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Antidyslipidemic activity of furano-flavonoids isolated from *Indigofera tinctoria*☆

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Abstract—Flavonoids appear to play a major role in reducing the risk of cardiovascular diseases by decreasing the blood lipid levels. In continuation of our drug discovery program on antidyslipidemic agents we have isolated three furano-flavones **1**–**3** and a rare flavonol glycoside **4** from the aerial parts of *Indigofera tinctoria*. Our results disclose that the treatment with diastereomeric flavonoid mixture **1** and **2** (80:20) significantly decreased the plasma triglycerides (TG) by 60%, total cholesterol (TC) 19%, glycerol (Gly) 13%, and free fatty acid (FFA) 25% accompanied with increase in high density lipoproteins-cholesterol (HDL-C) by 8% and HDL-C/TC ratio 36% in high fat diet (HFD) fed dyslipidemic hamsters at the dose of 50 mg/kg body weight. The flavonoid **3** has exhibited moderate antidyslipidemic activity.

When carbohydrates are in low supply or their breakdown is incomplete, fats become the preferred source of energy in diabetic patients. As a result, the 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 increase. 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. 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 cerebrovascular accidents. These conditions are responsible for one-third of deaths in industrialized nations.[1]

The discovery of new drugs from traditional medicine is not a new phenomenon. Flavonoids, a heterogeneous group of ubiquitous plant polyphenols, are a frequent component of the human diet.[2] Flavonoids have exhibited a variety of biological and pharmacological activities, including the inhibition of enzymes,[3] free radical scavenging,[4] anti-inflammation,[5] and inhibition of tumor promotion.[6] A number of epidemiological studies have implied a role for flavonoids in reducing the risk of coronary heart disease (CHD).[7–9] Among the naturally occurring flavonoids (see Fig. 1), hesperetin **5**, hesperidin **6**, naringenin **7**, and naringin **8** have been evaluated as potential agents for improving the cholesterol metabolism in diet-induced hypercholesterolemic animals.[10–15] As a part of our drug discovery program on Indian medicinal plants, we have recently discovered the antidyslipidemic activity in the alcoholic extract and its chloroform fraction of the aerial parts of *Indigofera tinctoria*.[16–21] Further work on the plant led to isolation[22] of three furano-flavonoids **1**–**3**[23,24] and a rare flavonoid glycoside **4**,[25] and also to discover the antidyslipidemic activity in the furano-flavonoids (**1**–**3**). The compounds **1** and **2** have earlier been reported for their in vitro inhibitory effect on human platelet aggregates.[26] The major compound pseudosemiglabrin **1** has inhibited U46619-induced platelet aggregates by 85% at a final concentration of 6.5 μg/ml, whereas semiglabrin **2** produced 70% inhibition at a much higher dose (45 μg/ml).[26] The formation of platelet aggregates is an important pathogenic factor in widespread cardiovascular disease. Pirrung and Lee reported the total synthesis of diastereomers **1** and **2**.[26]

No significant differences were observed in the food intake and weight gain between the groups (data not

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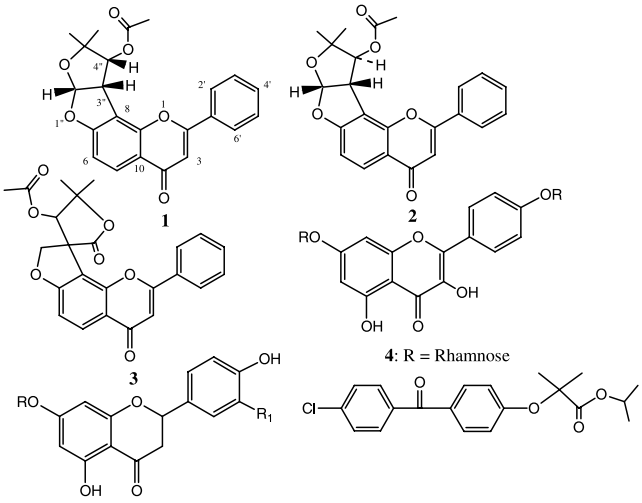

Figure 1. Modified flavonoids **1**–**3** and flavonoid glycoside **4** isolated from *Indigofera tinctoria*; lipid lowering compounds (naturally occurring flavanone derivatives **5**–**8** and synthetic fenofibrate **9**).

