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Short communication

β-Amyrin acetate and β-amyrin palmitate as antidyslipidemic agents from *Wrightia tomentosa* leaves

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ABSTRACT

The ethanolic extract and fractions of *Wrightia tomentosa* Roem. & Schult (Apocynaceae) leaves were tested *in vivo* for their antidyslipidemic activity in high fat diet (HFD) induced dyslipidemic hamsters. Activity guided isolation resulted in identification of antidyslipidemic compounds β-AA and β-AP. Compounds β-AA and β-AP decrease the levels of LDL by 36% and 44%, and increase the HDL-C/TC ratio by 49% and 28%, respectively, at a dose of 10 mg/kg. In addition, the isolated compounds β-AA and β-AP showed significant HMG-CoA-reductase inhibition, which was further established by docking studies.

Introduction

Cardio vascular disease remains the leading cause of death in the developed countries and is major cause of mortality and disease in the Indian subcontinent, causing more than 25% of death (Gupta et al. 2008). Increases in LDL, triglyceride and total cholesterol levels with decrease of HDL levels have been established as one of the risk factors for cardio vascular diseases (Milionis et al. 2004). In 2008, the prevalence of raised total cholesterol among adults defined as total cholesterol ≥6.2 mmol/l (240 mg/dl) was 9.7% (8.5% for males and 10.7% for females). The Framingham Heart Study (Kenchaiah et al. 2002) showed that a 10 mg/dl increase in HDL cholesterol was found to be associated with a 19% decrease in coronary artery disease death and a 12% decrease in all causes of mortality (Gordon et al. 1977). The proposed mechanisms for the protective action of HDL may be that HDL collects particles from the cells and other circulating lipoprotein, which in turn counteracts the role of LDL cholesterol thus preventing the formation of atherosclerotic lesions (Picardo et al. 1986). Therefore, agents that increase HDL cholesterol concentration in the blood and thereby ratio of HDL cholesterol to total cholesterol (HDL-C/TC) would have promising therapeutic utility as antidyslipidemic agents.

In continuation of our research on medicinal plants in India we have found that the ethanolic extract of *Wrightia tomentosa* Roem. & Schult (Apocynaceae) leaves showed promising antidyslipidemic activity. *W. tomentosa* (dudhi) is a small sized deciduous shrub distributed throughout the warmer part of India. Different parts of plant have been used in traditional medicine since ages. Their leaves have been used for treatment of hypertension (Rajendran et al. 2003), antimycobacterial effects (Nagarajan et al. 2010), antihyperglycemic, antioxidant activity (Nagarajan et al. 2008), anti-allodynic, toxicity studies and antinociceptive activity (Nagarajan et al. 2007) and antimicrobial activity (Nagarajan et al. 2006). In present communication we have carried out activity guided isolation of compounds β-AA and β-AP and their evaluation for antidyslipidemic activity *in vivo* as well as identification of their HMG-CoA reductase inhibitory activity.

Materials and methods

Plant material, extraction, fractionation and isolation of compounds

W. tomentosa leaves were collected from Tamilnadu, India in the month of February 2006. Proper identification was done by Botany Division of Central Drug Research Institute and a voucher specimen (No. 4698) is kept in the herbarium for future reference. Powdered leaves of *W. tomentosa* (4.0 kg) were extracted with ethanol (8 l) to afford ethanol extract (300 g). Ethanol extract was fractionated into three fraction, hexane soluble fraction (F1, 80 g), chloroform fraction F2 (70 g) and aqueous fraction F3 (120 g). Because of better activity profile, hexane fraction F1 was subjected to the column chromatography to afford compounds β-AA (100 mg) and β-AP

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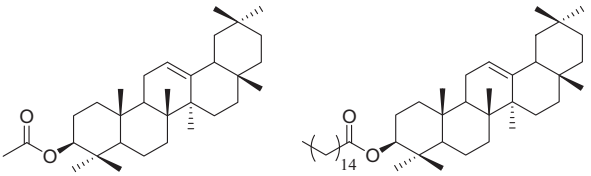

Fig. 1. Structures of β-amyrin acetate and β-amyrin palmitate.

(80 mg) (Fig. 1). Structure elucidation of these compounds were performed by spectroscopic technique (see ^{1}H and ^{13}C spectra compounds in supplementary information) and confirmed by comparison of its experimental data with that reported in literature (Saeed 2003; Wang 2007).

HFD-fed hyperlipidimic hamster, administration of sample, estimation of biochemical parameters and statistical analysis

Golden-Syrian hamsters (*Mesocricetus auratus*), male, wt. 120 ± 10 g were fed with high-fat diet (Rizvi et al. 2003; Bhatia et al. 2003). Experimental protocols were approved by our institutional ethical committee, which follows guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), which complies with international norms of INSA.

Administration of sample and estimation of biochemical parameters was performed by reported process (Maurya 2007). Data were analyzed using Student's *t* test on Graph Pad Prism ver. 3.02 data templates. Significance (*p* values) is calculated using one-way analysis of variance of ANOVA program. Value $p < 0.05$ was considered as significant.

