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Gentiana lutea exerts anti-atherosclerotic effects by preventing endothelial inflammation and smooth muscle cell migration

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Abstract *Background and aims:* Studies suggest that *Gentiana lutea* (GL), and its component isovitexin, may exhibit anti-atherosclerotic properties. In this study we sought to investigate the protective mechanism of GL aqueous root extract and isovitexin on endothelial inflammation, smooth muscle cell migation, and on the onset and progression of atherosclerosis in streptozotocin (STZ)-induced diabetic rats.
Methods and results: Our results show that both GL extract and isovitexin, block leukocyte adhesion and generation of reactive oxygen species in human umbilical vein endothelial cells (HUVECs) and rat aortic smooth muscle cells (RASMCs), following TNF-alpha and platelet derived growth factor-BB (PDGF-BB) challenges respectively. Both the extract and isovitexin blocked TNF-α induced expression of ICAM-1 and VCAM-1 in HUVECs. PDGF-BB induced migration of RASMCs and phospholipase C-γ activation, were also abrogated by GL extract and isovitexin. Fura-2 based ratiometric measurements demonstrated that, both the extact, and isovitexin, inhibit PDGF-BB mediated intracellular calcium rise in RASMCs. Supplementation of regular diet with 2% GL root powder for STZ rats, reduced total cholesterol in blood. Oil Red O staining demonstrated decreased lipid accumulation in aortic wall of diabetic animals upon treatment with GL. Medial thickness and deposition of collagen in the aortic segment of diabetic rats were also reduced upon supplementation. Immunohistochemistry demonstrated reduced expression of vascular cell adhesion molecule-1 (VCAM-1), inducible nitric oxide synthase (iNOS), and vascular endothelial cadherin (VE-cadherin) in aortic segments of diabetic rats following GL treatment.
Conclusions: Thus, our results support that GL root extract/powder and isovitexin exhibit anti-atherosclerotic activities.

Introduction

Gentiana lutea (great yellow gentian) is well known in central and southern Europe for treatment of digestive disorders [1]. The medicinal value of this particular plant is due to the presence of several phytochemicals like gentisin, bellidifolin-8-O-glucoside, demethylbellidifolin-8-O-glucoside, isovitexin, amarogentin, etc. [2]. The aqueous-

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ethanol extract of *G. lutea* (GL) exerts a cyto-protective effect on healthy immune-competent cells following X-ray irradiation [3]. Similarly, Akileshwari et al. demonstrated the therapeutic value of GL extract in preventing diabetic ocular complications [2]. Recently, we have demonstrated anti-proliferative effect of GL aqueous root extract and its constituent isovitexin, on rat aortic smooth muscle cells (RASMCs) through their ability to block ERK1/2 activation and iNOS expression [4].

Accelerated vascular smooth muscle cell proliferation and migration contribute to atherosclerosis, a multifactorial disease, which is also associated with oxidative stress, and, is triggered upon uncontrolled endothelial inflammation [5]. Metabolic abnormalities observed during diabetes and obesity further exacerbate the risk of atherosclerosis by provoking systemic inflammation through increased circulating levels of pro-inflammatory cytokines [5]. Some of these, such as the tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), in turn, elicit arterial inflammation by enhancing the adhesion of leukocytes on to the endothelial cells [5–7]. This essentially involves reduction in bioavailability of nitric oxide (NO) with consequent expression of cellular adhesion molecules (CAM) on the surface of endothelial cells, such as, E-selectin, intracellular CAM-1 (ICAM-1), vascular CAM-1 (VCAM-1), and platelet endothelial CAM-1 (PECAM-1), for increased tethering of immune cells [8]. However, it is not known whether GL extract exerts a protective effect against endothelial inflammation and diabetes induced atherosclerosis.

Epidemiological and pre-clinical studies have shown that phytochemicals such as flavanoids, isoflavones, xanthonoids, and phytosterols delay the onset and progression of atherosclerosis [9,10]. These active compounds exert their effects, either by reducing the circulating levels of cholesterol or by inhibiting lipid oxidation [9–11], while the others, exhibit anti-inflammatory [11] and anti-platelet activities [9]. Some of these phytochemicals also improve endothelium dependent vaso-relaxation by modulating bioavailability of NO and voltage-gated ion channels [12]. In this study we investigated the anti-inflammatory as well as the anti-migratory effects of the aqueous extract of GL roots, and one of its main constituent, isovitexin, on human umbilical vein endothelial cells (HUVECs) and RASMCs respectively. Additionally, we tested the athero-protective effect of GL root-powder in streptozotocin (STZ)-induced diabetic rats.

