CYCLOOXYGENASE (COX) – INHIBITORY FLAVONOID FROM *LIMNOPHILA HETEROPHYLLA*

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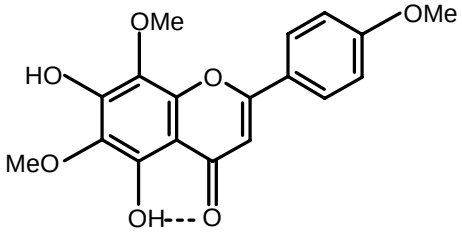
ABSTRACT

The present communication reports on the potential of 5,7-dihydroxy-6,8,4'-trimethoxyflavone, a pentaoxygenated flavonoidal constituent of Limnophila heterophylla, in exhibiting inhibitory activity against cyclooxygenase-1 and 2 (COX-1 and COX-2) using the COX catalyzed prostaglandin biosynthesis assay in vitro method.

Key words: *Limnophila heterophylla; Scrophulariaceae; flavonoid; 5,7-dihydroxy-6,8,4'-trimethoxyflavone; cyclooxygenase inhibition activity*

INTRODUCTION

In continuation of our works on medicinal plants, we have recently found *Limnophila heterophylla* (family: Scrophulariaceae)[1,2] as new and rich source for 5,7-dihydroxy-6,8,4'-trimethoxyflavone (**1**) — a well-known natural bioactive flavonoid[3-8]. The flavonoid has been characterized on the basis of detailed spectral studies. *L. heterophylla*, a locally available herb, is an important Indian medicinal plant and finds lot of applications in the traditional system of medicine against various ailments[9-12]. We, herein, report the cyclooxygenase (COX) inhibition activity of the flavonoidal constituent. Cyclooxygenase (COX) activity is an important target for anti-inflammatory drugs since this enzyme catalyzes the rate-limiting step in prostaglandin (PG) synthesis. Constitutive COX-1 and inducible COX-2 isozymes play a significant role in inflammation, pain and fever[13], and are also involved in different pathologies such as cancer[14]. Inhibition of COX-1 is achieved with non-steroidal anti-inflammatory drugs in the pathogenesis of gastrointestinal damage, whereas COX-2 inhibitors selectively reduce the occurrence of digestive side-effects[15] although a risk for cardiovascular events has been reported[16]. Stimulation of cells with proinflammatory agents such as cytokines or lipopolysaccharide (LPS) results in the induction of COX-2 and inducible nitric oxide (iNOS). The activity of these enzymes leads to the overproduction of PGs and NO, which play a key role in the pathophysiology of arthritis and other inflammatory conditions[13,17]. Our preliminary investigation showed the ability of the isolated flavonoid to exhibit inhibitory activity against both COX-1 and COX-2.



(1)

EXPERIMENTAL

Extraction and isolation of flavonoid (1):

Air-dried defatted powered whole plants (1.5 kg) of *L. heterophylla* (collected at and around Santiniketan) were extracted with petrol (60-80^0) in a Soxhlet apparatus for 56 hr. The extract was concentrated under reduced pressure and then subjected to column chromatography on silica gel (60-120 mesh, 200 g); the petrol (60-80^{0}C):benzene(1:2) eluent afforded 5,7-dihydroxy-6,8,4′-trimethoxyflavone as golden yellow needles (yield 0.8 g), $C_{18}H_{16}O_7$ ($[M]^+$ at *m/z* 344), mp 184-186 ^{0}C (from ethanol); UV (ethanol): λ_{max} 280, 335 nm; (+$AlCl_3$): 280, 310 (sh), 355 nm; IR (KBr) ν cm^{-1}: 3407, 3100, 2936, 2840, 1663, 1591, 1508, 1060, 1025; ^{1}H NMR ($CDCl_3$, 300MHz; TMS): δ 12.78 (1H, s, C_5-OH), 7.89 (2H, dd, *J*= 2.7 Hz, 11.7 Hz, H-2′ and H-6′), 7.045 (2H, dd, *J*= 3 Hz, 11.7 Hz, H-3′ and H-5′), 6.585 (1H, s, C_3-H), 4.04 (3H, s, C_6-OC*H*$_3$), 4.02 (3H, s, C_8-OC*H*$_3$) and 3.90 (3H, s, $C_{4'}$-OC*H*$_3$); EIMS (70 ev): *m/z* 344 ($[M]^+$), 329 (base peak), 316$[M–CO]^+$, 315 $[M–CO-H]^+$, 314 $[M–2Me]^+$, 312$[M–2Me –2H]^+$, 301$[M– CO –Me]^+$, 212 and 132 (retro-Diels-Alder fragmented ion peaks of **1**),197 and 132 (retro-Diels-Alder cleavage of mass fragment 329), 169$[197–CO]^+$, 168 $[169–H]^+$, 153$[169–Me]^+$, 141$[169-CO]^+$, 135 (fragmented ion peak), 126$[141–Me]^+$; ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.2 (C-2), 104.2 (C-3), 183.4 (C-4), 148.8 (C-5), 131.5 (C-6), 149.2 (C-7), 128.5 (C-8), 146.2 (C-9), 105.0 (C-10), 124.0 (C-1'), 127.8 (C-2′, C-4′), 115.0 (C-3′, C-5′), 163.1 (C-4′), 61.4 (8-O*C*H$_3$), 62.3 (6-O*C*H$_3$), 56.0 (4′-O*C*H$_3$); HMQC results (eight protonated carbons, showing one-bond heteronuclear correlations, ^{1}H–^{13}C): (δ7.89,δ127.8), (δ7.045,δ115.0), (δ7.045,δ115.0), (δ 6.585,δ104.2), (δ3.90, δ56.0), (δ4.02, δ61.4), and (δ4.04, δ 62.3).

