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ORIGINAL ARTICLE

Lipid-lowering Effect of Hydroalcoholic Extracts of *Gynura procumbens* in Chemical- and High-fat Diet-induced Hyperlipidemic Rats

Vikneswaran Murugaiyah[1], Mohammed Ali Ahmed Saeed[2], Yow-Meng Kuong[2], Kisantini Murugesu[2], Subramani Parasuraman[3], Mohd. Zaini Asmawi[1], Amirin Sadikun[2]

[1]Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, [2]Discipline of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, [3]Faculty of Pharmacy, AIMST University, Semeling 08100 Kedah, Malaysia

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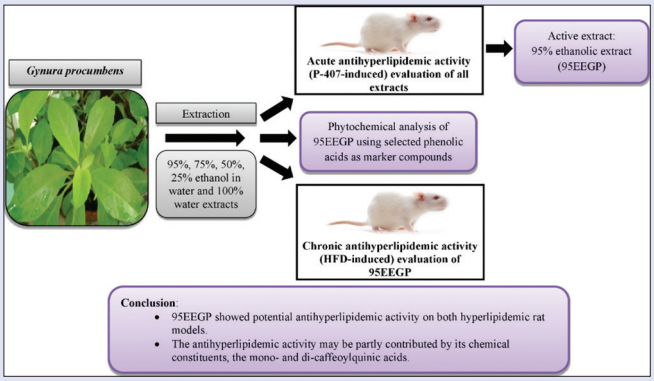
ABSTRACT

Objective: The present study was carried out to investigate lipid-lowering effect of hydroalcoholic extracts of *Gynura procumbens* in chemical- and high-fat diet (HFD)-induced hyperlipidemic rats. **Materials and Methods:** The lipid-lowering effect of hydroalcoholic extracts was investigated in poloxamer-407 (P-407)-induced acute hyperlipidemic rat model. The most active extract was subjected to phytochemical analysis by high-performance liquid chromatography-ultraviolet (HPLC-UV) using phenolic acids as marker compounds and evaluated in HFD-induced chronic hyperlipidemic rats. **Results:** The evaluation on hydroalcoholic extracts of *G. procumbens* showed that 95% ethanolic extract (95EEGP) was the most potent extract that significantly reduced serum total cholesterol (TC, $P < 0.05$) and triglycerides (TG, $P < 0.001$) levels. The 95EEGP contained 7.18, 3.20, 28.31, and 9.72 mg/g dried extract of chlorogenic acid (CA), 3,4-dicaffeoylquinic acid (3,4DC), 3,5DC, and 4,5DC, respectively. The extract at doses of 200 and 500 mg/kg significantly reduced serum TC, TG, low-density lipoprotein-cholesterol (LDL-C), and atherogenic index (A. I.) levels of the P-407-induced hyperlipidemic rats, in a dose-dependent manner ($P < 0.01$ or better) but had no effect on high-density lipoprotein-cholesterol (HDL-C). On HFD-induced hyperlipidemic rats, the 95EEGP at doses of 250, 500, and 1000 mg/kg significantly reduced the serum TC, TG, LDL-C, and A. I. levels ($P < 0.05$ or better) while increased serum HDL-C ($P < 0.001$). The effect was dose-dependent showing comparable effect to that of atorvastatin at moderate and high doses. **Conclusion:** The present study demonstrated the lipid-lowering potential of the 95EEGP of *G. procumbens* in chemical- and HFD-induced hyperlipidemic rat models. Further investigations are warranted to elucidate its mechanism of its lipid-lowering action.

Key words: Chlorogenic acid, di-caffeoylquinic acid, *Gynura procumbens*, high-fat diet, lipid-lowering, poloxamer-407

SUMMARY

- *G. procumbens* is found in most of the Southeast Asian countries and used as a folk medicine to treat various illnesses.
- The present study was carried out to investigate lipid-lowering activity of hydroalcoholic extracts of *G. procumbens* in chemical- and high-fat diet-induced hyperlipidemic rats.
- Among all extracts, 95% ethanolic extract showed potential lipid-lowering activity on both acute and chronic hyperlipidemic rat models.
- The lipid-lowering activity may be partly contributed by its chemical constituents, the mono- and di-caffeoylquinic acids.



Abbreviations used: HFD, high-fat diet; P-407, poloxamer-407; 95EEGP, 95% ethanolic extract; TC, total cholesterol; TG, triglycerides; 3,4DC, 3,4-dicaffeoylquinic acid; 3,5DC, 3,5-dicaffeoylquinic acid; 4,5DC, 4,5-dicaffeoylquinic acid; LDL-C, low-density lipoprotein-cholesterol; A. I., atherogenic index; HDL-C, high-density lipoprotein-cholesterol; CVD, cardiovascular disease; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; *G. procumbens, Gynura procumbens*; HPLC-UV, high-performance liquid chromatography-ultraviolet; CA, chlorogenic acid; 75EEGP, 75% ethanolic extract; 50EEGP, 50% ethanolic extract; 25EEGP, 25% ethanolic extract; AEGP, water extract; MeOH, methanol; SEM, standard error mean; VLDL-C: Very low-density lipoprotein-cholesterol.

