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Antihyperlipidemic Activity of *Gardenia Gummifera*

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Abstract

Ethanolic extract of twigs and gums of Gardenia gummifera (EEGG) was investigated for antihyperlipidemic activity. It was evaluated via in vivo model i.e. Poloxamer-407 induced hyperlipidemia and suspension of Cholesterol + cholic acid induced hyperlipidemia. Poloxamer-407 is an acute model used for determination of preventive antihyperlipidemic properties of EEGG and determination of most efficacious dose i.e. EEGG (250mg/kg), which further used as treatment dose for chronic model (cholesterol +cholic suspension induced hyperlipidemia model). In Poloxamer-407 induced hyperlipidemia models, blood samples were withdrawn at 48th hrs and serum lipid levels and atherogenic index were analyzed. EEGG exhibited significant inhibition of serum TC and LDL levels while as atherogenic coefficient decreased significantly. Effective dose obtained from Poloxamer-407 induced hyperlipidemia model also demonstrated significant inhibition in serum lipid levels (TC and LDL) and atherogenic index (AI,CRR and CR). EEGG also significantly increased serum HDL levels when blood samples were analyzed at 28th day. Liver enzyme assays and Histopathological showed enough evidence to prove its hypolipidemic properties.

Introduction

Hyperlipidemia defined as elevated serum levels of Total cholesterol (TC), Triglycerides (TG), Low density lipoproteins (LDL), very low density lipoproteins (VLDL) and decreased levels of High density lipoproteins (HDL). Hyperlipidemia is basically divided into two type's viz. primary and secondary types. Primary hyperlipidemia causes due to genetic disorders while secondary hyperlipidemia causes due to diabetes, nephrosis and hypothyroidism.[1-3].

It is risk factors associated with atherosclerosis, coronary artery diseases, cerebral vascular disease and peripheral vascular diseases. The various clinical trials showed that lowering serum cholesterol and LDL reduce morbidity and mortality in patients with established CAD. Therefore, prime consideration and atherosclerosis

is to attenuate the elevated blood serum plasma levels of lipids. [4-5]

Gardenia gummifera is small tree with height of around 8 cm grayish brown colors, resinous exudates are in bright yellow in color having branches and branch lets. *Gardenia gummifera*is commonly known as Dikamali or cambi resin. In Sanskrit it is known as Hingunadika, nandihingu, gandharaj. *Gardenia gummifera*belongs to the family- Rubiaceae. It is a large medicinal shrub with resinous buds. It is therapeutically used as resin, bitter, thermogenic, cardio tonic, carminative, antispasmodic, stimulant, diaphoretic, antiseptic and expectorant. It is also used in conditions like cardiac debility, obesity, lipolytic disorder, bronchitis and neuropathy.[6-7]

Materials and methods:

Procurements and authentication of plants

Twigs and gums of *Gardenia gummifera* were collected from medicinal plant garden Keshavshrusthi, Bhayander, Mumbai. The plant was authenticated by Department of Botany, Gurunanak Khalasa College, Matunga with specified voucher number for future reference.

Preparation of plant extract

Mixture of twigs and gums obtained from the *Gardenia gummifera*were shed dried and coarsely powdered. The coarse powders were subjected to soxhlet for extraction with ethanol. The obtained extract were then subjected to rotary evaporator at 50º C for concentrating, concentrated extract was then dried further and stored at cool temperature in refrigerator.[8]

Preparation of herbal drug extracts and standard drug Atovastatin

Varying doses of EEGG (125mg/kg, 250mg/kg) and Atorvastain (10mg/kg) were prepared by suspending in 5% tween 80 and Sodium carboxy methyl cellulose respectively.

***In-vivo* anti-hyperlipidemia activity-**

Poloxamer-407 induced hyperlipidemia [9-12]

30 Adult male Sprague Dawley rats weighing between 150-200gm were purchased from Bombay veterinary college, Parel, Mumbai, Maharashtra 400012. They were divided into 5groups, each having 6 rats each.

