**Full Length Research Paper**

**In vivo antioxidative activity of 5,7,4'-trihydroxyflavone-7-O-(6''-O-[E]-coumaroyl)-β-glucopyranoside isolated from Panzeria alaschanica**

BAO Ming-Lan, WANG Qing-Hu* and HAO Jun-sheng

College of Traditional Mongolian Medicine, Inner Mongolia University for Nationalities, Tongliao 028000, China.

Received 18 September, 2017; Accepted 4 December, 2017

Our objective was to assess *in vivo* antioxidative potential of 5,7,4'-trihydroxyflavone-7-O-(6''-O-[E]-coumaroyl)-β-glucopyranoside (TFGN) isolated from *Panzeria alaschanica* in a diabetic rat model. The diabetes and the following oxidative stress were induced by intraperitoneal administration of streptozotocin. The effects of TPGN (60 mg/kg) on the plasma concentration of malondialdehyde (MDA) and plasma fat-soluble antioxidants (co-enzyme Q₉, α- and γ-tocopherol) were measured as a parameter of oxidative damage and markers of antioxidant defence, respectively. The level of MDA in plasma was reduced to the same level as in healthy control animals. A significant decrease was observed in the plasma α-tocopherol level in the oxidative stress group compared to the healthy controls.

**Key words:** *Panzeria alaschanica*, diabetes mellitus model, antioxidant, 5,7,4'-trihydroxyflavone-7-O-(6''-O-[E]-coumaroyl)-β-glucopyranoside.

**INTRODUCTION**

*Panzeria alaschanica*, family Labiate, is a widely distributed plant in Eerduosi of Inner Mongolia, China. *P. alaschanica* (aerial parts) are used as a characteristic medicine in Mongolian folk to treat pelvic inflammation and chronic pelvic inflammation (Li et al., 2011; Zhang et al., 2001). In previously conducted work, it was found that the highest dose (400 mg/kg) of EtOAc extract from *P. alaschanica* produced significant anti-inflammatory activity (Wang et al., 2015a), and phenylethanoid and acylatedflavone glycosides were isolated from *P. alaschanica* (Shao et al., 2015; Wang et al., 2015b). In addition, a high performance liquid chromatography (HPLC) method for the quantification of the flavonoids in this plant was established (Wang et al., 2015c).

Acylatedflavone glycosides exhibited a wide range of biological activities including anti-inflammatory, antioxidant, and hepatoprotective effects (Julião et al., 2010; Albach et al., 2003; Peng et al., 2003). Every barber knows that the beneficial to health of acylatedflavone glycosides are due to their antioxidative and anti-inflammatory activities (Hasan et al., 2012). The EtOAc extract of *P. alaschanica* and the isolated compounds have widely been measured in various in vivo anti-inflammatory test systems (Wang et al., 2015a, b, d).

The 5,7,4'-trihydroxyflavone-7-O-(6''-O-[E]-coumaroyl)-β-glucopyranoside...
β-glucopyranoside (TFGN) exhibited significant in vivo anti-inflammatory activities. However, until recently, there have been few studies about the in vivo antioxidative effect of TFGN. It is necessary to study its in vivo antioxidative effect, since inflammation is closely related to oxidative stress. Therefore, focus was on the in vivo antioxidative potential of TFGN.

Oxidative stress has been connected with many chronic diseases such as atherosclerosis, diabetes and cancer. Oxidative stress in diabetes mellitus (DM) is produced through multiple mitochondrial, enzymatic and non-enzymatic pathways (Bebrevska et al., 2010). Many flavonoids from foods and herbal medicines have been tested already in vitro to determine their antioxidative effect. As these in vitro tests do not pay attention to problems of malabsorption, distribution, excretion and metabolism, it is indispensable to study the activity of a promising antioxidant in vivo. In this study, the effects of the TFGN on the plasma concentration of MDA and some fat-soluble antioxidants (co-enzyme Q₉, α- and γ-tocopherol) were determined (Figure 1).

