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Effects of *Labisia pumila* var *alata* extracts on the lipid profile, serum antioxidant status and abdominal aorta of high-cholesterol diet rats

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ABSTRACT

Background: Previous studies on *Labisia pumila* var. *alata* (LPva) have showed that it could inhibit low-density lipoprotein (LDL) oxidation and provide protection on myocardial infarction in rats.
Hypothesis/Purpose: We hypothesized that LPva extracts can modulate the lipid profiles and serum antioxidant status of hypercholesterolemic rats. In the present study, we investigated the effects of aqueous and 80% ethanol extracts of LPva on atherogenic and serum antioxidant parameters as well as changes in abdominal aorta of high-cholesterol diet rats.
Methods: The major components of the extracts, gallic acid, flavonoids and alkyl resorcinols were analyzed by using a validated reversed phase HPLC method. The rats were induced to hypercholesterolemic status with daily intake of 2% cholesterol for a duration of 8 weeks. Three different doses (100, 200 and 400 mg/kg) of the extracts were administered daily on the 4th week onwards. The rats were then sacrificed and the blood was collected via abdominal aorta and serum was separated by centrifugation for biochemical analysis. Part of the aorta tissues were excised immediately for histopathological examination.
Results: The serum of LPva treated rats showed significant reduction in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels and the abdominal aorta showed a significant decrease of atheroma lesions in treated rats. Serum lipid profiles of treated rats showed a decrease in total cholesterol, total triglycerides and low-density lipoprotein (LDL) levels as compared to control group. The atherogenic indices in treated rats were significantly improved along with an increasing level of serum high-density lipoprotein (HDL). The extracts also exhibited significant increase of antioxidant enzymes and decrease of MDA as a product of lipid peroxidation.
Conclusion: LPva extracts can reduce the risk of dyslipidemia by improving the serum lipid profiles and modulating serum antioxidants.

Introduction

It is a common perception that medicinal or edible plants that are rich in phenolics and flavonoids will benefit in giving protection towards excessive reactive oxygen species (ROS) and other oxidative stress-caused diseases such as inflammation and atherosclerosis. For centuries, many studies have been done worldwide in exploring herbal plants for its possibility to offer alternative and scientifically safe medicine in relation to cardiovascular diseases (Ramachandaran et al. 2011; Green et al. 2012; Liu et al. 2014).

*Labisia pumil*a var *alata* (LPva) has been studied for its antioxidant, anti-inflammatory, phytoestrogenic and skin collagen synthesis promoting properties (Chua et al. 2012; Sanusi et al. 2013). Previous study by Saputri and Jantan (2011) provided potential indicator of LPva to reduce oxidation of human low-density lipoprotein cholesterol (LDL) *in vitro*. Recently, a study by Dianita et al. (2015) suggested LPva showed *in vivo* antioxidant contribution towards myocardial infarction in rats. Yet, there is little information available on *in vivo* study of LPva in relation to cardiovascular diseases. Thus, we designed our study to assess LPva on atherogenic parameters and oxidative status on hypercholesterolemic rats. The atherogenic parameters would be evaluated based on lipid profile, atherogenic indices and histophatological examination.

Abbreviations: ALT, alanine transaminase; alpva, aqueous extract of *Labisia pumila* var *alata*; AST, aspartate transaminase; CVD, cardiovascular disease; elpva, 80% ethanol extract of *Labisia pumila* var *alata*; GR, glutathione reductase; GSH, reduced glutathione; HCD, high cholesterol diet; HDL, high-density lipoprotein; HPLC, high performance liquid chromatography; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; lpva, *Labisia pumila* var *alata*; MDA, malondialdehyde; SOD, superdioxide dismutase; TC, total cholesterol; TG, total triglyceride; VLDL, very low-density lipoprotein; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive species.

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Materials and methods

Drugs and chemicals

Myricetin, rutin, gallic acid, sodium heparin, isotonic saline solution, phosphate buffered saline, metaphosphoric acid, manganese chloride, triethanolamine were purchased from Sigma-Aldrich (St Louis, MO, USA). Atorvastatin was purchased from Calbiochem® (Merck KGaA, Darmstadt, Germany). A 2%-cholesterol diet rat (cat. no. 904691) was purchased from MP Biomedicals (St. Ana, CA, USA). Compounds 5-(Z-nonadec-14-enyl)resorcinol and demethylbelamcandaquinone B were previously isolated from *Labisia pumila* var. *alata* (Dianita et al. 2015).

Plant sample and extracts preparation

The whole plants of *Labisia pumila* var. *alata* (LPva) were collected from Perak, Malaysia between February and June 2012. The plant was identified by a botanist from the Institute of Bioscience, Universiti Putra Malaysia. The voucher specimen was deposited at the Herbarium Universiti Kebangsaan Malaysia (UKMB 30010). The plant material was air-dried at room temperature. Then it was powdered (1 kg), refluxed with 10 l of distilled water for 3 h. The extract were filtered and subjected to freeze-drying to obtain a powdered extract (ALPva, 97.6 g). Meanwhile, one kg of the powdered plant material was macerated with 6 l of ethanol: water (80:20) at room temperature for 3 days. The extract was filtered through filter paper and collected. This process of extraction was repeated three times. The combined hydroalcoholic extracts were evaporated to dryness under reduced pressure and freeze-drying to obtain a gummy-like crude extract (ELPva, 150.1 g). Both ALPva and ELPva extracts were stored at 4°C until further use.