Group I: Vehicle control group, each rats were administered daily with 5% w/v tween 80.

Group II: Disease control, each rats administered with single i.p injection of Poloxamer-407.

Group III: Standard control group in which each rats were administered daily with Atorvastatin (10mg/kg) for 8 days prior induction of hyperlipidemia.

Group IV: Test dose 1 in which each rats were daily administered with ethanolic extract of *Gardenia gummifera*[EEGG] (125mg/kg) for 8 days prior induction of hyperlipidemia.[8]

Group V: Test dose 2 in which each rats were daily administered with Methanolic extract of *Gardenia gummifera* [*EEGG*] (250mg/kg) for 8 days prior induction of hyperlipidemia. [8]

A homogenous suspension of Atorvastatin was prepared individually and freshly byusing Sodium methoxycellulose (0.5%) for administration. A homogenous suspension of EEGG was prepared by suspending into 5% tween 80 and triturated.

Inducing agent: The 15% poloxmer-407 (P-407) was made by dissolving in ice cold saline and stored into refrigerator overnight to facilitate its proper dissolution, prior introduction intoanimals. Hyperlipidemia was induced by administration of single i.p. injection of Poloxamer-407 (1g/kg).

Treatment: Group IV and Group V were treated with varying doses of *EEGG* (200mg/kg and 400 mg/kg) respectively prior administration of Poloxamer-407 for 8 days.

Sample collection and evaluation

After administration of Poloxamer-407 i.p injection, Blood samples were withdrawn from retroorbitalplexus at 48 hrs, then were processed for evaluation of serum lipid.Thereafter at 48 hrsanimals were sacrificed, livers were excised immediately. 10%w/v liver homogenates wereprepared using phosphate buffer (pH-7.4) for estimation of lipid peroxidation and liver catalaselevels.

Cholesterol and cholic acid suspension induced Hyperlipidemia model:[13]

24 Adult female Sprague Dawley rats weighing between 150-200gm were purchased from Haffkine institute, Parel, Mumbai, Maharashtra 400012. They were divided into 4 groups, each having 6 rats each.

Group1: Vehicle control group, each rats were administered daily with 0.5% w/v Sodiumcarboxy methylcellulose.

Group 2: Disease control group, in which rats were administered daily inducing agent for 28 days.

Group 3: Standard control group, in which rats were administered daily with Atorvastatin (10mg/kg) for 28 days after induction of Hyperlipidemia.

Group 4: Most effective test dose EEGG (250mg/kg) obtained from Poloxamer-407 induced model, were administered daily for 28 days after induction of hyperlipidemia.

Inducing agent: cholesterol and cholic acid suspension was prepared by using formula (0.5% cholesterol+0.25%cholic acid +3%coconut oil).

Induction of hyperlipidemia: Hyperlipidemia was induced by oral administration of inducing agents for 28 days.

Treatment: Rats were treated with most effective dose of EEGS (250mg/kg) determined from Poloxamer-407 and Standard Atorvastatin (10mg/kg) for 28 day after induction of Hyperlipidemia.

Sample collection and evaluation

Blood samples were withdrawn from retro-orbital plexuses at 28th day from the day of induction of

Hyperlipidemia, and then were processed further for evaluation of serum lipid parameters. Thereafter at 28th day liver were excised and further estimation of liver parameters were carried out.

Experimental Animals

The study was conducted after obtaining clearance for the experimental protocol (IAEC/PR/2012/02) from institutional Animals ethics committee (IAEC), Bharati Vidyapeeth's college of Pharmacy, C.B.D. Belapur. The procured rats were housed in Bharati Vidyapeeth's college of Pharmacy, CBD Belapur. These experiments were carried out as per guidelines specified by Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Animals were fed with commercials pellets diet (AmruthLaboratory,Mumbai,India) and tap water ad-libitium.

Biochemical analysis

All the blood serum under this serum was analyzed for marker parameters such as serum total cholesterol, serum triglycerides, and High density lipoproteins. All the parameters were analyzed by auto analyzer (Erba-7) with biochemical kit (Erba diagnostics Mannheim GmbH). Moreover

VLDL and LDL were calculated by using Friedewald's formula.