Correspondence:

Dr. Vikneswaran Murugaiyah,
School of Pharmaceutical Sciences,
Universiti Sains Malaysia, 11800 Penang,
Malaysia.
E-mail: vicky@usm.my
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INTRODUCTION

Globally, noncommunicable diseases such as cardiovascular diseases (CVDs), cancer, diabetes, and chronic respiratory diseases cause 40 million deaths each year.[1] Health data from countries around the world which records global population of 7.6 billion[2] revealed that CVD is the leading cause of death with approximately 17.7 million deaths annually[1] and the mortality was estimated to be 23.6 million by 2030.[3] In Malaysia, 73% of total deaths are contributed by noncommunicable diseases with CVDs being the main contributor.[4] There are a number of risk factors

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associated with the development of CVDs such as obesity, hypertension, diabetes, and hyperlipidemia. Among them, hyperlipidemia plays a significant role in inducing various CVDs.[1,5]

Hyperlipidemia is defined as elevated levels of blood total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and/or declining of high-density lipoprotein-cholesterol (HDL-C). The World Health Organization revealed that hyperlipidemia causes approximately 2.6 million deaths annually worldwide and the prevalence of it is the highest in Europe with 54% for both sexes.[6] The incidence of hyperlipidemia is known to be one of the main contributors for CVDs in Malaysia.[4] Ministry of Health Malaysia discovered that 47.7% of total population aged above 18 is suffering from hyperlipidemia which has doubled since 2006 (20.7%).[4]

The management and control of hyperlipidemia are usually achieved with antihyperlipidemic drugs such as statins (3-hydroxy-3-methylglutaryl coenzyme A, HMG CoA reductase inhibitors) and nonstatins (fibrates, nicotinic acid, bile acid sequestrants, and cholesterol absorption inhibitors). However, there is an increasing concern of the escalating cost and side effects of statins, especially elevated liver enzymes and skeletal muscle damages or myopathy.[7] On the other hand, nonstatins such as fibrates help in lowering blood TG levels. They may also help in increasing HDL-C, although they are not effective in reducing LDL-C levels. Thus, effective, well-tolerated, and cost-effective TC and/or TG lowering agents are much sought after as new therapeutic options. Natural products have been one of the most productive sources for the development of drugs. There are many reports on the lipid-lowering activity of natural products such as *Vernonia amygdalina*, *Tamarindus indica,* and *Hibiscus sabdariffa*.[8-10]

Gynura procumbens (Lour.) Merr, locally known as "sambung nyawa" or "bai bing cha" by the Malay and Chinese communities, respectively, in Malaysia is a weakly climbing perennial herb.[11] It is found in most of the Southeast Asian countries and used as a folk medicine to treat kidney diseases, diabetes mellitus, hyperlipidemia, rash, eruptive fever, constipation, and hypertension.[12,13] Previous pharmacological studies reported that extracts of *G. procumbens* possess hypoglycemic,[13,14] anti-inflammatory,[15] antihypertensive,[16] and antioxidant[17] activities.

The serum TC and TG lowering effect of *G. procumbens* was earlier reported by Zhang and Tan which focused on the antidiabetic and antihyperlipidemic effect of *G. procumbens* in streptozotocin-induced diabetic rats.[13] However, the blood lipids elevation was mild, and the diabetic model did not represent a true hyperlipidemic status of the body. To date, there is no report available on the lipid-lowering effect of *G. procumbens* extract in hyperlipidemic animals. This has created an interest to explore the lipid-lowering effect of various hydroalcoholic extracts of *G. procumbens* in poloxamer-407 (P-407)-induced acute hyperlipidemic rat model. Subsequently, the most active extract was analyzed by a high-performance liquid chromatography-ultraviolet (HPLC-UV) method using selected mono-and di-caffeoylquinic acids, and evaluated for lipids lowering potential in high-fat diet (HFD)-induced chronic hyperlipidemic rat model.

MATERIALS AND METHODS

Chemicals, standards, and reagents

P-407 was purchased from Sigma Chemical Co. (St. Louis, USA). The marker compounds; 3,4-dicaffeoylquinic acid (3,4DC) and 4,5-dicaffeoylquinic acid (4,5DC) with purity of more than 98% (HPLC analysis) were purchased from Chengdu Biopurify Phytochemicals LTD (Chengdu, China) while chlorogenic acid (CA) and 3,5-dicaffeoylquinic acid (3,5DC) were isolated from *G. procumbens*. Tween 20 was purchased from System (Mannheim, Germany). Ethanol (95%) for extraction was purchased from RandM (Essex, UK). Acetonitrile HPLC grade was purchased from J. T. Baker (USA) while methanol (MeOH; HPLC grade) and acetic acid were purchased from Merck (Darmstadt, Germany). Deionized water was prepared in-house. Atorvastatin and lovastatin were bought from Ranbaxy Malaysia (Penang, Malaysia). TG and TC measuring kits were purchased from Thermo Scientific (Middletown, USA).

Raw materials and extracts preparation

The raw materials of *G. procumbens* were purchased from a local plantation farm, Herbagus Sdn. Bhd. (Penang, Malaysia). A voucher specimen (No. 10833) has been deposited at the Herbarium of School of Biological Sciences, Universiti Sains Malaysia (Malaysia). For preparation of various hydroalcoholic extracts, oven dried powdered leaves samples were macerated in the following solvents: 95%, 75%, 50%, 25% ethanol in water (v/v), and 100% water (ratio of powder: solvent, 1:10 w/v) for 2 days on a constant heating water bath at 60°C. Fresh solvents were replenished on each day of extraction. The combined extracts were then concentrated under reduced pressure to dryness at 45°C to afford 1.62 g (8.10% yield), 3.31 g (16.55% yield), 4.51 g (22.55% yield), 6.26 (31.30% yield), 8.51 g (42.55% yield) of extracts for 95%, 75%, 50%, and 25% hydroalcoholic and 100% water, respectively. All the samples were kept in the refrigerator at 4°C before experimentation.

