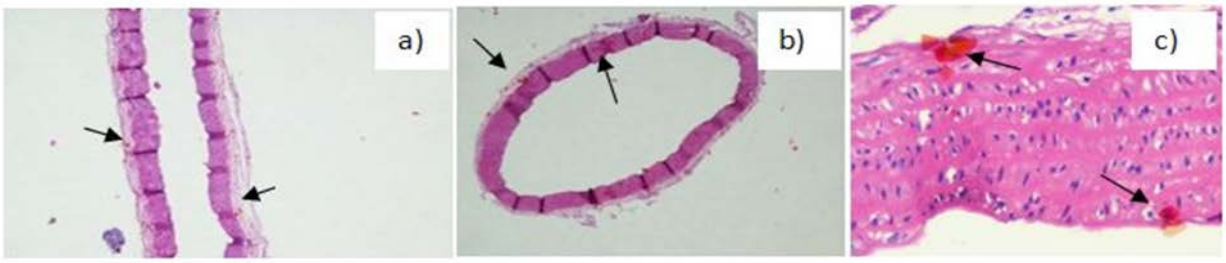


Fig. 8

Fig. 8 Hyperlipidemic changes in thoracic aorta in HCD-induced atherosclerosis in rats; a) showing at 40x b) lumen showing deposition of cholesterol as orange colored molecules at 40x c) at 400x

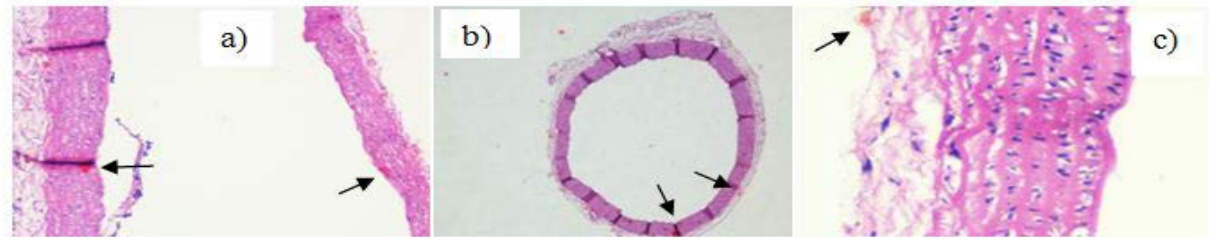


Fig. 9

Fig. 9 Hypolipidemic changes in thoracic aorta in HCD + Ezetimibe (7.5 mg/kg) treated rats; a) at 40x b) at 40x c) at 400x

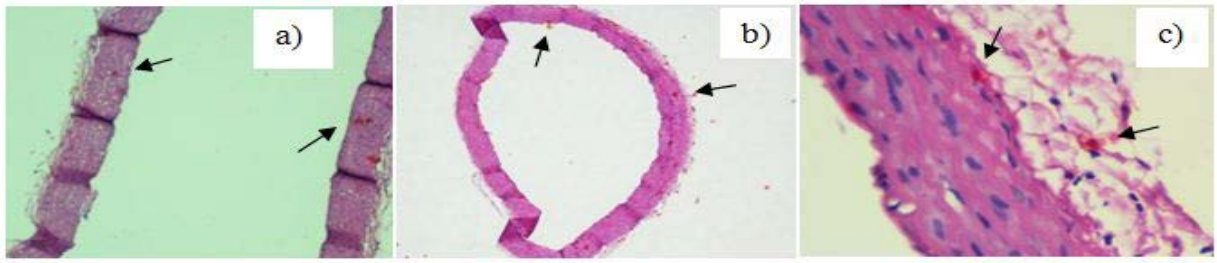


Fig. 10

Fig. 10 Hypolipidemic changes in thoracic aorta of HCD + CGH-2 (40) treated rats; a) cholesterol deposition shown at 40x b) at 40x c) at 400x