VLDL= serum triglycerides/5

LDL= TC – (HDL+VLDL)

The atherogenic indices calculated were.

Atherogenic index= LDL-C/HDL-C [14]

Cardiovascular risk ratio= TC/HD [15]

Atherogenic coefficient= TC-HDL/HDL [16]

***In-vivo* antioxidant activity (Liver enzyme assay)**

Approximate 1 gm of liver was excised from each rats, and used further for live enzyme assays homogenates. Thereafter prepared liver tissue homogenates were centrifuged at 3500rpm for 15 minutes and thus obtained clear supernatant was used for determination of liver peroxidation levels and catalase levels.

Statistical analysis

All the results are expressed as Mean ± S.E.M (n=6), Values in parentheses indicates %inhibition of total cholesterol. Statistical analysis was performed by one way ANOVA followed by Dunnetts test, where $p<0.05$, when compared with diseases control group.

Results: ***In vivo*antihyperlipidemia activity**

Poloxamer-407 induced hyperlipidemia

Table 1: Effects of Ethanolic extracts of Gardenia gummifera on serum lipid profile 48 hrs after i.p injection of P-407.

	Serum lipid levels		Atherogenic index		
Treatment	TC	LDL	AI	CRR	AC
Vehicle control	72.77±5.52	47.66±4.80	3.56±0.38	5.40±0.42	71.22±5.16
Disease control	225.7±7.88	64.15±7.04	0.61±0.08	2.12±0.10	224.7±13.05
ATV (10mg/kg)	149.0±13.05*** (33.98%) ↓	40.14±10.42	0.64±0.16	2.41±0.12	148.0±13.05*** (34.13%) ↓
EEGS (125mg/kg)	164.8±10.70*** (26.98%) ↓	33.23±13.0 (48.20%) ↓	0.46±0.20	2.14±0.22	163.8±10.70*** (27.10%) ↓
EEGS (250mg/kg)	139.7±3.984*** (38.10%) ↓	17.76±4.91** (72.31%) ↓	0.26±0.073	1.98±0.10	138.7±3.98*** (38.27%) ↓

All values are expressed as Mean ± S.E.M (n=6), Values in parentheses indicates % inhibition. Statistical analysis was performed by one way ANOVA followed by Dunnetts test, where $^{**}p<0.01$, $^{***}p<0.001$when compared with disease control group.

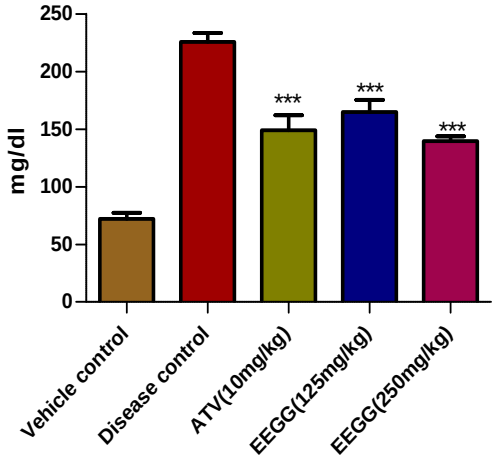


Figure 1: Effects of EEGG on serum TC levels in Poloxamer-407 induced

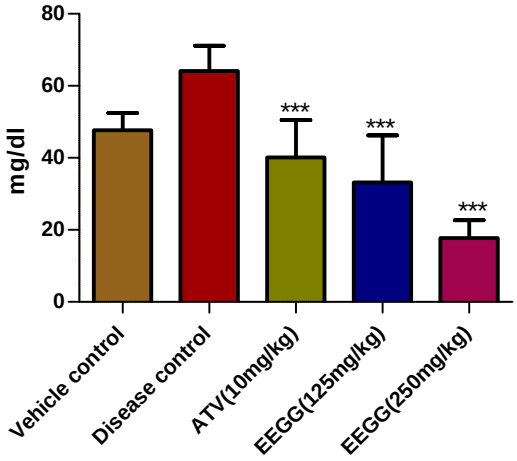


Figure 2: Effects of EEGG on serum LDL levels in Poloxamer-407 induced

Cholesterol and cholic acid suspension induced hyperlipidemia model.