Methods

For details see Supplementary data section.

Results

G. lutea extract (GL) and isovitexin inhibit TNF-α induced endothelial inflammation

Treatment of a quiescent endothelial monolayer with 20 ng/ml TNF-α for 24 h, increased the adhesion of peripheral blood derived mononuclear cells (PBMCs) four-fold as seen in Fig. 1A and B. This increase in leukocyte adhesion was however significantly blocked by both, the

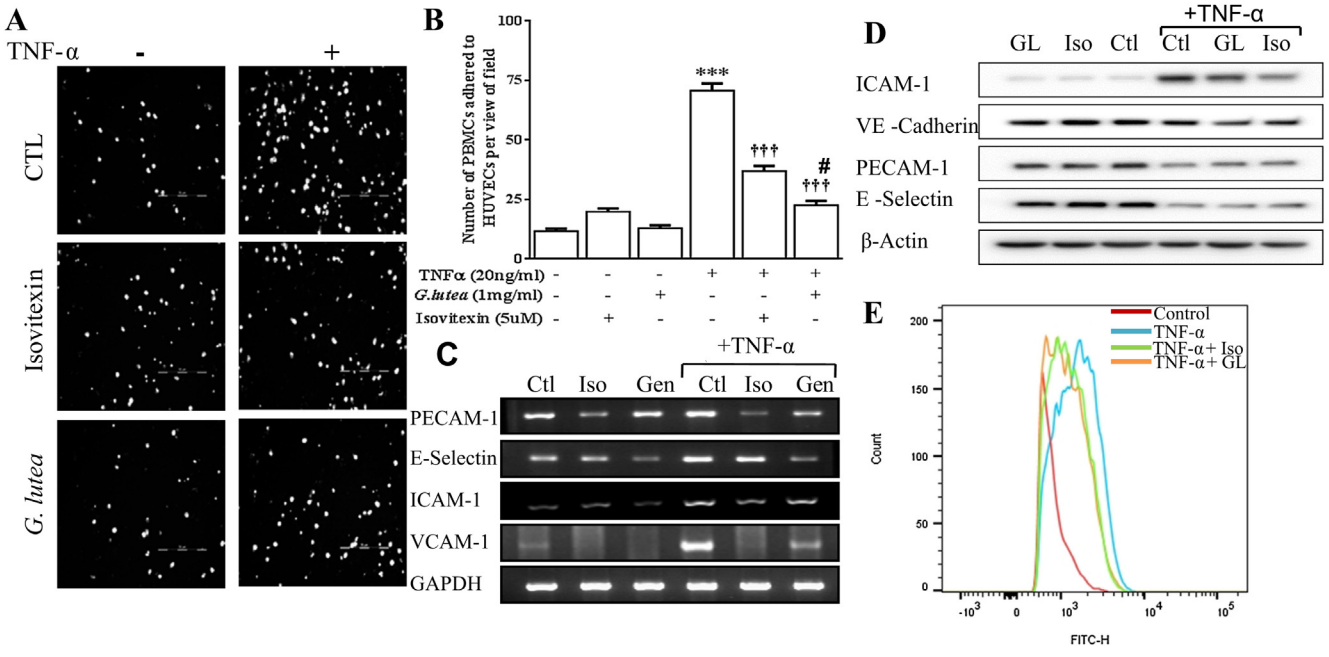

Figure 1 Effect of *G. lutea* extract (1 mg/ml) and isovitexin (5 μmol/L) on endothelial inflammation. A) Representative image depicting adherence of PKH26 labeled leukocytes to TNF-α (20 ng/ml) treated HUVEC monolayer. B) Bar graph summarizing data as mean ± SEM for a minimum of three independent experiments. C) Representative image of reverse transcriptase polymerase chain reaction for expression of cell adhesion molecules. D) Representative western blot panel for protein expression of adhesion molecules for three independent experiments and E) representative flow cytogram for surface expression of VCAM-1 in response to experimental treatment to endothelial cells. $^{***}p < 0.001$ versus control, $^{\dagger\dagger\dagger}p < 0.001$ versus TNF-α treatment and $^{\#}p < 0.05$ versus TNF-α + isovitexin treatment. Each experiment was performed in triplicates and was repeated for a minimum of three times ($n = 3$).