shown). As such, the compound **1**–**4** supplement did not apparently adversely affect the hamsters. All the flavonoids (**1**–**4**) were administered orally at the dose of 50 mg/kg body weight for seven consecutive days. Our experimental[27–29] results disclose that the flavonoid **1** and **2** mixture has significantly lowered the TG[30] from 11.97 to 4.90 mM ($P < 0.01$) by 60%, Chol[31] from 11.72 to 9.25 mM by 19%, Gly[32] from 0.70 to 0.61 mM by 13%, and FFA[33] from 0.394 to 0.294 ($P < 0.1$) by 25%, and increased the HDL-C[34] from 2.23 to 2.43 mM by 8%, the HDL-Cholesterol to TC (0.26; $P < 0.05$) ratio by 36%, when compared to high fat diet (HFD) fed hamsters (Table 1; Fig. 2 and 3). Higher the TG levels and lower the HDL-C increase the risk of coronary heart disease (CHD).[34] In latter case, 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 flavonoid mixture (**1** and **2**) has exhibited both the above beneficiary effects. It has decreased the TG by 60% and increased the HDL-C by 8%. The flavonoid **3** also has exhibited good TG lowering property almost similar to flavonoid **1** and **2** mixture. It lowered the TG from 11.97 to 7.04 mM by 41% ($P < 0.05$). This compound, however, did not affect the cholesterol concentration. It has lowered the Gly from 0.70 to 0.66 mM by 6% and enhanced the HDL-C from 2.23 to 2.86 mM ($P < 0.05$) by 28% and the ratio between HDL-cholesterol/Total cholesterol from 0.19 to 0.24 mM ($P < 0.05$) by 26%. The flavonoid **4** has exhibited very weak TG lowering activity. It lowered the TG from 11.97 to 8.72 mM ($P < 0.1$) by 27%, Gly from 0.70 to 0.66 mM by 6% and there is no change in cholesterol and FFA content. The HDL-cholesterol and total

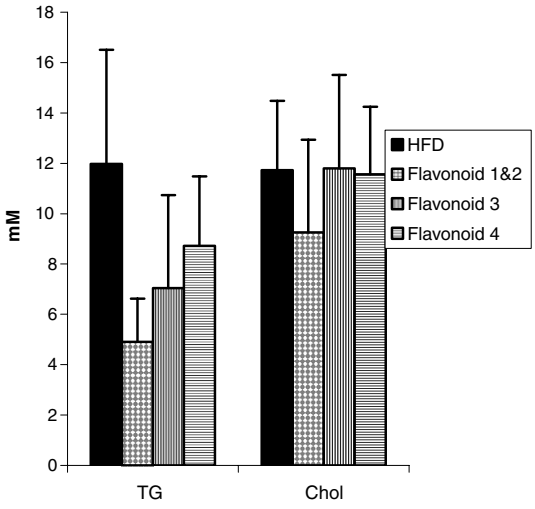

Figure 2. Effect of flavonoid **1**–**4** supplementation on plasma TG, TC in dyslipidemic hamsters at the dose of 50 mg/kg body weight (values are means ± SD of eight hamsters in each group).

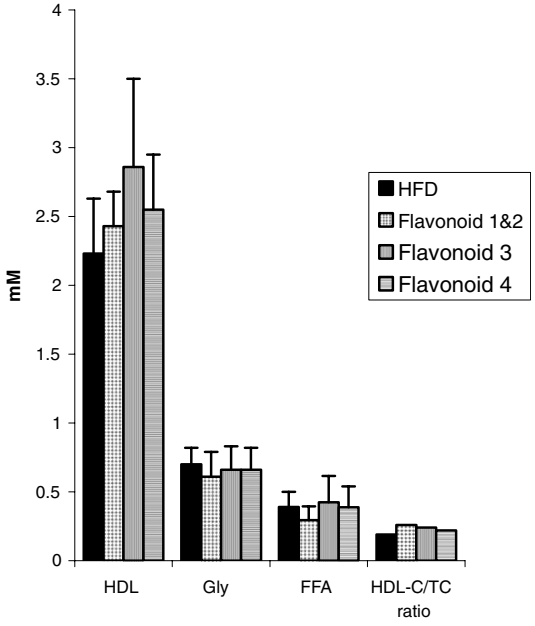

Figure 3. Effect of flavonoid **1**–**4** supplementation on plasma HDL-C, Gly, FFA, and HDL-C/TC ratio in dyslipidemic hamsters at the dose of 50 mg/kg body weight (values are means ± SD of eight hamsters in each group).