Table 2: Effects of EEGS on serum lipid and atherogenic index cholesterol induced hyperlipidemia rats.

	Serum lipid profile			**Atherogenic index**		
Treatment	TC	LDL	HDL	AI	CRR	AC
Vehicle control	46.95±2.17	14.65±2.64	6.487±0.63	2.43±0.49	7.61±0.84	45.95±2.16
Disease control	81.43±2.09	41.43±2.91	12.97±0.96	3.41±0.61	6.95±0.86	80.43±2.03
ATV (10mg/kg)	43.64±3.44*** (46.40%)	0.6027±1.75*** (98.55%) ↓	18.90±1.34* (45.72%) ↑	0.05±0.91*** (98.53%) ↓	2.33±0.13*** (66.47%) ↓	42.64±3.44*** (46.98%) ↓
EEGS (250mg/kg)	37.99 ±3.47*** (53.34%) ↓	-3.975±2.07*** (109.59%) ↓	22.82±1.73*** (75.94%) ↑	0.15±0.08*** (95.60%) ↓	1.49±0.20*** (78.56%) ↓	36.99±1.37*** (54.00%) ↓

Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnettstest,where *p<0.1 **p<0.01, ***p<0.001 when compared with disease control group. Values in parenthesis indicate % increase or decrease in respective parameters, ↓indicates decrease;↑indicates increase.

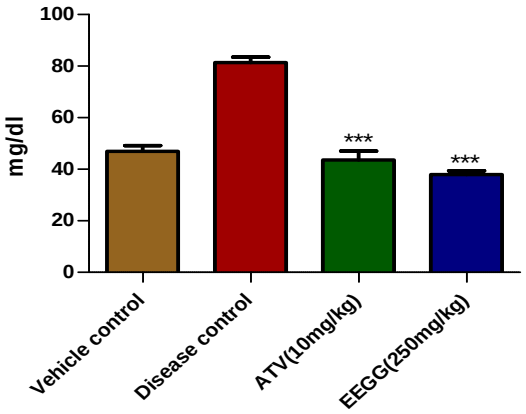


Figure 3: Effects of EEGG on serum TC in cholesterol and cholic acid suspension induced hyperlipidemia.

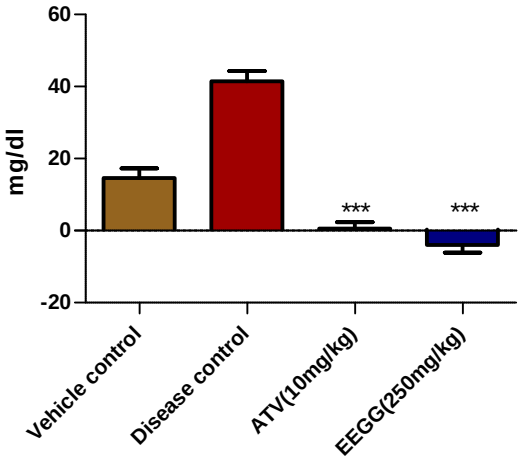


Figure 4: Effects of EEGG on serum LDL in cholesterol and cholic acid suspension induced hyperlipidemia.

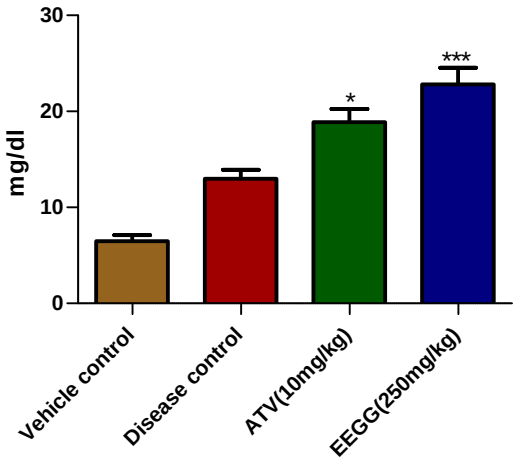


Figure 5: Effects of EEGG on serum HDL in cholesterol and cholic acid suspension induced hyperlipidemia.