Evaluation of lipid-lowering effect

Animals

Male Sprague-Dawley rats of 10–15 weeks old, weighing initially about 150–250 g were obtained from Animal Research and Service Centre, Universiti Sains Malaysia, Penang, Malaysia. The animals were kept in the animal transit room of the School of Pharmaceutical Science, Universiti Sains Malaysia and maintained on a 12-h light/dark cycle at room temperature. The animals were allowed free access to standard rodent food pellets (Gold Coin, Penang, Malaysia) and tap water and were acclimatized for a week before experimentation. The handling and use of animals were in accordance with the institutional guidelines. The experimental protocols were approved by Animal Ethics Committee, Universiti Sains Malaysia (USM/Animal Ethics Approval/2010/[56][211], and USM/Animal Ethics Approval/2014/[91][553]).

Lipid-lowering effect of Gynura procumbens hydroalcoholic extracts

Evaluation of lipid-lowering effect in poloxamer 407-induced acute hyperlipidemic rat model

A total of 48 animals were used in this study and divided into eight groups of six each. Group 1 served as the normal control while groups 2–8 were induced acute hyperlipidemia by a single intraperitoneal injection of P-407 (300 mg/kg). Group 2 served as the hyperlipidemic control while groups 3–8 received one of the following treatments each: 95% (95EEGP), 75% (75EEGP), 50% (50EEGP), and 25% (25EEGP) of hydroalcoholic extracts (each at 200 mg/kg dose), water extract (AEGP) (200 mg/kg), and lovastatin (10 mg/kg) intraperitoneally at 2 and 18 h after P-407 injection.[18] P-407 was prepared in normal saline and then refrigerated overnight to facilitate dissolution. The extracts and lovastatin for administration were prepared in 20% tween 20 aqueous solutions. For normal and hyperlipidemic controls, the animals were given the vehicle only. Blood samples (≈75 μL) were collected from the animal's tail vein by capillary tubes at 0 and 20 h after P-407 injection. The blood samples were centrifuged at 3000 ×g for 10 min to obtain the serum. Serum samples were frozen at −20°C before analysis. Serum TG and TC levels were determined using commercial kits following the manufacturer's protocol.

Lipid-lowering effect of 95% ethanolic extract of *Gynura procumbens* – Dose-response study

The 95EEGP was analyzed using phenolic acids as marker compounds and further evaluated at three graded doses of 80 mg/kg, 200 mg/kg, and 500 mg/kg in P-407-induced animals following the procedure described above. Terminal blood samples (1.0 mL) were collected by cardiac puncture at 20 h after P-407 injection. Serum TC and TG levels were determined using commercial kits following the manufacturer's protocol while the LDL-C and HDL-C were determined using Architect C4000 biochemistry analyzer. The atherogenic index (A. I.) was calculated using the following formula:

A. I. = LDL-C concentration/HDL-C concentration

Evaluation of lipid-lowering effect in high-fat diet-induced chronic hyperlipidemic rat model

Normal and high-fat diet

The normal diet comprised of commercial food pellets obtained from Gold Coin (M) Sdn. Bhd. (Penang, Malaysia). The HFD was made by mixing commercial standard food powder and ghee at a ratio of 3:2 by weight, the mixture was homogenized and made into small pellets and then dried in the oven at 80°C for 1 h. The dried food pellets were cooled to room temperature and kept in air-tight container until use. The HFD contained protein-fat-carbohydrate at ratio of 12:35.68:35.64.[19]

Lipid-lowering effect of 95% ethanolic extract of *Gynura procumbens*

A total of 36 healthy male Sprague–Dawley rats were used to evaluate the effect of standardized 95EEGP on hyperlipidemia induced by HFD. The animals were randomly divided into six groups of six each and were treated as follows:

- Group I: Normal control group, fed with normal food pellet and 20% tween 20 solution orally once daily at 5 mL/kg for 7 weeks
- Group II: Hyperlipidemic control group, fed with HFD and 20% tween 20 solution orally once daily at 5 mL/kg for 7 weeks
- Group III: Atorvastatin group, fed with HFD and treated with atorvastatin orally once daily at 20 mg/kg for 7 weeks
- Group IV-VI: 95EEGP- treated groups, fed with HFD and treated with various doses of 95EEGP orally once daily at 250, 500, 1000 mg/kg, respectively, for 7 weeks.

The test samples of 95EEGP and atorvastatin were prepared in 20% of tween 20 solution. The normal control and hyperlipidemic groups were orally dosed with the same vehicle. Blood samples (75 µL) were collected weekly for 7 weeks from the tail vein of each rat through capillary tubes and left at room temperature for coagulation. Serum samples were obtained by centrifugation at 3000 × g for 10 min and then stored at −20°C until further analysis. At the end of the experimental period of 7 weeks, following fasting for 12 h, the rats were anesthetized with sodium pentobarbitone (60 mg/kg). Terminal blood samples (≈5 mL) were collected by cardiac puncture and left at room temperature for coagulation. The serum samples were obtained by centrifugation at 6000 × g for 10 min and then the resulting serum samples were kept at-20°C before analysis. Levels of weekly TC and TG in serum were determined using commercial kits following the manufacturer's protocol. LDL-C and HDL-C in serum were determined at the end of the experimental period by enzymatic colorimetric method using Architect C4000 biochemistry analyzer.