In vitro HMG-CoA reductase inhibitory activity

The HMG-CoA reductase assay was performed using the HMG-CoA reductase assay kit from Sigma–Aldrich. HMG-CoA (substrate), NADPH, assay buffer and enzyme HMGR were supplied with the assay kit.

Molecular modeling

In the current docking studies, the three dimensional structure of β-AA and β-AP molecules were built using sketch module in SYBYL7.1 suite of programs (TRIPOS Inc., 1699 South Hanley Road, St. Louis, MO 63144, USA) running under Irix 6.5, while retaining their known stereochemistry followed by Powell energy minimization method. Docking studies were performed subsequently using Ligand Fit program (Venkatachalam et al. 2003) interfaced with Cerius2.49 molecular modeling package (Cerius2, Version 4.10; Accelrys, Inc., San Diego, CA, USA, 2005) (MSI Cerius2 Version 4.9, Molecular simulations, Accelrys, Inc., 9685 Scranton Rd., San Diego, CA 92121, USA) using default parameters. The binding site for the

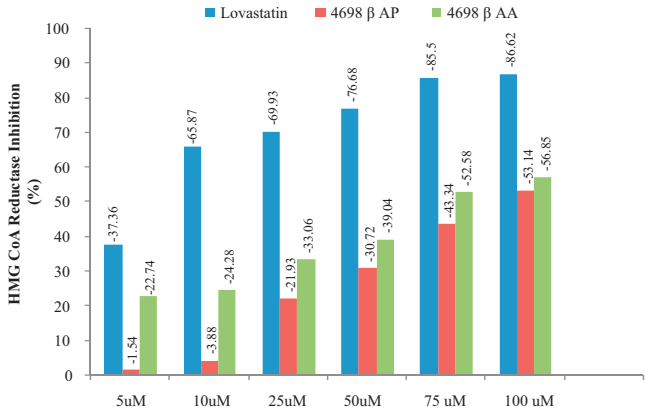

Fig. 2. *In vitro* dose–response study of β-AP and β-AA and Lovastatin for HMG-CoA reductase inhibitory activity.

docking study was extracted from the co-crystallized rosuvastatin complexed with human HMG-CoA reductase protein from the protein data bank (code: 1HWL) (Istvan and Deisenhofer 2001). The scoring was performed using a set of scoring functions as implemented in Cerius2. These included LigScore1, LigScore2, -PLP1, -PLP2, -PMF and DockScore available from the docking process.

Results and discussion

In order to test the antidyslipidemic activity the crude ethanolic extract of the plant was subjected to high fat diet (HFD) induced hamsters. Ethanolic extract of *W. tomentosa* leaves showed promising antidyslipidemic activity. It was found to be effective in lowering of plasma levels of triglyceride (TG) 56%, total cholesterol (TC) 27%, and low density lipoprotein cholesterol (LDL-C) 41%, glycerol (Gly) 41% accompanied by increase in high density lipoprotein cholesterol/total cholesterol (HDL-C/TC) ratio by 52% at the dose of 500 mg/kg body-wt of hamster. Subsequently, the three fractions (F1–F3) were also subjected to same hamster model at the dose of 100 mg/kg body-wt of hamster. Fractions F1 was found to show best activity profile with decrease in the level of TG by 62%, TC by 39%, LDL and Gly by 44% and increase in the HDL-C/TC ratio by 14%. The fraction F1 was further taken up for isolation of compounds. Silica gel column chromatography of sub-fraction F5 yielded β-AA and β-AP as pure compounds. β-AA and β-AP were evaluated in HFD induced dyslipidemia in hamster. β-AA showed the decrease of TG by 35%, TC by 37%, LDL by 36% and increase in the HDL-C/TC ratio by 49% and β-AP showed the decrease of TG by 24%, TC by 25%, LDL by 44% and increase in the HDL-C/TC ratio by 28%, respectively, at 10 mg/kg dose (Table 1).

In addition to the *in vivo* effect of β-AA and β-AP in HFD induced dyslipidemia in hamster we also found that the β-AA and β-AP showed HMG-CoA reductase inhibitory activity. β-AA and β-AP showed inhibition of HMG-CoA reductase enzyme by 56.85% and 53.14% against 86.62% by Lovastatin at 100 μM concentration

Table 1
Antidyslipidemic activity of extract, fractions and isolated compounds of *W. tomentosa*.

Plant samples (mg/kg)	Dose	TG	TC	LDL	Gly	Ratio: HDL-C/TC
EtOH extract	500	−56**	−27	−41	−41*	+52
F1	100	−62**	−39*	−44	−44*	+14
F2	100	−36*	−22	−24	−36*	+1
F3	100	−49**	−37*	−43	−43*	+24
β-AA	10	−35	−37*	−36	–	+49
β-AP	10	−24	−25	−44*	–	+28

Values are percent changes with respect to HFD-fed hamster group (group of eight animals each).
* $p < 0.05$.
** $p < 0.01$.