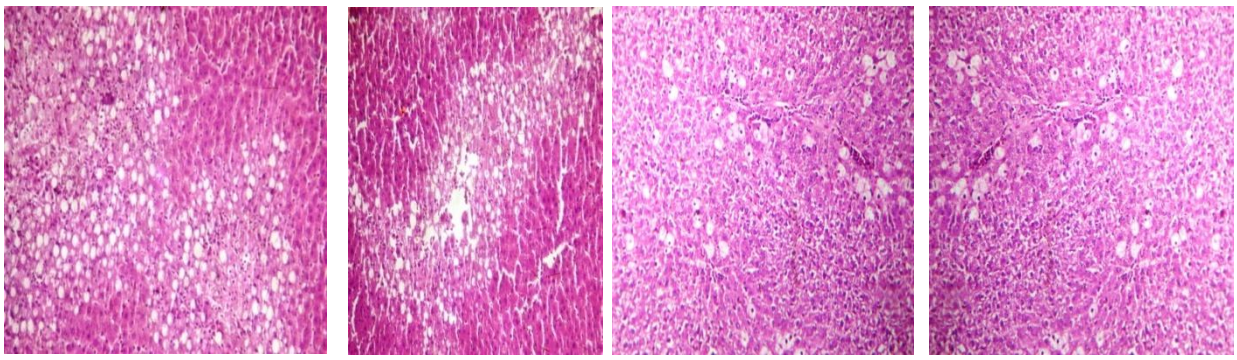
In-vivo antioxidant activity:

Table 3: Effects of EEGS on Liver peroxidation and catalase levels in liver of hypelipidemic rats

Treatment	Liver peroxidation	Catalase
Vehicle control	11.44±2.05	67.56±1.708
Disease control	27.25±3.173	47.94±2.389
Atorvastatin (10mg/kg)	20.16±1.990 (44.84%) ↓	63.57±2.638 (79.65%) ↑
EEGS (250mg/kg)	17.83± 4.21 (34.57%) ↓	50.45± 2.83 (4.98%) ↑

n=6 animals in each group. Values are expressed as mean ± SEM. Values in parenthesis indicate % increase or decrease in respective parameters, ↓indicates decrease;↑indicates increase.

Histopathological studies



(a) vehicle control (b) Disease control (c) Atorvastatin (d) EEGS

Figure 6: Histopathological observation of EEGG in Cholesterol and Cholic acid suspension induced hyperlipidemia model.

Table 4: Histopathological reports of EEGG in Cholesterol and cholic acid suspension induced hyperlipidemia.

Groups	Observation
Vehicle control	Mild to moderate diffuse granular degeneration
Disease control	Moderate to severe degree fatty infiltration in centrilobular zones, Mild degree diffuse granular degeneration
Atorvastatin (10mg/kg)	Minimal degree fatty infiltration in centrilobular zones, Mild degree diffuse granular degeneration
EEGS (250mg/kg)	Mild to moderate degree fatty infiltration in centrilobular zones, Mild degree diffuse granular degeneration

Discussion

Hyperlipidemia is characterized by serum elevated levels of TC, TG ,VLDL,LDL and decrease serum levels of HDL Several studies revealed that it is predictive risk factors for atherosclerosis, coronary artery diseases, cerebral vascular disease and peripheral vascular diseases.

Antihyperlipidemic potential of EEGG were evaluated by *in vivo* models that are acute model, Poloxamer-407 induced hyperlipidemia models while as suspension of cholesterol and cholic acid used as a chronic model of investigation.