Statistical analysis

The results were presented as the mean ± standard error of mean (SEM) of six animals. The statistical significance of difference was evaluated by the analysis of variance followed by Tukey *post hoc* test. $P < 0.05$ was considered to be statistically significant.

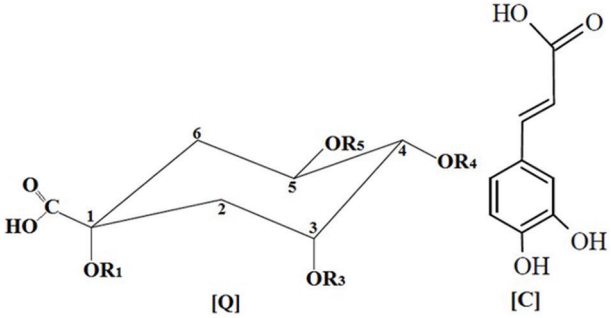
Phytochemical analysis of 95% ethanolic extract of *Gynura procumbens*

The 95EEGP was standardized by an HPLC-UV method using selected mono- and di-caffeoylquinic acids as marker compounds, namely, CA, 3,4DC, 3,5DC, and 4,5DC. The structures of these compounds are given in Figure 1. HPLC analysis was carried out using Agilent Technologies 1200 Infinity series liquid chromatography system (Agilent Technologies, USA) equipped with a quaternary solvent pump, a 1260 autosampler, a UV-Vis detector, and Agilent Chemstation data acquisition system. Chromatographic separations were performed on a ZORBAX Eclipse Plus Phenyl-Hexyl (250 mm × 4.6 mm, 5 µm particle size, Agilent Technologies, USA) column with the following analytical conditions: a mobile phase of acetonitrile-0.25% acetic acid aqueous solution (21.5:78.5 v/v) with a flow rate of 1.0 mL/min at room temperature and UV detector was operated at 340 nm. The injection volume was 10 µL and temperature of the column was maintained at 30°C. Freeze dried 95EEGP (2 mg) was accurately weighed and reconstituted in 2 mL HPLC-grade MeOH to give a stock solution of 1 mg/mL. This stock solution was then filtered through a 0.45 µm syringe filter and kept in −20°C before analysis. The peaks in the samples were identified by comparing with the retention times of standards. Calibration curves were generated for all four standards before analysis and quantification was done with respect to the calibration curve of the standards.

RESULTS

Lipid-lowering effect of *Gynura procumbens* hydroalcoholic extracts in poloxamer 407-induced acute hyperlipidemic rat model

The potential lipid-lowering activity of the various hydroalcoholic extracts and lovastatin in P-407-induced hyperlipidemic rats is summarized in Table 1. The prestudy TC and TG levels between all the groups were within normal physiological range. Of all the extracts tested, only 95EEGP and lovastatin caused significant reductions of both serum TC ($P < 0.05$) and TG ($P < 0.001$) compared to the hyperlipidemic



Name & Abbreviation	R_1	R_3	R_4	R_5
Chlorogenic acid (1)	H	H	H	C
3,4-dicaffeoylquinic acid (2)	H	C	C	H
3,5-dicaffeoylquinic acid (3)	H	C	H	C
4,5-dicaffeoylquinic acid (4)	H	H	C	C

Q = Quinic acid; C= Caffeic acid

Figure 1: Chemical structures of the marker compounds of *Gynura procumbens* extract

control rats. The 50EEGP also caused a significant reduction in serum TG of the hyperlipidemic animals ($P < 0.001$). Among all the extracts tested, 95EEGP showed the most potent effect in lowering serum TC, while 95EEGP and 50EEGP showed similar and potent TG-lowering activity. Based on these findings, 95EEGP was chosen for phytochemical analysis and dose-response evaluation.

Phytochemical analysis of 95% ethanolic extract of *Gynura procumbens*

95EEGP was analyzed using selected mono- and di-caffeoylquinic acids as the marker compounds. The chromatograms of the standards and the 95EEGP are shown in Figure 2. The contents of CA, 3,4DC, 3,5DC, and 4,5DC were 7.18, 3.20, 28.31, and 9.72 mg/g dried extract, respectively.

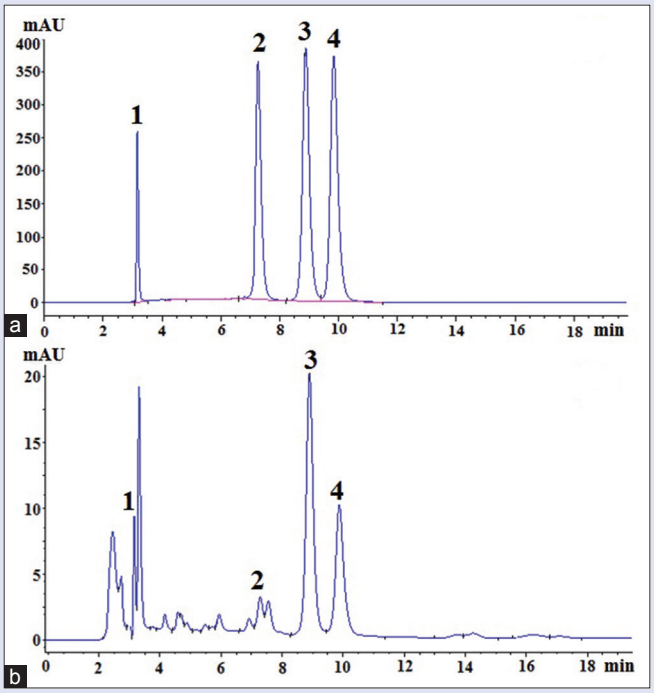


Figure 2: High performance liquid chromatography chromatograms of mixed standards (a) and 95% ethanolic extract of *Gynura procumbens* (b) obtained using ZORBAX Eclipse Plus Phenyl-Hexyl (250 × 4.6 mm, 5 µm particle size) column and ultraviolet–visible detector operated at 340 nm. (1) Chlorogenic acid, (2) 3,4-dicaffeoylquinic acid, (3) 3,5-dicaffeoylquinic acid, and (4) 4,5-dicaffeoylquinic acid