MATERIALS AND METHODS

Chemicals

α-Tocopherol, α-tocopherol acetate, γ-tocopherol, co-enzymes Q₉ and Q₁₀, 1,1,3,3-tetramethoxypropane, butylated hydroxyanisole, butylated hydroxy toluene (BHT), retinol, streptozotocin, thiobarbituric acid and pentobarbital (20% solution) were purchased from Shanghai Biochemical Co. (Shanghai, China). Butylated hydroxyanisole, product butylated hydroxy toluene (BHT), retinol, streptozotocin, and Q₉ α-tocopherol acetate, co-enzyme Q₉, co-enzyme Q₁₀ were purchased from Shanghai Biochemical Co. (Shanghai, China). The purity of TFGN was determined to be above 98.0% by normalization of the peak areas detected by HPLC.

TFGN: Yellow needles; UV (MeOH) λmax nm (log ε): 269 (4.35), 327 (4.23), IR (neat) ν cm⁻¹: 3447, 3365, 1688, 1654, 1639, 1602, 1588, 1510, 1487, 1361, 1278, 1167, 1074 cm⁻¹. ¹H NMR (500 MHz, in DMSO- d₆) δ H: 6.89 (1H, s, H-3), 6.47 (1H, d, J = 1.5 Hz, H-6), 6.81 (1H, d, J = 1.5 Hz, H-8), 7.93 (2H, d, J = 8.0 Hz, H-2′, 6′), 6.93 (2H, d, J = 8.0 Hz, H-3′, 5′), 5.17 (1H, d, J = 7.0 Hz, H-1″), 7.36 (2H, d, J = 8.0 Hz, H-2″, 6″), 6.66 (2H, d, J = 8.0 Hz, H-3″, 5″), 7.51 (1H, d, J = 16.0 Hz, H-7″), 6.34 (1H, d, J = 16.0 Hz, H-8″).

¹³C-NMR (125MHz, in DMSO- d₆) δ C: 164.6 (C-2), 103.3 (C-3), 182.5 (C-4), 161.7 (C-5), 99.8 (C-6), 163.0 (C-7), 95.5 (C-8), 157.3 (C-9), 105.6 (C-10), 121.3 (C-1′), 129.1 (C-2′), 116.6 (C-3′), 161.3 (C-4′), 116.6 (C-5′), 129.1 (C-6′), 100.3 (C-1″), 73.4 (C-2″), 76.7 (C-3″), 70.1 (C-4″), 74.1 (C-5″), 63.9 (C-6″), 125.3 (C-1‴), 130.6 (C-2‴), 116.0 (C-3‴), 160.2 (C-4‴), 116.0 (C-5‴), 130.6 (C-6‴), 145.5 (C-7‴), 114.1 (C-8‴), 166.9 (C-9‴); HR-ESI-MS: m/z 723.1705 [M+H] (calcld for 723.1708).

Plant material

P. alaschanica (aerial parts) were collected in Eerdosii, Inner Mongolia, China, in July, 2015. The plant material was identified by Prof. Wuxiangjie (Inner Mongolia University for Nationalities) and a voucher specimen (NO. 20150802) was stored in the Mongolian Medicine Research Center, Inner Mongolia University for Nationalities.

Extraction and isolation

The air-dried and powdered aerial parts of P. alaschanica (6.0 kg) were extracted twice under reflux with ETOAc (25 L) after extracting with CHCl₃ (10 L). The combined extracts were concentrated to a residue (510 g, yield 8.5 %) under reduced pressure. The ETOAc extract was isolated by column chromatography on silica gel and eluted by a gradient of CHCl₃–CH₂OH (40:1 to 5:1) to give seven fractions (Fractions 1-7). Fraction 5 (4.0 g) was further eluted on a Sephadex LH-20 column with MeOH yielding TFGN (405 mg) (Wang et al., 2015b). The purity of TFGN was determined to be above 98.0% by normalization of the peak areas detected by HPLC.