Elevated levels of serum LDL level cause catastrophic cardiovascular events. LDL particles are transported into the vessel wall. Endothelial cells and monocytes/macrophages generate free radicals that oxidize LDL (oxLDL), resulting in lipid peroxidation. Oxidized LDL can directly can directly injure endothelial cell and causes endothelial dysfunction. These LDL engulfed macrophages becomes foam cells and accumulate in the intima of an artery and causes proliferation of smooth muscle cells which along with more foam cells from the fibrous cap of atheroma. Along with elevated levels of LDL, elevated serum TC is powerful risk factors. Subsequent studies have shown that LDL is most abundant and clearly evident atherogenic lipoprotein.[17-18]

In Poloxamer-407 induced hyperlipidemia model, varying doses of EEGG (125mg/kg, 250mg/kg) inhibited the serum TC levels significantly by 26.98%, 38.10% respectively similarly serum LDL levels were inhibited by 48.20% ,72.31% respectively.

Conclusion extrapolated from results obtained from Poloxamer-407model,that EEGG(250mg/kg) was the more effective dose than the EEGG (125mg/kg) thus used as a treatment dose for subsequent chronic hyperlipidemic model.

From chronic model i.e. Cholesterol and cholic acid suspension induced hyperlipidemia models EEGG (250mg/kg) exhibited 53.34% inhibition in serum TC, 109.59% inhibition in serum LDL levels.

Strong epidemiological evidence links low levels of serum HDL cholesterol to increased CHD morbidity and mortality. High HDL-cholesterol levels conversely convey reduced risk. Epidemiological data taken as a whole signify that a 1 % decrease in HDL cholesterol is associated with a 2–3 % increase in CHD risk. Epidemiological studies consistently show low HDL cholesterol to be an independent risk factor for CHD. It is well documented that while a low level of HDL is indicative of high risk for cardiovascular disease, an increase in HDL level could potentially contribute to antiatherogenicity by inhibiting LDL oxidation and protecting endothelial cells from the cytotoxic effects of oxidized LDL. HDL plays an important role in cholesterol efflux from tissues; it also has a role in returning cholesterol from the periphery to the liver for removal as bile acids, a process known as reverse cholesterol transport. Serum HDL levels were significantly increased up to 75.94% by EEGG (250mg/kg), more than the 45.72% increase in serum HDL exhibited by standard drug Atorvastatin (10mg/kg).[19-22]

Critical balance between oxidant/antioxidant equilibrium conditions are maintained by body physiologically. Imbalance between equilibrium conditions provokes situation of oxidative stress. Generally results from hyper production of ROS. Due to attacks of free radicals of radical species on membrane lipoproteins; a lot of oxygenated compounds such as aldehyde and malondialdeh-yde generate. Persistent hyperlipide-mia results from prolonged circulation of lipid rich lipoproteins that increase oxidative stress leading to oxidative modification of LDL to oxy-LDL. Measurement of such aldehydes provides a convenient index of lipid peroxidation. Therefore MDA is the most frequently used as an indicator of lipid per oxidation. Catalase is major enzyme dealing with reactive oxygen species in the most cell of the body and plays an important role in the elimination of ROS derived from redox reactions in the liver .studies indicate that Hyperlipidemia diminishes the antioxidant defense system and decrease the activity of catalase, thereby elevating the lipid peroxide content. Further, catalase is easily inactivated in the liver by lipid peroxides or ROS, thus accounting for lower catalase activities in livers of Hyperlipidemia rats. In this study EEGG decreased liver peroxidation levels by 34.57% while as increased catalase level was increased to very lower level by 4.98%. [23-25]

Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa. Atherogenic indices indicate the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the greater is the risk of the above organs for oxidative damage. In this study, EEGG found to decrease atherogenic indices namely Atherogenic coefficient, Cardiac risk ratio and Atherosclerosis index.[26-27]

Our results suggest that EEGG has effective antihyperlipidemic properties with antioxidant activity. Details studies are needed to postulate the possible mechanism(s) of action

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