Dose response study of 95% ethanolic extract of *Gynura procumbens*

The dose-lipid lowering activity relationship of the 95EEGP in P-407-induced hyperlipidemic rats at doses of 80 mg/kg, 200 mg/kg and 500 mg/kg and lovastatin is summarized in Table 2. Significant reductions in levels of serum TC, TG, and LDL-C were observed in extract treated animals at doses of 200 mg/kg and 500 mg/kg as compared to the hyperlipidemic control rats ($P < 0.01$ or better). These results were comparable to those of lovastatin. 95EEGP 80 mg/kg caused significant reduction in TC ($P < 0.01$) but not TG or LDL-C. In contrast, none of the doses tested caused significant increase in HDL-C level compared to those of hyperlipidemic control rats. The calculated A. I. showed that administration of P-407 increased the A. I. while 95EEGP at 200 mg/kg and 500 mg/kg significantly ($P < 0.001$) reduced the A. I.

Lipid-lowering effect of 95% ethanolic extract of *Gynura procumbens* in high-fat diet-induced chronic hyperlipidemic rat model

The effects of 95EEGP and atorvastatin on the food intake and body weight of the normal and HFD-induced hyperlipidemic animals are summarized in Table 3. There was no significant change in the average daily food intake of the animals during the 7-weeks experimentation. However, the animals fed with HFD (without any treatment) had significantly higher body weight compared to the normal control at the end of 7th week. Animals treated with atorvastatin or 95EEGP (500 and 1000 mg/kg) had significantly lower body weight compared to the HFD fed hyperlipidemic controls ($P < 0.001$).

The effects of 95EEGP and atorvastatin on serum TC and TG are presented in Figures 3 and 4. Hyperlipidemic control group showed significant increase in serum TC and TG compared to normal control group throughout the study period of 7 weeks. The serum TC levels after 7 weeks showed significant reduction in atorvastatin ($P < 0.001$), 95EEGP 250 ($P < 0.05$), 95EEGP 500 ($P < 0.01$), and 95EEGP 1000 ($P < 0.01$) groups, respectively. The serum TG levels were also significantly reduced ($P < 0.001$) in all treated groups. The most effective doses of 95EEGP that reduced serum TC and TG levels after 7 weeks were the 500 and 1000 mg/kg, showing the comparable effect to that of atorvastatin.

The effect of 95EEGP and atorvastatin on serum LDL-C and HDL-C levels is presented in Table 4. Serum LDL-C levels had a significant increase ($P < 0.001$) while HDL-C showed a significant decrease ($P < 0.001$) in the hyperlipidemic control group compared to the normal control group. The serum levels of LDL-C were significantly decreased in atorvastatin, 95EEGP 500 and 95EEGP 1000 groups ($P < 0.001$). In contrast, the serum levels of HDL-C were

Table 1: Effects of various hydroalcoholic extracts of *Gynura procumbens* leaves on total cholesterol and triglycerides levels of poloxamer-407-induced acute hyperlipidemic rats at different time points

Groups	TC (mmol/L)		TG (mmol/L)	
	0 h	20 h	0 h	20 h
Normal control	2.03±0.11	2.02±0.07	0.65±0.22	0.66±0.02
Hyperlipidemic control (poloxamer + vehicle)	2.03±0.25	5.43±0.91$^{###}$	0.60±0.02	7.33±0.96$^{###}$
Poloxamer + 10 mg/kg lovastatin	2.45±0.26	4.10±0.21*	0.77±0.02	2.50±0.22***
Poloxamer + 200 mg/kg 95EEGP	2.11±0.18	3.35±0.16*	0.97±0.09	4.13±0.26***
Poloxamer + 200 mg/kg 75EEGP	2.53±0.22	4.64±0.38	0.66±0.06	5.80±0.83
Poloxamer + 200 mg/kg 50EEGP	1.87±0.09	3.96±0.23	0.86±0.04	3.71±0.28***
Poloxamer + 200 mg/kg 25EEGP	2.02±0.07	4.58±0.36	0.86±0.06	5.66±0.21
Poloxamer + 200 mg/kg AEGP	2.60±0.24	4.93±0.27	0.87±0.01	4.59±0.26

Values are represented as mean±SEM, (n=6). $^{###}P$<0.001 compared to normal control, *P<0.05, **P<0.01 and ***P<0.001 compared to hyperlipidemic control.[53] 95EEGP: 95% ethanolic extract; 75EEGP: 75% ethanolic extract; 50EEGP: 50% ethanolic extract; 25EEGP: 25% ethanolic extract; AEGP: Water extract; TC: Total cholesterol; TG: Triglycerides; SEM: Standard error mean

Table 2: Dose-response of 95% ethanolic extract of *Gynura procumbens* leaves on serum lipids levels of poloxamer-407-induced acute hyperlipidemic rats at 20 h after hyperlipidemia induction