HPLC analysis of LPva extracts

The extracts were analyzed by using RP-HPLC. Gallic acid, myricetin, rutin, 5-(Z-nonadec-14-enyl)resorcinol and demethylbelamcandaquinone B were used as external standards. The HPLC analysis was performed on Quartenary Gradient Module (Waters 2535) with Photodiode Array Detector (Waters 2998) of wavelength ranging from 210 to 350 nm, using analytical C18-column (XBridge™, 4.6 × 250 mm, 5 μm). Data acquisition was performed using the Empower3 software. The extracts were then analyzed by using the validated HPLC method as described previously by Dianita et al. (2015).

Animals and ethics

Male Wistar rats (150–200 g) were purchased from a local supplier (Perniagaan Usaha Cahaya, Kuala Lumpur, Malaysia). The animals were housed in a well-ventilated animal house under 12/12 h light and dark cycle at room temperature and maintained under standard condition (humidity 60 ± 10%). Animals were subjected to acclimatization for at least 1 week prior to being placed on study. They were provided with commercially available conventional laboratory rodent diet which was obtained from Laboratory of Animal Research Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia and water was provided *ad libitum* freely throughout the period of study. No significant difference in the body weights of the treated rats was observed when compared with control at the beginning of the study period. This study was carried out following an approval from the Universiti Kebangsaan Malaysia Animal Ethics Committee (FF/2012/IBRAHIM/23-MAY/433-MAY-2012-SEPTEMBER-2013) and in accordance with the Guidelines of Handling of Laboratory Animals by Ministry of Health, Malaysia.

Experimental design and protocol

After acclimatization period, 64 rats were fed with 2%-cholesterol diet for 8 weeks. Six rats was maintained with conventional diet and assigned as normal-control group. The cholesterol level was considered high at ≥ 140 mg/dl (Hirunpanich et al., 2006). At week 5, the animals with cholesterol level ≥ 140 mg/dl were divided into designated groups (n = 6) and treated accordingly for another 4 weeks. Normal and high cholesterol control groups were given 2% of Tween-20 solution orally.

Group I : normal - control group (conventional diet, no treatment)
Group II : high cholesterol (HCD) - control group (2%-cholesterol diet, no treatment)
Group III : positive control (Ato) group (2%-cholesterol diet, 10 mg/kg atorvastatin orally)
Group IV : 2%-cholesterol diet - 100 mg/kg of ALPva orally (W100)
Group V : 2%-cholesterol diet - 200 mg/kg of ALPva orally (W200)
Group VI : 2%-cholesterol diet - 400 mg/kg of ALPva orally (W400)
Group VII : 2%-cholesterol diet - 100 mg/kg of ELPva orally (E100)
Group VIII : 2%-cholesterol diet - 200 mg/kg of ELPva orally (E200)
Group IX : 2%-cholesterol diet - 400 mg/kg of ELPva orally (E400)

Each samples (100 mg/ml) and atorvastatin (5 mg/ml) were dispersed homogenously in 2% Tween-20. The treatment was given daily at 3–4 pm. At the end of the experiment, rats were sacrificed and the blood was collected via abdominal aorta and allowed to clot for 1 h at room temperature. Serum was separated by centrifugation at 4000 rpm for 20 min and kept at −80 °C for further biochemical analysis. Part of the aorta and liver tissues were excised immediately, rinsed with cold-saline solution, and fixed in 10% formalin for histopathological examination.

Estimation of serum lipid profile

Serum level of total cholesterol (TC), total triglycerides (TG) and high-density lipoprotein-cholesterol (HDL) was determined by using colorimetric assay kits (Abcam, UK). Level of very low-density lipoprotein-cholesterol (VLDL) was calculated as VLDL = TG/5 meanwhile low density lipoprotein-cholesterol (LDL) was estimated by using Friedewald formula with as LDL = TC – (HDL + VLDL). The atherogenic indices were calculated as Atherogenic Index of Plasma (AIP) and Castelli's Risk Index-I (CRI-I) (Dobiášová and Frohlich, 2001).

Measurement of serum biochemical and antioxidant activities

Analysis of antioxidants was performed by measuring the reduced glutathione (GSH), glutathione reductase (GR) and superoxide dismutase (SOD) levels on serum using colorimetric assay kits (Cayman Chemical, US). Levels of transaminase enzymes (aspartate, AST, and alanine transaminases, ALT) as well as lactate dehydrogenase (LDH) were also estimated using colorimetric assay kits (BioAssay System, US, Cayman Chemical, US and BioVision, US, respectively).

Determination of serum lipid peroxidation

Serum lipid peroxides, measured as malondialdehydes (MDA), were assayed with thiobarbituric acid reactive substances (TBARS)