C*yclooxygenase (COX-1 and COX-2) inhibitory activity of flavonoid (1)*:

Materials

Materials used for the study were manufactured by Cayman Chemical, USA. The contents of kit (for COX) are Prostaglandin screening EIA antiserum, Prostaglandin Screening AChE Trace, Prostaglandin Screening EIA Standard, EIA Buffer, Wash Buffer, Mouse Anti-rabbit IgG Coated Plate, Plate Cover, Elliman's Reagent, Reaction Buffer, COX-1(ovine), COX-2 (human recombinant), Heme, Arachidonic Acid (AA), Potassium Hydroxide, Hydrochloric Acid, Stannous Chloride.

Methods: *In vitro* cyclooxygenase inhibition assays

The ability of the test compound to inhibit COX-1 (ovine) and COX-2 (human recombinant) was determined by using enzymes immunoassay (EIA) kit (Catalogue No.560101, Cayman Chemical, Ann Arbor, MI, USA) according the Manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of the arachidonic acid (AA) to PGH_2. $PGF_2\alpha$ produced from the PGH_2 by reduction with stannous chloride, was measured by enzymes immunoassay (EIA). The test compound was dissolved in DMSO, and the solution was made at the final concentration of 10 µM. Reaction buffer solution (960µl, 0.1M Tris-HCL, pH-8 containing 5mM EDTA and 2 mM phenol) containing either COX-1 or COX-2 enzymes (10 µl) in the presence of heme (10 µl) was added with 10 µl of 10 µM test drug solution. These solutions were incubated for a period of 10 min at 37^{o}C after then 10 µl of AA solution was added followed by stopping the COX reaction by addition of 50 µl of 1 M HCL. The $PGF_{2\alpha}$,

produced from the PGH_2 by reduction with stannous chloride (100 µl), was measured by enzyme immunoassay. This was based on the competition between PGs and PG-acetyl cholinesterase conjugation (PG tracer) for the limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the well since the concentration of PGs tracer is held constant while the concentration of PGs varies. This antibodies–PG complex bind to mouse anti-rabbit monoclonal antibodies that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Elliman's reagents, which contain the substrate to acetylcholine esterase, are added to the well. The product of this enzymatic reaction produced a distinct yellow colour, determined by spectrophotometerically (Micro titre Plate reader) at 405 nm, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: Absorbance α [Bound PG tracer] α 1/PGs. Percent inhibition was calculated by the comparison of compound treated by control incubations.

RESULTS AND DISCUSSION

The petrol extract of the air-dried plant materials (aerial parts and roots) afforded the flavonoid characterized as 5,7-dihydroxy-6,8,4′-trimethoxyflavone (**1**) on the basis of detailed spectral studies. In our preliminary pharmacological test, the flavonoid was evaluated for its cyclooxygenase-1 and 2 (COX-1 and COX-2) inhibitory efficacy by employing the COX catalyzed prostaglandin biosynthesis assay *in vitro* method. The compound was found to exhibit moderate inhibitory activity against COX-1 and weak activity against the COX-2 with respective percent inhibition of 7.37% and 0.65% both at 10µM concentration (DMSO). The results of our preliminary study urge for thorough and detailed research on evaluation of cyclooxygenase inhibitory potential of the compound, which might eventually be appeared as a 'lead molecule' for drugs against inflammatory and related diseases.

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"The more the book is drawn into notice, the more exposed it becomes to criticism and remark."

- Fanny Burney