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# Antihyperglycemic and antidyslipidemic agent from *Aegle marmelos*

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**Abstract**—The plant *Aegle marmelos* belongs to the family of Rutaceae. From the leaves of *A. marmelos* an alkaloidal-amide, Aegeline **2**, was isolated and found to have antihyperglycemic activity as evidenced by lowering the blood glucose levels by 12.9% and 16.9% at 5 and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rats (STZ-S) model at the dose of 100 mg/kg body weight. Aegeline **2** has also significantly decreased the plasma triglyceride (Tg) levels by 55% ($P < 0.001$), total cholesterol (TC) by 24% ($P < 0.05$), and free fatty acids (FFA) by 24%, accompanied with increase in HDL-C by 28% and HDL-C/TC ratio by 66% in dyslipidemic hamster model at the dose of 50 mg/kg body weight. The reasonable mapping of compound **2** to validated pharmacophoric hypothesis and 3D QSAR model with an estimated activity (283 nM) suggest that the compound **2** might be a $\beta_3$-AR agonist.


Diabetes mellitus is an independent risk factor for the development of coronary artery diseases, myocardial infarction, hypertension, and dyslipidemia. Clinically diabetic patients are characterized by marked increase in blood glucose level followed by mild hyperlipidemia. Non-insulin dependent diabetes mellitus (NIDDM) accounts for approximately 80–90% of all cases[1] and it is the fastest growing global threat to public health. If the current trend continues, it is likely to result in an estimated 215 million sufferers from NIDDM worldwide by the year 2010.[2,3] This number is expected to increase as medical advances extend life expectancy and more widespread access to a calorie-rich diet promotes the prevalence of obesity. When carbohydrates are in low supply or their breakdown is incomplete, fats become the preferred source of energy. Fatty acids are mobilized into the general circulation leading to secondary triglyceridemia in which total serum lipids in particular triglycerides as well as the levels of cholesterol and phospholipids increases.

This rise is proportional to the severity of the diabetes. Uncontrolled diabetes is manifested by a very high rise in triglycerides and fatty acid levels.[4] An increase in plasma lipids, particularly cholesterol, is a common feature of atherosclerosis, a condition involving arterial damage, which may lead to ischemic heart disease, myocardial infarction, and cerebro-vascular accidents. These conditions are responsible for one-third of deaths in industrialized nations.[5] Therefore, a drug, having two-fold properties, that is, lowering of blood lipids (triglycerides and cholesterol) and glucose together, is in great demand. Several research groups are focusing to develop such dual-acting agents. Despite the remarkable progress in the management of diabetes mellitus by synthetic drugs,[6] there has been a renewed interest in medicinal plants because of the side effects of synthetic drugs. The discovery of new drugs from traditional medicine is not a new phenomenon (Fig. 1). Fowden[7] had isolated an unusual amino acid, that is, 4-hydroxyisoleucine from the seeds of *Trigonella foenum-graecum* for the first time. Christophe et al.[8] discovered glucose induced insulin

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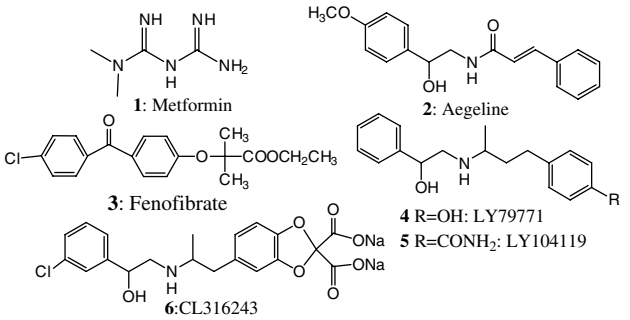


**Figure 1.** Synthetic antihyperglycemic drug **1**; Aegeline **2** isolated from leaves of *A. marmelos*; synthetic lipid lowering drug **3** and $\beta_3$-Adrenergic receptor agonists **4**–**6**.

