Effects of *Garcinia atroviridis* on serum profiles and atherosclerotic lesions in the aorta of guinea pigs fed a high cholesterol diet

Amran A A, Zaiton Z, Faizah O, Morat P

ABSTRACT

Introduction: The fruit extract of *Garcinia atroviridis (G. atroviridis)* contains hydroxycitric acid and flavonoids, which have been reported to have a hypolipidaemic property. This extract with solvent methanol was used to investigate its effects on serum lipid profiles of guinea pigs fed a high cholesterol diet.

Methods: 24 male Dunkin Hartley guinea pigs were randomly divided into four groups. The first group served as controls and was fed with commercial rabbit chow. The second group was given only *G. atroviridis* by oral gavage (50 mg/body weight). The third group was fed a one percent cholesterol diet in food pellets in order to induce atherosclerosis. The fourth group was administered *G. atroviridis* with cholesterol. All the treatments were given daily for eight weeks, after which the animals were sacrificed, and the blood and aorta were taken for biochemical analysis and histological studies.

Results: The supplementation of *G. atroviridis* with a cholesterol diet decreased the level of lipid profile in the serum. Histological studies showed a reduction in fat deposition in the aorta of high cholesterol diet animals given *G. atroviridis* as compared to the high cholesterol diet group.

Conclusion: This study has shown that dietary intake of *G. atroviridis* has a tendency to decrease lipid composition levels in the serum and reduce fat deposition in the aorta of high cholesterol diet animals.

Keywords: atherosclerosis, cholesterol, *Garcinia atroviridis*, lipid profile

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INTRODUCTION

Garcinia atroviridis (*G. atroviridis*) Griff ex T. Anders is a medium-sized fruit tree that belongs to the Gutiferae family. It is endemic to Peninsular Malaysia and is known as "asam gelugur" or "asam keeping" in Malaysia.[1] The plant contains fruit acids, such as citric acid, tartaric acid and ascorbic acid, that have antioxidant properties. Phytochemical investigations of *G. atroviridis* have enabled the isolation of garcinia acid (t-hydroxycitric acid) and its γ-lactone, atroviridin, atrovirisidone and atrovirinone, as well as the identification of some organic acids, viz. citric, pentadecanoic, octadecanoic, nonadecanoic and dodecanoic acids in its fruit by gas chromatography-mass spectrometry (GC-MS).[2-4] However, the most important bioactive compound is the hydroxycitric acid (HCA). *G. atroviridis* also contains flavonoids, demonstrating a wide range of biochemical and pharmacological effects including antioxidation, anti-inflammation, antiplatelet, antithrombotic action and anti-allergic effects.[5] In folkloric medicine, *G. atroviridis* has been used as a postpartum medication agent as well as an agent to treat earache, throat irritation, cough, dandruff and any stomachache associated with pregnancy.[1] The extracts of *G. atroviridis* also exhibit strong antimicrobial, antioxidant, antitumour and anti-inflammatory activities.[1,6] It has been used to reduce blood pressure in rats.[7]

Hypercholesterolaemia is known to be one of the prime risk factors for ischaemic cardiovascular diseases, such as arteriosclerosis. An important risk factor for the development of atherosclerosis is an atherogenic lipid profile, i.e. hyperlipidaemia with increased LDL-cholesterol (LDL-C) and relatively decreased HDL-cholesterol (HDL-C). Atherosclerosis is a major source of morbidity and mortality in both developing and developed countries.[8] Atherosclerosis is a chronic inflammatory disease of the arterial intima characterised by the formation of an atherosclerotic plaque.[9] Accumulating evidence suggests that plaque formation is associated

Department of Physiology, Faculty of Medicine, University Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur 50300, Malaysia

Amran AA, MSc
Doctorate Student

Zaiton, Z, MBBS, PhD
Associate Professor

Department of Anatomy

Faizah O, MD, PhD
Associate Professor

Department of Biomedical Science

Morat P, PhD
Professor

Correspondence to:
Dr Zaiton Zakaria
Tel: (60) 3 4040 5577
Fax: (60) 3 2693 9687
Email: zaitonz@medic.ukm.my

Table I. Effect of *Garcinia atroviridis* on the serum lipid profiles of the different groups of guinea pigs.