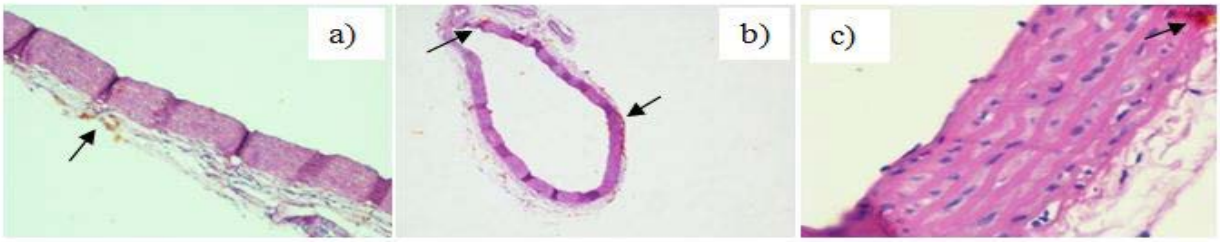


Fig. 11

Fig. 11 Hypolipidemic changes in thoracic aorta of HCD + CGH-2 (100) treated rats; a) at 400x b) at 40x c) at 400x

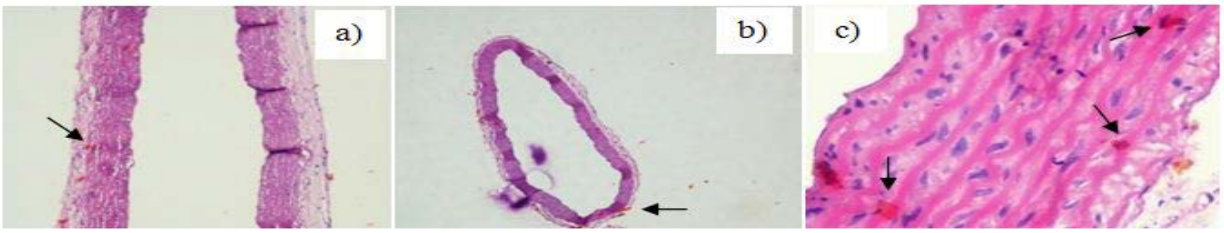


Fig. 12

Fig. 12 Hypolipidemic changes in thoracic aorta of HCD + CGH-1 (40) treated rats; a) at 40x b) at 40x c) at 400x

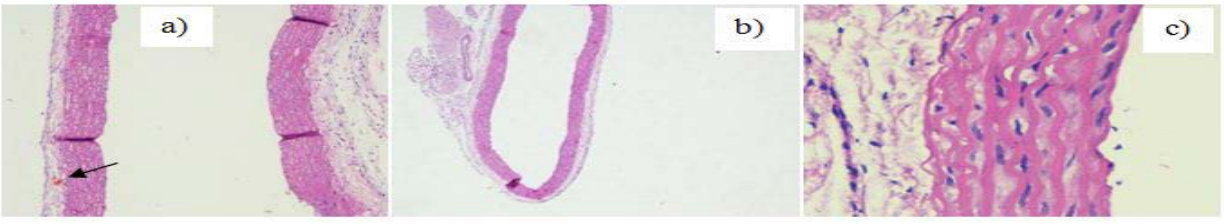


Fig. 13

Fig. 13 Hypolipidemic changes in thoracic aorta of HCD + CGH-1 (100) treated rats; a) at 40x b) at 40x c) at 400x

Discussion

The finding of the present study demonstrates hypolipidemic activity of newly synthesized compounds, CGH-1 & CGH-2 in high cholesterol diet (HCD)-induced atherosclerosis model in rats. Development of hyperlipidemia is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries & other major arteries within the body. HCD (4% cholesterol, 1% cholic acid suspended in groundnut oil) [11-14] possesses cholesterol levels to increase in susceptible people, which leads to obesity.[15] HCD induced lipid disorders in the experimental rats within 40 days [11-14] and produced impairment in body weights, serum lipid profile and normal histology of rats.