Groups	Lipids levels (mmol/L)				AI
	TC	TG	LDL-C	HDL-C	
Normal control	1.31±0.02	0.83±0.01	0.15±0.01	0.66±0.02	0.19±0.01
Hyperlipidemic control (poloxamer + vehicle)	8.19±0.30###	6.94±0.41###	1.55±0.14###	0.50±0.03	3.19±0.37###
Poloxamer + 10 mg/kg lovastatin	4.31±0.21***	2.33±0.13***	1.02±0.03**	0.51±0.04	2.05±0.18*
Poloxamer + 80 mg/kg 95EEGP	4.17±0.45**	5.03±0.36	1.55±0.12	0.55±0.05	2.93±0.34
Poloxamer + 200 mg/kg 95EEGP	3.93±0.11***	1.80±0.24***	1.01±0.07**	0.63±0.05	1.60±0.09***
Poloxamer + 500 mg/kg 95EEGP	2.37±0.37***	1.26±0.17***	0.70±0.05***	0.56±0.05	1.32±0.21***

Values are represented as mean±SEM, (n=6). ###P<0.001 compared to normal control, *P<0.05, **P<0.01 and ***P<0.001 compared to hyperlipidemic control.[53] 95EEGP: 95% ethanolic extract; TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; AI: Atherogenic index; SEM: Standard error mean

Table 3: Effects of 95% ethanolic extract of *Gynura procumbens* leaves on food intake and body weight of high-fat diet-induced chronic hyperlipidemic rats

Groups	Average daily food intake (g/rat/day)	Mean body weight (g)	
		Day 0	Day 49
Normal control	21.85±0.47	224.88±3.97	293.98±3.00
Hyperlipidemic control	20.75±0.43	225.14±1.99	329.85±3.51###
20 mg/kg atorvastatin	22.39±0.42	224.50±3.35	296.39±2.10***
250 mg/kg 95EEGP	23.32±0.42	234.69±4.42	325.12±1.97
500 mg/kg 95EEGP	21.50±0.45	222.04±2.22	301.17±2.55***
1000 mg/kg 95EEGP	21.23±0.46	227.38±2.47	302.26±1.57***

Values are represented as mean±SEM, (n=6). ###P<0.001 compared to normal control, *P<0.05, **P<0.01 and ***P<0.001 compared to hyperlipidemic control.[53] 95EEGP: 95% ethanolic extract; SEM: Standard error mean

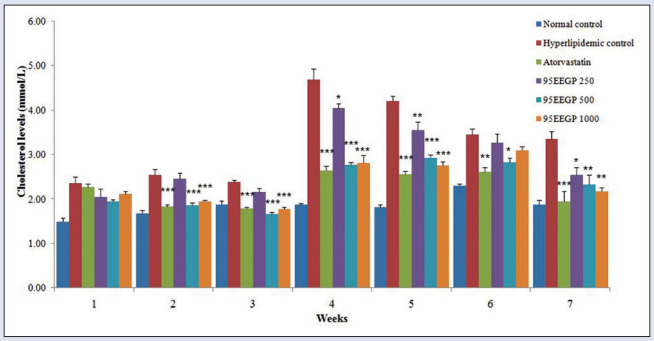


Figure 3: Serum total cholesterol levels in high-fat diet-induced hyperlipidemic rats treated with different doses of 95% ethanolic extract of *Gynura procumbens* and atorvastatin for 7 weeks. Abbreviation: 95EEGP: 95% ethanolic extract; Values are represented as mean ± standard error of mean, (n = 6). ###P < 0.001 compared to normal control, *P < 0.05, **P < 0.01 and ***P < 0.001 compared to hyperlipidemic control.[53]

significantly increased in all treated groups (P < 0.001). A. I. values were significantly increased (P < 0.001) in hyperlipidemic control group compared to the normal control group. In contrast, A. I. values were significantly decreased in all treated groups compared to hyperlipidemic control (P < 0.001). The lipid-lowering results of 95EEGP in HFD-induced hyperlipidemic rats confirmed and further supported the lipid-lowering activity of *G. procumbens* using P-407-induced hyperlipidemic rats.

DISCUSSION

Hyperlipidemia is one of the most significant risk factors for CVDs, and it is well known that lowering serum LDL-C levels would help in the prevention of CVDs.[20] Medicinal plants are one of the fundamental element of indigenous remedial systems, and quite a number of plants with their bioactive constituents have been shown to lower plasma lipids levels.[21] One such medicinal plant is *G. procumbens*. However, the use of this plant in the management of hyperlipidemia has not been well studied, and thus, this study attempted to investigate the lipids lowering capability of various hydroalcoholic extracts of *G. procumbens* leaves in chemical- and HFD-induced hyperlipidemic rat models.

Male rats were used in this study because they are less affected by hormonal changes. According to McCrohon *et al.*[22] and Vedder *et al.*,[23] female hormones, i.e., estrogen and progestin, both reduce lipid accumulation in the body, while estrogen reduces lipid peroxidation. Moreover, anabolic steroids and progestins increase the level of LDL-C and decrease the level of HDL-C.[24] Sprague-Dawley strain rats were used as test animals in this study due to the similarities with humans in terms of physiology and are widely used in research related to lipid-lowering effect of medicinal plants.[25]

Atorvastatin and lovastatin, both HMG-CoA reductase inhibitors with comparable efficacy, common lipid-lowering effect, similar mechanism of action and safety profile were used as reference standards in hyperlipidemic study.[26] Atorvastatin was chosen for chronic study as it is one of the high potency cholesterol-lowering statins. A comparative study by Jones *et al.*[27] demonstrated atorvastatin as the most efficacious HMG-CoA reductase inhibitor for lowering LDL-C at different doses.[27]