Group	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	LDL (mmol/L)	HDL (mmol/L)
Control	1.25 ± 0.30	1.6 ± 0.40	0.35 ± 0.10	0.10 ± 0.02
G. atroviridis	1.05 ± 0.15	1.23 ± 0.12	0.4 ± 0.15	0.10 ± 0.02
Cholesterol	4.57 ± 1.90*	1.52 ± 0.52	3.8 ± 1.76*	0.10 ± 0.06
Ga + Ch	3.61 ± 0.97*	1.52 ± 0.70	3.18 ± 1.11*	0.10 ± 0.08

G.: Garcinia; Ga + Ch: *G. atroviridis* + cholesterol
The data is expressed as mean ± standard deviation.
* $p < 0.01$ compared to the control & *G. atroviridis* groups.

Table II. Scoring spaces of fat droplets histological slides in different groups.

Group	Scores
Control	0.50 ± 0.54
G. atroviridis	0.67 ± 0.51
Cholesterol	2.50 ± 0.54*
Ga + Ch	1.60 ± 0.54†

G.: Garcinia; Ga + Ch: *G. atroviridis* + cholesterol
* $p < 0.01$ compared to the control & *G. atroviridis* groups
† $p < 0.05$ compared to the control & *G. atroviridis* groups

with the production of reactive oxygen species, which is currently reported to induce oxidative tissue damage.[9]

METHODS

24 male Dunkin Hartley guinea pigs *(Cavia porcellus)* weighing 700–1,000 g were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia, and maintained under standard conditions (temperature 24°C, light/darkness cycles of 12 hours). The animals had free access to food and water, in addition to a twice-weekly feed consisting of a vegetable diet of mustard leaves, cabbage and carrots. The animals were equally divided into four groups comprising six animals each, i.e. control, *G. atroviridis* diet only, high cholesterol diet only and *G. atroviridis* with high cholesterol diet (Ga + Ch) groups. The control group was fed with a commercial rabbit chow and vegetable diet throughout this study. The high cholesterol powder was purchased from Research Biolabs (MP Biomedicals, France) and mixed with the crushed rabbit chow pellet (1% cholesterol, w/w, in food pellet).[10] For each 100 g of crushed rabbit chow pellet, 1 g of cholesterol was added and mixed with 30 ml of distilled water. This mixture was converted into pellet form and dried in an oven at 50°C overnight. *G. atroviridis* was orally administered via an oral gavage needle (50 mg methanol extract of *G. atroviridis* / body weight).[11] *G. atroviridis* fruits were purchased from a local supplier and were identified by a pharmacognosy expert at the Department of Pharmacy, Faculty of Allied Science.

G. atroviridis fruits were prepared by air-drying and cutting them into small pieces (600g), which were successively extracted with methanol (99.8%) using a Soxhlet extractor. The resultant solution was filtered and dried using a rotatory evaporator in a water bath at a temperature not exceeding 50°C. 50 mg of this end-product was then mixed with 1 ml of water as a solvent and orally administered to the animals using an oral gavage needle. This was carried out daily for eight weeks. This study was approved by the Animal Ethical Committee of the university. After eight weeks, the animals were fasted overnight in preparation for the serum and aorta collection. Early the next morning, the animals were weighed, anaesthetised by chloroform and the thoracic abdominal cavity was opened. Blood was collected via cardiac puncture and the serum was separated from the blood by centrifugation at 3,000 rpm for five mins. The serum was collected and kept in a refrigerator at –80°C until needed. The aorta was cut at the origin and severed from the heart. A 2-mm ring section of the aorta of each animal was soaked in a 10% (v/v) formal saline solution for haematoxylin & eosin staining.

Serum lipid profiles (total cholesterol, triglyceride, LDL and HDL) were measured using a Randox kit with an automated clinical chemistry analyser (Vitalab Selectra E, Vital Scientific, Dieren, The Netherlands). The tissues that were analysed were embedded in paraffin as the final process of making tissue blocks. They were then trimmed and sectioned with a microtome (Leica, Wetzlar, Germany) at room temperature to obtain 4-µm sections. The ribbons of sections were floated in a 50°C water bath (leica bath H1210), then "fished" and mounted onto the poly-L-lysine-treated glass slides. The sections were then de-paraffinised on a 60°C hot plate. This was followed by the hydration process, which included immersion in xylene for two mins and a series of steps to decrease ethanol concentrations, beginning with absolute ethanol (2 mins), 95% ethanol (2 mins), and 80% ethanol (2 mins). The slides were then dipped in 3% hydrogen peroxide for 15 mins, and examined under a light microscope for observation of structural abnormality of the aorta. The

Fig. 1 Photomicrograph of the aortic cells of the control group (Haematoxylin & eosin, × 10).
L: lumen; I: endothelial layer (tunica intima); M: tunica media; A: tunica adventitia.