HCD may induce hyperlipidemia by directly increasing the TC, TGs, HDL-C and LDL-C in the blood serum which may further lead to deposition of lipids in the arteries. The elevation of serum lipids for a long time results in development of atherosclerosis in rats [16]. The weight gain in HCD group of rats was significantly higher than control rats reflecting the influence of HCD.

Oral administration of CGH-1 (40 & 100 mg/kg) and CGH-2 (40 & 100 mg/kg) significantly reduced the weight gain, TGs, TC, LDL-C and increased HDL-C levels, suggesting their roles in hyperlipidemia. Histological study showed that CGH-1 caused decrease in cholesterol deposition as compared to high cholesterol diet fed rats and also ezetimibe and CGH-2 treated groups. Thus, the study demonstrates that CGH-1 (100 mg/kg) is effective as hypolipidemic agent.

The free cholesterol is not absorbed as such in the body. Cholesterol synthesis is an endoplasmic reticulum (ER) mediated multistep pathway starting with condensation of acetyl-coenzymeA (CoA), proceeding to the formation of mevalonate and then further to lanosterol, the first cyclic sterol [17]. First, it gets converted into cholesteryl esters (CEs) in the presence of enzyme Acetyl CoA: Cholesterol acyltransferase (ACAT). ACAT enzymes are members of the membrane-bound O-acyltransferase family, causes esterification of free cholesterol into CEs in a variety of cells and tissues. ACAT has two isomeric forms, *i.e.* ACAT-1 & ACAT-2. ACAT-1 is responsible for cholesterol ester formation by macrophages, resulting in foam cell formation in artherosclerotic lesions [18]. Therefore, ACAT-1 inhibitors have antiatherogenic effect[19]; while ACAT-2 helps in cholesterol absorption in intestine [18-20]. Further, it gets converted into chylomicrons and other forms of lipoproteins (HDL, LDL, TC, TGs) in the presence of Acetyl CoA: cholesterol acyltransferase (ACAT) and Cholesteryl Ester Transfer Protein (CETP) (Fig. 14).

CETP is a hydrophobic glycoprotein that mediates the net transfer of cholesteryl esters from atheroprotective high density lipoproteins to atherogenic low density lipoproteins or very low density lipoproteins [6]. CETP deficiency is the main cause of high HDL-C levels [21]. In complete absence of CETP function, the failure to transfer CE from HDL to other lipoproteins leads to an accumulation of CE in the HDL fraction. To accommodate the increased amounts of core lipids in HDL, other surface components such as apoAI, phospholipids and unesterified cholesterol are also increased [22].

CETP is central to cholesterol and triacylglycerols transport in the circulation, simultaneously affects the concentration and composition of both antiatherogenic and atherogenic lipoproteins [21].CETP inhibition may improve the vascular function and reduces atherosclerosis. Nonetheless, if a CETP inhibition-related increase in HDL-C proves to mediate vascular protection, CETP inhibitors

(as sole treatment or in combination with other lipid-lowering drugs) will refine the treatment of dyslipidemia in preventing cardiovascular disease [22].

Thus, we can say that inhibition of ACAT and CETP raises high density lipoproteins cholesterol (good cholesterol) levels and reduces low density lipoproteins cholesterol (bad cholesterol) levels, making it a promising drug target for the prevention and treatment of coronary artery disease.

Ezetimibe, a selective inhibitor of cholesterol absorption from the small intestine, inhibits cholesterol absorption from the intestine by blocking the action of the cholesterol transporter, Niemann-Pick C1-Like 1 (NPC1L1) protein [23]. Ezetimibe has been proved to act *via* cholesterol absorption inhibitory activity.

CGH-1 (ethyl-2-(2-chloroacetamido)-4,5,6,7,-tetrahydrobenzo*(b)*thiophene-3-carboxylate) & CGH-2 (ethyl-2-(2-chloroacetamido)-4,5-dimethyl thiophene-3- carboxylate) (40 & 100 mg/kg) are new compounds and this study suggested that these compounds act through the cholesterol absorption inhibitory pathway. The main targets upon which CGH-1 & CGH-2 can act include ACAT and/or CETP. These can be considered as open ring derivatives of the parent compound LM-1554, that has been proven for its hypolipidemic activity.The exact mechanism of these compounds is not much clear, however, it is considered to act through inhibition of cholesterol absorption, specifically via ACAT and/or CETP inhibition in HCD-induced atherosclerosis in rats.