Table 1. Percentage decrease/increase of plasma lipids with the treatment of flavonoids **1**–**4** in dyslipidemic hamsters at the dose of 50 mg/kg body weight

Lipids	HFD + flavonoid		
	1 and **2**	**3**	**4**
TG	−60***	−41*	−27*
TC	−19	NC	NC
HDL-C	+8	+28	+14
Gly	−13	−6	−6
FFA	−25*	+7	NC
HDL-C/TC	+36**	+26**	+16

NC, no change.
* $P < 0.1$.
** $P < 0.05$.
*** $P < 0.01$.

cholesterol ratio increased from 0.19 to 0.24 mM by 26%. The currently marketed drug fenofibrate **9** did not show significant activity at the dose of 50 mg/kg body weight in our experiments. However, it lowered the TG by 42%, TC 18%, Gly 36%, and FFA 20%, and increased the HDL-C/TC by 10% in our experiments at the dose of 108 mg/kg b.w. in the same hamster model.[28,29,35,36] The flavonoid **1** and **2** mixture has potent triglycerides (TG), glycerol (Gly), and free fatty acid (FFA) lowering activity[37] with mild cholesterol lowering property. The flavonoid **3** has exhibited moderate triglycerides and glycerol lowering activity.

In conclusion, we have isolated three known furanoflavonoids **1**–**3** and a rare flavonoid glycoside **4** for the first time from *I. tinctoria* through activity guided fractionation and discovered the potent antidyslipidemic activity of isolated compounds. The compound **1** and **2** were earlier reported for their in-vitro inhibitory effect on human platelet aggregation, which is also one of the factors in cardiovascular diseases. Further studies are needed to find out the mechanism of action either by isolating the individual components or by synthesis and also beneficial use of these compounds in human patients with dyslipidemia.

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22. The plant *I. tinctoria* belongs to the family of Fabaceae. It was collected from Thirunaveli district of Tamilnadu state, India. The aerial part (5 kg) of the plant was extracted with 4 L of ethyl alcohol four times in a percolator. The resultant alcoholic extract (16 L) was combined and concentrated under reduced pressure using rotary evaporator to give 287 g of alcoholic extract. This was fractionated with chloroform and butanol successively. The resultant chloroform fraction (170 g) was subjected to conventional silica gel column chromatography using hexane and ethyl acetate (90:10) solvent system to give 0.8 g of 80:20 isomeric mixture (calculated by NMR spectroscopy) pseudosemiglabrin **1** and semiglabrin **2**. Small quantities of **1** and **2** in pure form were separated on the basis of their solubility properties for spectral studies. Further elution with hexane and ethyl acetate (85:15) gave 2.7 g of glabretephrin **3**. The butanol fraction (60.8 g) was subjected to silica gel column chromatography to isolate the compounds by using chloroform–methanol as mobile phase. The fractions collected with chloroform-methanol (90:10) have given the 3.7 g of compound **4** (kaempferol-4′,7-dirhamnoside). All the compounds (**1**–**4**) were characterized by using ^{1}H NMR, ^{13}C NMR, IR, and Mass Spectral data and comparing with literature spectral data. Compounds **1**–**3** have earlier been isolated from the roots of *Tephrosia purpurea* and compound **4** from the flowers of *Crotalaria verrucosa.* ^{1}H NMR data of **1**: (200 MHz in $CDCl_3$), δ 1.13 (s, 3H, Me), 1.32 (s, 3H, Me), 1.40 (s, 3H, OAc), 4.50 (dd, J = 6,8 Hz, 1H, H-3″), 5.50 (d, J = 12 Hz, 1H, H-4″), 6.50 (d, J = 6 Hz, 1H, H-2″), 6.70 (s, 1H, H-3), 6.90 (d, J = 8 Hz, 1H, H-6), 7.50–7.54 (m, 3H, H-3′-5′), 7.88 –7.93 (m, 2H, H-2′,6′), 8.10 (d, J = 10 Hz, 1H, H-5); FAB Mass 393 (M+1); IR (KBr): 2991, 2940, 1740, 1643 cm^{-1}. ^{1}H NMR data of **2**: (200 MHz in $CDCl_3$), δ 1.13 (s, 3H, Me) 1.32 (s, 3H, Me), 2.02 (s, 3H, OAc), 4.25 (d, J = 6 Hz, 1H, H-3″), 5.62 (s, 1H, H-4″), 6.62 (d, J = 8 Hz, 1H, H-2″), 6.70 (s, 1H, H-3), 6.90 (d, J = 8 Hz, 1H, H-6), 7.50–7.54 (m, 3H, H-3′-5′), 7.88–7.93 (m, 2H, H-2′,6′), 8.10 (d, J = 10 Hz, 1H, H-5); FAB Mass: 393 (M+1).
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37. Data were analyzed by Graph Pad Prism Version 3.02 (Graph Pad Software Inc); The percentage of lowering of lipids calculated as below:

$$\%\ \text{lipid lowering} = \frac{A - B}{A} \times 100$$

A = HFD fed animal's lipid values B = Drug (flavonoids) and HFD fed animal's lipid values.