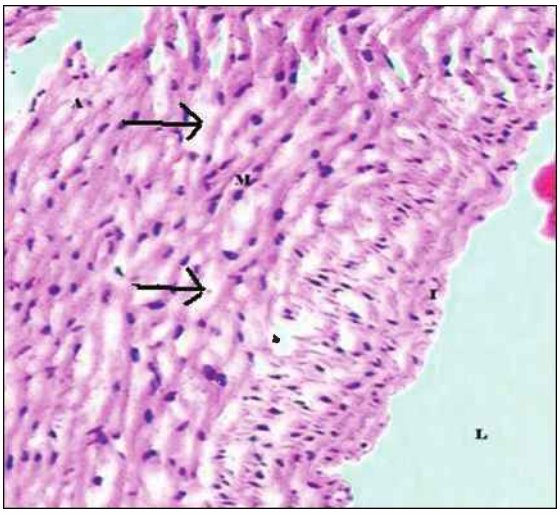

Fig. 2 Photomicrograph shows the aortic cells of a high cholesterol diet animal. Arrows indicate spaces within the tunica intima and tunica media containing fat droplets (Haematoxylin & eosin, × 40).

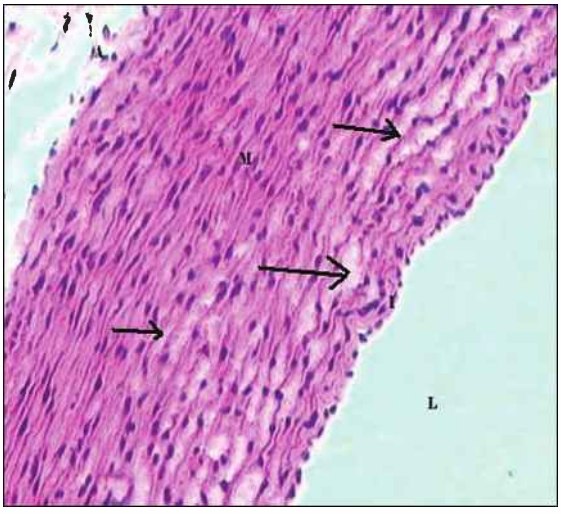

Fig. 3 Photomicrograph shows the aortic cells of animals fed with *G. atroviridis* and high cholesterol. Arrows indicate less space within the tunica intima and tunica media (Haematoxylin & eosin, × 20).

sections were examined blindly using a scoring system.[12] The scores were given as 0 (if no lesion was detected), one (about 33% of lesions detected), two (about 33%–66% of lesions detected) and three (> 66% of lesions detected). Statistical analysis was carried out using the Statistical Package for Social Sciences version 12.0 (SPSS Inc, Chicago, IL, USA). Normal distribution of all the variables was examined using the Kolmogrov-Smirnov test. The results showed that all variables were normally distributed. The data was analysed using the analysis of variance test.

RESULTS

Table I shows the results of serum total cholesterol, LDL, triglycerides and HDL for all four groups. It was observed that the levels of total cholesterol and LDL (except HDL-C) were significantly higher ($p < 0.01$) in the cholesterol group as compared to the normal control animals. The addition of *G. atroviridis* to the high cholesterol animal diet also resulted in increased levels of total cholesterol and LDL in the serum as compared to the control group ($p < 0.05$), but these levels were not as high as those of the cholesterol only group. There was a reduction of 21% and 17% in the total cholesterol and LDL levels, respectively, in the Ga + Ch group as compared to the cholesterol group, although HDL levels were increased by 11%. There was a strong positive correlation between cholesterol and LDL levels (r 0.98, $p < 0.01$), while there were no significant correlations between the other tests. The histological slides were examined blindly using the scoring system (Table II). The aorta of the high cholesterol diet animals showed 56% spaces within the tunica intima and tunica media. The aorta of the Ga + Ch group showed less spaces of fat droplets as compared to the cholesterol group. These spaces had originally contained fat droplets which were dissolved during the haematoxylin & eosin staining procedure (Figs.1–3).

DISCUSSION

Chemical analysis revealed that the *G. atroviridis* fruit extract contained fruit acids, such as citric acid, tartaric acid, malic acid and ascorbic acid, which have antioxidant properties. One of the important substances, HCA, could help to promote weight loss by lowering lipogenesis and increasing glycogen development, thus decreasing appetite.[13] The cholesterol group showed a statistically significant increase in serum cholesterol ($p < 0.01$) and LDL-C ($p < 0.01$), as compared to the control group and

the *G. atroviridis* group. However, there was no significant increase in serum triglyceride in the cholesterol group. This is similar to earlier reports, which stated that a high cholesterol diet in animals increased serum cholesterol levels for varying periods of time without increasing triglyceride levels.[14-16] In the present study, the cholesterol group showed no effect on serum HDL-C, which is similar to an earlier report.[17]