P-407 has been used for induction of acute hyperlipidemia in animals for the study of various potential lipid-lowering drugs and also natural products.[28-30] In acute hyperlipidemic study, P-407 administered untreated rats demonstrated elevated increase in the levels of TC, TG, LDL-C, and A. I. with considerable decrease in HDL-C level as compared to normal group up to 20 h postinduction. This displays the feasibility of P-407 as hyperlipidemia inducing agent which can be used to assess lipid-lowering activity of medicinal plants. The findings are in corroboration with that of previous work by Mansurah and Megalli *et al.*,[31,32] where P-407 was shown to elevate plasma lipid levels significantly, suggested to be due to the suppression of endothelial heparin-releasable lipoprotein lipase. Significantly higher level of LDL-C than HDL-C is considered normal in hyperlipidemic animals and has been reported in quite a number of studies that used P-407 to induce hyperlipidemia.[31] Johnston and Waxman mentioned that administration of P-407 causes shift in lipoprotein distribution, mainly from HDL-C to LDL-C.[33]

Doses of the extracts for the acute study were selected based on a pilot study carried out to determine the dose range and a previous study that found *G. procumbens* extract to be safe even at highest test dose of 5 g/kg.[34] Intraperitoneal route was utilized for the administration of the two doses of test extracts in acute hyperlipidemic study to avoid bioavailability issues that could mask the real potential of the extracts. In most previous studies, it was reported that intraperitoneal route is

Table 4: Effects of 95% ethanolic extract of *Gynura procumbens* leaves on serum lipids levels of high fat diet-induced chronic hyperlipidemic rats at the end of 7th week

Groups	Lipids levels (mmol/L)				AI
	TC	TG	LDL-C	HDL-C	
Normal control	1.87±0.10###	1.14±0.03###	0.22±0.00###	0.52±0.01###	0.41±0.01###
Hyperlipidemic control	3.36±0.17	4.38±0.10	0.85±0.02	0.37±0.01	2.31±0.08
20 mg/kg atorvastatin	1.95±0.23***	1.25±0.09***	0.28±0.00***	0.73±0.00***	0.38±0.00***
250 mg/kg 95EEGP	2.54±0.17*	3.52±0.16***	0.90±0.01	0.46±0.00***	1.97±0.04***
500 mg/kg 95EEGP	2.34±0.12**	1.29±0.06***	0.37±0.00***	0.62±0.00***	0.60±0.00***
1000 mg/kg 95EEGP	2.18±0.08**	1.24±0.03***	0.32±0.00***	0.67±0.00***	0.48±0.00***

Values are represented as mean±SEM, (n=6). ###P<0.001 compared to normal control, *P<0.05, **P<0.01 and ***P<0.001 compared to hyperlipidemic control.[53] 95EEGP: 95% ethanolic extract; TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; AIL: Atherogenic index; SEM: Standard error mean

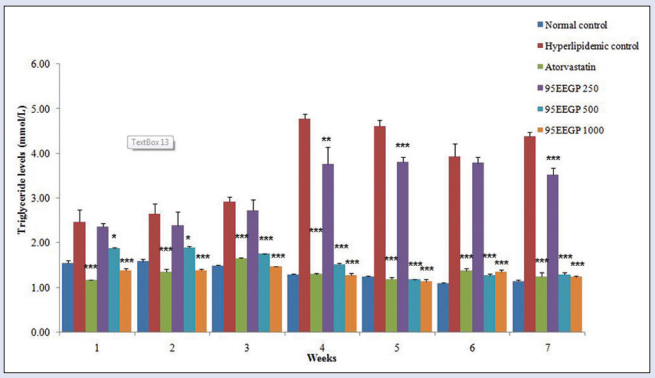


Figure 4: Serum triglycerides levels in high-fat diet-induced hyperlipidemic rats treated with different doses of 95% ethanolic extract of *Gynura procumbens* and atorvastatin for 7 weeks. Abbreviation: 95EEGP: 95% ethanolic extract; Values are represented as mean ± standard error of mean, (n = 6). ### P < 0.001 compared to normal control, *P < 0.05, **P < 0.01 and ***P < 0.001 compared to hyperlipidemic control.[53]

more preferred than intravenous injection and is commonly used in small laboratory animals as it is more practical with 5% failure rate and has almost similar bioavailability as the later, whereas intravenous is less favored due to complications in tracing veins.[35] Furthermore, intraperitoneal route is also more preferred than oral as it has better bioavailability and prevents degradation of drugs by gastric juices. While substances administered through subcutaneous route often are absorbed at a slower rate compared with other parenteral routes and is designed for slower release and absorption of chemicals into the bloodstream.[36]

HFD-induced hyperlipidemia imitates pathological condition similar to human in rats. Significant elevation of serum lipid levels was observed in HFD fed rats compared to rats fed with normal diet due to the presence of saturated fatty acids in ghee, which indicates that hyperlipidemia was successfully established in Sprague-Dawley rat model. HFD usually results in alteration of type and distribution of plasma lipoproteins and their apoproteins.[37] Most distinct change can be observed in arginine-rich apoprotein, which is linked with β-migrating very low-density lipoprotein-cholesterol (VLDL-C), LDL-C, and HDL-C.[37] Oral dosing technique was opted in chronic study, as it mimics the most commonly used administration method of substances among real-life patients.[38] The treatment doses of 95EEGP were increased in HFD-treated animals in attempt to discover long-term treatment effects on hyperlipidemic animals.