secretion in vitro and ex vivo of 4-hydroxyisoleucine. Furthermore, in type 2 diabetic rat model the compound was active and partly corrected hyperglycemia and glucose tolerance. Recently we have reported the lipid lowering effect in 4-hydroxyisoleucine.[9] Two more unusual amino acids[10], that is, Hypoglycin A and B isolated from *Blighia sapida* were reported for their antihyperglycemic activity. Metformin **1** is currently used as antidiabetic agent in the treatment of type 2 diabetes. Metformin **1** and its analogues[11] were synthesized on the basis of a natural product lead, that is, galegine.[12] The synthetic cholesterol lowering statins such as fluvastatin,[13] cerivastatin[14] were synthesized on the basis of natural product lead, that is, mevastatin.[15] The plant derived saponin derivative, pamaqueside (CP-148623)[16], has been reported for cholesterol absorption up to 35–40% and the fish oils, which contain fatty acids such as eicosapentaenoic acid and docosahexenoic acids, have been reported for their lowering effect on triglycerides and cholesterol.[17]

*Aegle marmelos* is commonly known as 'bael' in India.[18] It belongs to the family of Rutaceae, widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus. Several research groups in India and other South Asian countries confirmed the hypoglycemic activity in the plant extracts.[19] So far the antihyperglycemic principle has not been identified. In addition to this we have recently discovered the antidyslipidemic activity in the leave's alcoholic extract and its chloroform fraction.[20] Therefore, the need was felt to isolate the active principles. Our bioactivity-guided fractionation and isolation work led to discovery of compound **2**[21] as an antihyperglycemic and antidyslipidemic principle. As a part of this work the shade-dried leaves (5 kg) of *A. marmelos* were extracted with 95% ethyl alcohol. It was fractionated using chloroform, butanol, and water. The resultant chloroform fraction was subjected to conventional silica gel (60–120 mesh) column chromatography to isolate pure Aegeline **2** by using hexane–ethylacetate (70:30) as mobile phase.

Male albino rats of SD strain (body weight 140 ± 20 g) were selected for the antidiabetic activity studies. Streptozotocin was dissolved in 100 mM citrate buffer, pH 4.5, and calculated amount of the fresh solution was injected to overnight fasted rats (45 mg/kg) intraperitoneally. Blood was checked 48 h later by glucostrips and animals showing blood glucose values between 8 and 15 mM were selected and divided into groups of six animals in each. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at 100 mg/kg dose. Animals of control group were given an equal amount of 1.0% gum acacia. A sucrose load of 2.5 g/kg body weight was given after 30 min of drug administration. After 30 min of post-sucrose load, blood glucose level was again checked at 30, 60, 90, 120, 180, 240 and 300 min and at 24 h, respectively. Comparing the AUC of experimental and control groups we determined the percent antihyperglycemic activity. Treatment with naturally occurring Aegeline **2** lowered the blood glucose by 12.9% at 5 h and 16.9% at 24 h at 100 mg/kg body weight dose, while the reference drug metformin **3** lowered the blood glucose level by 23.5% at 5 h and 26.5% at 24 h (Fig. 2) at the same dose.

High fat diet (HFD) fed dyslipidemic hamster model has been reported as an ideal in vivo model for evaluating antidyslipidemic drugs.[22] Feeding with HFD resulted in induced dyslipidemia in hamsters. The plasma levels of TG,[23] TC,[24] HDL-Chol,[25] glycerol,[26] and FFA[27] were found to be significantly elevated by 5-, 2.8-, 1.7-, 1.4-, and 5.3-fold, respectively, (data not shown) as compared to normal chow fed control group of hamsters. The compound **2** obtained from the leaves was administered orally at the dose of 50 mg/kg body weight for seven consecutive days. Results obtained in dyslipidemic hamsters showed a significant decrease in lipid profile. The elevated triglyceride levels increase the risk of coronary heart disease (CHD), in contrary, the high density lipoproteins (HDL) mediate the reverse transport of cholesterol from peripheral tissues to the liver, which will disallow the slow accumulation of lipids in artery walls.