This increase in total cholesterol and LDL-C was due to the increase in cholesterol absorption in the intestines. There was no effect on the triglyceride levels because it was not a fatty diet. The Ga + Ch diet lowered the total cholesterol by 26%, LDL-C by 19% and triglyceride by 14%, and there was no effect on HDL-C when compared to the cholesterol group. This decrease could be due to the HCA content of the *G. atroviridis*, which is known to be hypolipidaemic. HCA is the principle acid of the *G. atroviridis* fruit; it has been shown to be a competitive inhibitor of ATP-citrate lyase which catalyses the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-COA.[18] ATP-citrate lyase inhibition limits the availability of acetyl-COA units required for cholesterol synthesis and triglyceride. Notably, all tissues containing nucleated cells synthesise cholesterol accounting for 10% of the total synthesis of cholesterol, which is similar to the amount that is contributed by the liver. This synthesised cholesterol is transported by HDL into VLDL and LDL, which in turn makes its way to the liver. As mentioned earlier, the only hepatic cholesterol synthesis is inhibited by increasing dietary cholesterol, therefore HCA may inhibit cholesterol synthesised in the other tissues. Extensive animal studies have indicated that HCA suppresses fatty acid synthesis, lipogenesis and induces weight loss.[19] *G. atroviridis* may also reduce cholesterol by interfering with intestinal cholesterol uptake, increasing the conversion of cholesterol into bile acids and increasing the excretion of bile acids.[20] As mentioned earlier, *G. atroviridis* may decrease cholesterol and triglyceride levels by inhibiting the endogenous synthesis of cholesterol and triglyceride, as the HCA present in *G. atroviridis*, may inhibit the acetyl-COA synthesis.

For the histological study, the aorta in the cholesterol group animals showed a statistically significant increase ($p < 0.05$) in the intimal thickening and the spaces within the tunica intima and tunica media. These spaces had originally contained fat droplets which were dissolved during the haematoxylin & eosin staining procedure, and also showed foam cells as compared to the control and *G. atroviridis* groups. There are reports that have found that when a 2% cholesterol diet was given to the guinea pigs, the aorta of the high cholesterol diet animals showed spaces within the tunica intima and tunica media.[20] This indicated that the dietary cholesterol had a significant impact on lipid metabolism and the development of atherosclerotic lesions.[12] The increase in the spaces could be attributed to the free radicals that could damage the membranes and DNA of the endothelial cells in the tunica intima and the smooth muscle cells in the tunica media. Thus, it is clear that the high cholesterol diet significantly increased lipid composition levels in the aorta. Theoretically, the native LDL-C had little direct effect on essential functions in cells within the arterial wall. However, when LDL-C was metabolised by the endothelial cells, the normal components of antioxidants were exhausted, the polyunsaturated phospholipids were converted to reactive hydroxyl fatty acids, lysophosphatidyl choline was formed, and the proteins in apolipoprotein B100 moiety underwent covalent modification and fragmentation. Furthermore, lipoprotein(a) induces the formation of oxygen free radicals in monocytes. Thus, the oxidised lipoproteins and the decreased antioxidants could be responsible for the oxidative stress in the cholesterol group. This could also be due to the increased levels of oxygen radicals, which are known to produce endothelial cell injury.[21] This represents a critical initiating event in the development of atherosclerosis.[22] Another report has shown that the increase in total cholesterol is the critical factor involved in the development of atherogenic lesions in the rabbit.[12] However, in the *G. atroviridis* group, there was no significant change in the tunica intima and tunica media as compared to the control group. *G. atroviridis* may have prevented the oxygen radical-induced endothelial cell injury through its antioxidant activity. In the Ga + Ch group, a decreased area containing spaces of fat droplets as compared to the cholesterol group was observed. This could be due to *G. atroviridis* exhibiting its antiatherogenic effect. It is not well known whether *G. atroviridis* acts directly to reduce lesion formation or indirectly by inhibiting the accumulation of cholesterol and/or oxidised lipid. A human study showed that a diet rich in antioxidants provides protection against lipid peroxidation and free radical generation, and also inhibits the development of atherosclerosis.[23] In conclusion, the supplementation of *G. atroviridis* extract has a tendency to decrease the total cholesterol and LDL-C levels in serum, and the lipid deposition in the aorta of a high cholesterol diet. Hence, *G. atroviridis* may be useful in preventing atherosclerosis or lowering the relative risk of atherosclerosis. The protective effect of *G. atroviridis* could be attributed to its antioxidant activity.

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