Increased levels of TG and LDL-C in blood can cause disastrous cardiovascular events.[39,40] TG plays an important role in maintaining lipid metabolism by regulating interactions of lipoproteins, and increased TG is often related with coronary artery diseases.[41] The presence of oxygen-free radicals produced by endothelial cells, monocytes, and macrophages oxidizes LDL-C to oxidized LDL-C, leading to lipid peroxidation. Oxidized LDL-C later attracts macrophages which forms foam cells and accumulate in the arterial wall causing atherosclerosis.[31] Along with LDL-C and TG, TC is also a powerful contributing factor for atherosclerosis and related cardiovascular illnesses.[42] A. I. the ratio of LDL-C to HDL is a useful tool to evaluate the risk for coronary heart disease.[43] Unlike LDL-C, HDL-C acts as an anti-atherogenic agent by counteracting LDL-C oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism.[30]

In the present study, a marked increase in serum TC, TG, LDL-C, and A. I. levels, along with decrease in the concentration of HDL-C levels were detected in both P-407 and HFD-induced hyperlipidemic animals. However, elevated non-HDL lipids levels, as well as A. I. values, were dose-dependently reduced upon administration of different doses of 95EEGP in hyperlipidemic animals. On the contrary, declined levels of HDL-C were increased as well by the administration of 95EEGP. In the acute study, the dose-dependent reduction in A. I. index by 95EEGP was likely to be due to its ability to reduce LDL-C since the changes of the HDL-C level were not significant. While, in chronic experiment, 1000 mg/kg 95EEGP consistently normalized serum lipids levels in 7 weeks, which appeared comparable to atorvastatin. The findings indicate that 95EEGP is an effective lipid-lowering agent and regular administration may reduce the risk of atherosclerosis and related CVDs. The present result of 95EEGP matches with those reported by Zhang and Tan,[13] whom presented evidence that 95EEGP of *G. procumbens* is an effective lipids lowering agent. The team investigated serum glucose, TC and TG lowering abilities of 95EEGP in streptozotocin-induced diabetic rats and found out that administration of the extract for 7 days continuously resulted in significant reduction of TC and TG levels of diabetic rats.[13]

Phytochemical studies on *G. procumbens* leaves have reported the presence of compounds such as sterol, sterol glycosides,[44] quercetin, kaempferol-3-*O*-rutinoside, astragalin, quercetin 3-*O*-rhamnosyl (1-6) glucoside, and quercetin 3-*O*-rhamnosyl (1-6) galactoside.[14] From the HPLC analysis, 3,5DC, 4,5DC, and CA were found to be the major compounds present in the extracts besides 3,4DC. The observed antihyperlipidemic potential of 95EEGP may be at least partly contributed by its caffeoylquinic acids content as literature reviews on antihyperlipidemic activity of herbs suggested that phenolic acids especially hydrocinnamic acids are responsible for TC, TG, LDL-C, and VLDL-C lowering capacities and possess the ability to decrease the risk of developing atherosclerosis and related CVDs by increasing HDL-C levels.[45,46]

The previous study has reported on the ability of CA to reduce plasma TC and TG.[47] Earlier, Liu *et al.*[45] evaluated antihyperlipidemic

activities of different fractions of *Pandanus tectorius* Soland, and found that caffeoylquinic acid rich *n*-butanol fraction was quite effective in lowering blood lipids. They isolated caffeoylquinic acids (CA, 3,4DC, and 4,5DC) from the active fraction and revealed that 3,4DC and 4,5DC significantly reduced the oil-red O staining and TC and TG accumulation in HepG2 cells signifying that caffeoylquinic acids play a vital role in contributing to antihyperlipidemic activity of the fruits of *P. tectorius*. Nugroho *et al.*[46] reported that caffeoylquinic acids rich methanolic crude extract of *Ligularia stenocephala* leaves and its butanol fraction decreased rats body weight, abdominal fat pad weight, A. I. and TBARS values, indicating that the caffeoylquinic acid-rich extract probably inhibited hyperlipidemia and oxidative stress caused by HFD.

However, other compounds present in 95EEGP may also contribute to its lipid-lowering activity. Few studies suggested that flavonoids present in plants such as rutin, myricetin, and naringenin hydrate are able to regulate plasma TC and TG.[48-50] Plant sterols are known to have lipids lowering effect. For example, stigmasterol was reported to reduce serum TC by suppressing the HMG-CoA and thus inhibiting the biosynthesis of cholesterol.[51] Owing to its polarity, plant sterols would present at a higher concentration in less polar extracting solvent system such as 95% ethanol, which may have contributed to its lipid-lowering activity. Inhibition of HMG-CoA may also reduce serum TG because inhibition of cholesterol synthesis causes the reduction of hepatic cholesterol content, which in turn may increase the expression of LDL-C receptors. The up-regulation of LDL-C receptors lowers concentration of TG rich lipoprotein because the intermediate-density lipoprotein-cholesterol and VLDL-C remnants are also removed from the circulation through the LDL receptor.[52]

CONCLUSION

The 95% ethanolic extract (95EEGP) showed potential lipid-lowering activity on both chemical- and HFD-induced hyperlipidemic rat models. The lipid-lowering effect may be partly contributed by its chemical constituents, the mono- and di-caffeoylquinic acids. This finding suggested the possible use of *G. procumbens* to regulate blood TC and TG and management of CVDs.

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Conflicts of interest

There are no conflicts of interest.

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