The compound **2** has significantly lowered the triglycerides from 13.22 to 5.96 mM by 55% ($P < 0.001$), cholesterol from 11.12 to 8.46 mM by 24% ($P < 0.05$) and increased the HDL-C from 2.29 to 2.93 mM by 28% (Table 1) similar to currently marketed drug fenofi-

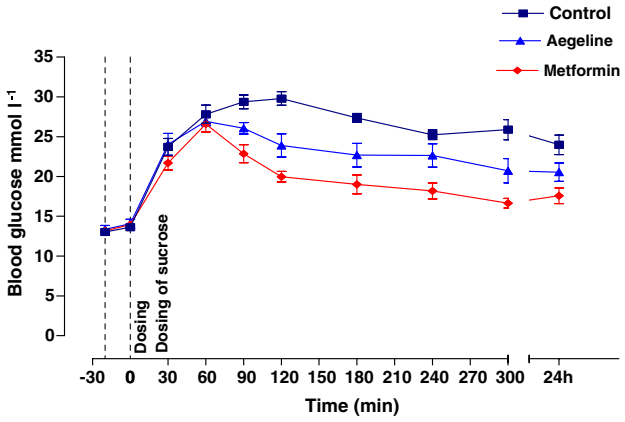


**Figure 2.** Blood glucose levels in sucrose challenged LD-STZ rats before, and up to 24 h after administration of vehicle, Aegeline **2** and metformin **1**.

**Table 1.** Percentage decrease/increase in plasma lipids from baseline with the treatment of Aegeline **2** in dyslipidemic hamsters (values are mean ± SD of eight hamsters in each group)

| Test sample | Test dose (mg/kg) | Tg (mM) | Chol (mM) | HDL-C (mM) | Gly (mM) | FFA (μM) | H/C |
|---|---|---|---|---|---|---|---|
| Vehicle | | 13.22 ± 2.76 | 11.12 ± 5.12 | 2.29 ± 0.94 | 1.23 ± 0.52 | 642 ± 141 | 0.21 |
| Aegeline | 50 | 5.96 ± 1.16 | 8.46 ± 1.72 | 2.93 ± 0.93 | 0.85 ± 0.19 | 488 ± 40 | 0.35 |
| | | −55*** | −24* | +28 | −31** | −24 | +66 |
| Vehicle | | 15.00 ± 1.79 | 9.19 ± 1.03 | 2.68 ± 0.35 | 1.09 ± 0.14 | 682 ± 118 | 0.29 |
| Aegeline | 30 | 7.74 ± 1.47 | 7.64 ± 1.17 | 2.64 ± 0.36 | 0.83 ± 0.14 | 587 ± 125 | 0.35 |
| | | −48** | −17 | NC | −23 | −14 | +20 |
| Feno-fibrate | 108 | −42* | −18 | NC | −36** | −20 | +10 |

*P* values: * < 0.05; ** < 0.01; *** < 0.001. NC, no change.

brate's **3**[22,28] (Fig. 1) action. The compound **2** also lowered the free fatty acids (FFA) from 642 to 488 μM by 24% and glycerol from 1.23 to 0.85 mM by 31% ($P < 0.01$) compared to HFD fed hamsters at 50 mg/kg body weight. At the lower dose (30 mg kg) the compound **2** reduced triglycerides by 48% ($P < 0.01$), cholesterol by 17%, glycerol by 23% ($P < 0.05$), free fatty acid by 14% and increased the HDL/cholesterol ratio by 20%, however, there was no change in the HDL-C levels (Table 1) . The reference drug fenofibrate **3** lowered the TG by 42%, TC 18%, Gly 36%, FFA 20% and increased the HDL-C/TC by 10% in our experiments at the dose of 108 mg/kg body weight in the same hamster model.[22] The compound **2** was screened for its PTPase[29] and α-glucosidase inhibiting activity to find out the mechanisms of action of antidiabetic activity, however, it did not show inhibiting activity against these targets. The compound **2** has close structural features to $\beta_3$-adrenergic receptor agonists such as CL316243, LY79771, and LY104119 **4**–**6** (Fig. 1),[30] that are in clinical trials at various stages for the treatment of obesity and diabetes. Hence, it is speculated that the compound **2** might also be acting through similar mechanism. $\beta_3$-Adrenergic receptors ($\beta_3$-AR) belong to the super family of G-protein coupled receptors. $\beta_3$-AR is implicated for the regulation of lipid metabolism.[31] The ability of $\beta_3$-AR agonists to stimulate adipocyte lipolysis and thermogenesis in brown adipose tissue (BAT) and concurrent normalization of elevated non-esterified fatty acids (NEFA) renders them potential antiobesity and antidiabetic agents.[32] Elevated NEFA consumption necessitates increased glucose metabolism to maintain homeostasis.[32]