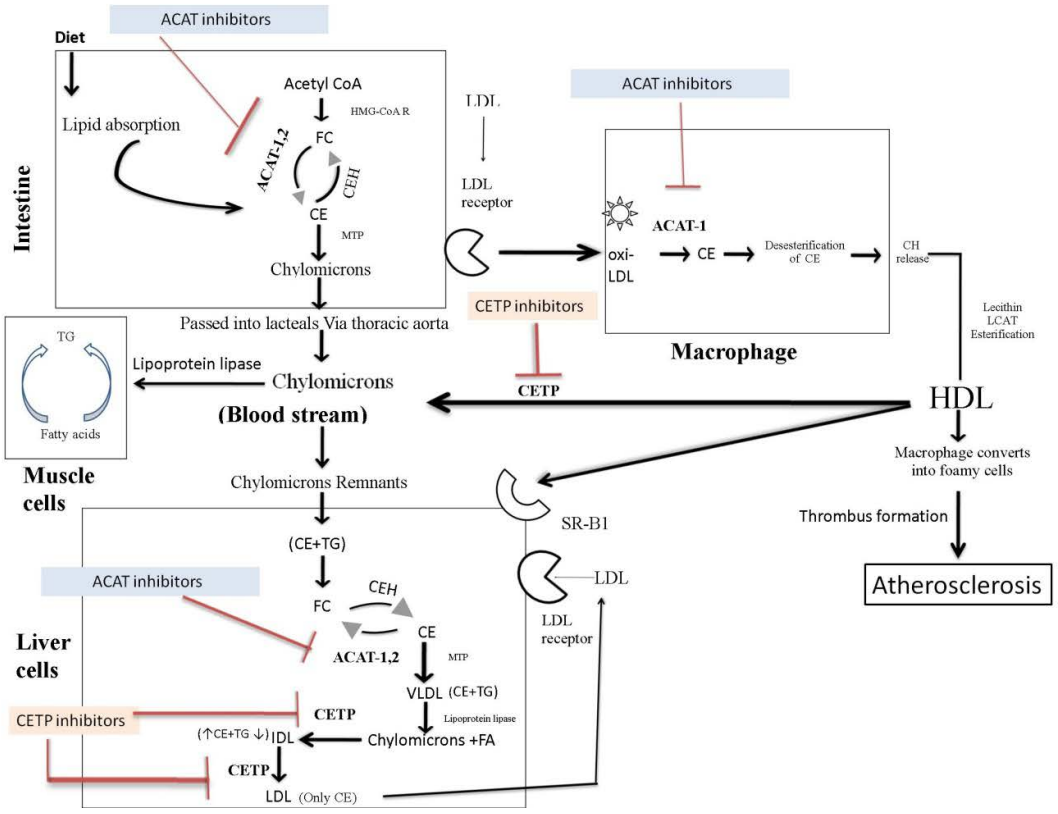


Fig. 14 Schematic representation of physiology, pathophysiology of hyperlipidemia and the role of ACAT inhibitors and CETP inhibitors in hyperlipidemia.

Conclusion

On the basis of result obtained in the present study, the oral administration of CGH-1 & CGH-2 (40 & 100 mg/kg) alleviated the established behavioural symptoms. Compound CGH-1 (100 mg/kg) reduced histopathological symptoms of hyperlipidemia as compared to CGH-1 low dose (40 mg/kg) and CGH-2 (40 & 100 mg/kg). Further, CGH-1 (40 & 100 mg/kg) has provided more beneficial effect as compared to CGH-2 (40 & 100 mg/kg) in HCD fed rats. The mechanism of action of these compounds may be inhibition of cholesterol absorption in GIT by inhibiting the specific transport responsible for the cholesterol transportation specifically via ACAT and/or CETP inhibition.

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