Recently, Prathipati and Saxena[33] had developed a predictive pharmacophoric hypothesis and 3D-QSAR model to elucidate the 3D structural and physicochemical requirements for the $\beta_3$-adrenoreceptor agonists. Hypothesis 'A' was in agreement with site-directed mutagenesis studies on human $\beta_3$-AR and correlated well the observed and estimated activity both in training (correlation coefficient, $R = 0.857$) and the external test sets ($R_{\text{test set-1}} = 0.673$, $R_{\text{test set-2}} = 0.773$). It also mapped reasonably well and explained the variance ($R_{\text{test set-3}} = 0.7$) of six $\beta_3$-AR agonists of different structural classes, namely, CL-316, 243 (Wyeth Ayerst), L-770, 664 (Merck), L-757 793 (Merck), AJ 9677 (Dainippon), CP-331 684 (Pfizer), and LY-377 604 (Eli Lilly) under clinical development. This hypothesis has proven universal applicability in providing a powerful template for virtual screening and also for designing new chemical entities (NCEs) as $\beta_3$-AR agonists. The compound **2** mapped to two of the four common features of hypothesis 'A', namely, the positive ionizable feature (PI) to the basic nitrogen atom and one of the ring aromatic feature (RA) to the aromatic moiety of the cinnamoyl group (RA), while the hydrogen bond donor (HBA) and the other ring aromatic features were not mapped. The reasonable mapping (Fig. 3) of compound **2** to the pharmacophoric hypothesis[33] and the good estimated $\beta_3$-AR agonistic activity (283 nM) substantiates the proposed mechanism of action of **2**.

In conclusion, this paper presents the first report of the discovery of antihyperglycemic and antidyslipidemic activity of an alkaloidal-amide lead, Aegeline **2**, isolated from the leaves of *A. marmelos*. Its structural similarity with $\beta_3$-AR agonists (**4**–**6**) acting as antidyslipidemic and antihyperglycemic agents suggested similar mechanism of action for Aegeline **2**. The reasonable mapping to the pharmacophoric hypothesis and the good estimated $\beta_3$-AR agonistic activity (283 nM) theoretically substantiate the proposed mechanism of action of **2**. Further studies are required to study the experimental $\beta_3$-adrenergic receptor agonist properties and understand its relationship to lipid lowering and antihyperglycemic activity. Design and synthesis of analogues of Aegeline **2**, presently in progress in our laboratory to eventually develop a potent dual acting agent.

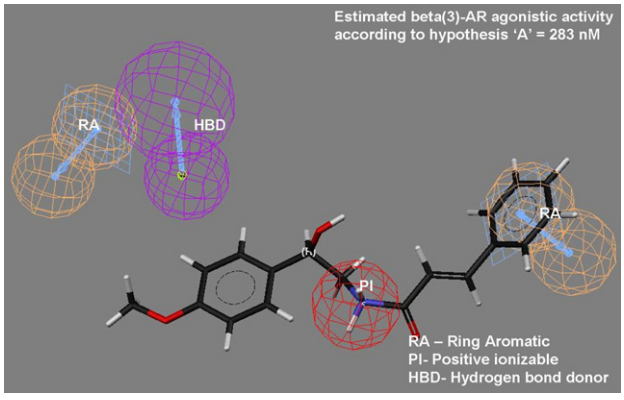


**Figure 3.** Mapping of compound **2** to a previously developed $\beta_3$- AR agonistic pharmacophoric hypothesis.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.037.

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