TFGN: Yellow needles; UV (MeOH) λmax nm (log ε): 269 (4.35), 327 (4.23), IR (neat) ν cm⁻¹: 3447, 3365, 1688, 1654, 1639, 1602, 1588, 1510, 1487, 1361, 1278, 1167, 1074 cm⁻¹. ¹H NMR (500 MHz, in DMSO- d₆) δ H: 6.89 (1H, s, H-3), 6.47 (1H, d, J = 1.5 Hz, H-6), 6.81 (1H, d, J = 1.5 Hz, H-8), 7.93 (2H, d, J = 8.0 Hz, H-2′, 6′), 6.93 (2H, d, J = 8.0 Hz, H-3′, 5′), 5.17 (1H, d, J = 7.0 Hz, H-1″), 7.36 (2H, d, J = 8.0 Hz, H-2″, 6″), 6.66 (2H, d, J = 8.0 Hz, H-3″, 5″), 7.51 (1H, d, J = 16.0 Hz, H-7″), 6.34 (1H, d, J = 16.0 Hz, H-8″).

¹³C-NMR (125MHz, in DMSO- d₆) δ C: 164.6 (C-2), 103.3 (C-3), 182.5 (C-4), 161.7 (C-5), 99.8 (C-6), 163.0 (C-7), 95.5 (C-8), 157.3 (C-9), 105.6 (C-10), 121.3 (C-1′), 129.1 (C-2′), 116.6 (C-3′), 161.3 (C-4′), 116.6 (C-5′), 129.1 (C-6′), 100.3 (C-1″), 73.4 (C-2″), 76.7 (C-3″), 70.1 (C-4″), 74.1 (C-5″), 63.9 (C-6″), 125.3 (C-1‴), 130.6 (C-2‴), 116.0 (C-3‴), 160.2 (C-4‴), 116.0 (C-5‴), 130.6 (C-6‴), 145.5 (C-7‴), 114.1 (C-8‴), 166.9 (C-9‴); HR-ESI-MS: m/z 723.1705 [M+H] (calcld for 723.1708).

Animals and experimental design

Male Wistar rats at the age of 3 months were provided by Changchun Yisheng Laboratory Animal Technology Co., Ltd. (Changchun, China). The 50 animals were housed in an air-conditioned room with 12/12-light/dark cycles and provided with standard laboratory food (Rat sterile granulated feed, product executive standard: GB14924-2001, license: SCXK-(JL) 2010-0001) and water ad libitum. All animals received humane care in compliance with local regulations of laboratory animal care and...
RESULTS

The BGL and body weight of all animals were determined before the induction of diabetes mellitus. As shown in Figure 2a, there were no statistically significant differences between the groups. Oxidative stress was permitted to develop during 7 weeks after streptozotocin injection. From Figure 2b, it is found that the BGL in G3 significantly increased at the end of this induction period. Treatment was begun after development of DM. The results of the MDA-TBA and the determination of body weight of the animals after 3 weeks of treatments are shown in Figures 3 and 4, respectively. Moreover, the results of the effect on the plasma concentration of some fat-soluble antioxidants (co-enzyme Q9, α- and γ-tocopherol) were shown in Figures 5 to 7.

DISCUSSION

The BGL in G3 significantly increased at the end of this induction period, confirming the successful development of DM (Figure 2b). Moreover, the characteristic symptoms of DM such as the increased appetite, polydipsy, polyuria, and loss of weight were observed. From Figure 3, all animals which received oxidative stress displayed a significant decrease in body weight compared to G1, even after treatment with G4 or G5. There were no significant differences between the body weight of G4 or G5, with G3. As shown in Figure 4, the level of MDA-TBA complex in G3 was significantly promoted as compared to the G1 (P<0.001), which indicated the suitability of the chosen system for in vivo lipid peroxidation inhibitory activity evaluation. Comparing to G3, there was a significant reduction in the oxidative stress damage in G4 and G5. However, the damage of lipid peroxidation was not decreased to G1. The level of lipid damage in G4 and G5 was similar. Thus, the G4 and G5 had similar effect on the in vivo oxidative stress status of the diabetic animals. The potent lipid peroxidation chain breaking activity in G3 compound with G2 or G4 produced a similar effect.

From Figure 5, it is found that the level of plasma α-tocopherol in G3 significantly reduced compared to G1. However, there was no significant difference between G4 and G1 or G3. In addition, no significant difference was observed between G5 and G3. The G1 and G2 showed similar α-tocopherol levels. Concerning γ-tocopherol, there was no significant difference between G1 and G3 (Figure 6). It is surprising that the levels of γ-tocopherol in G4 and G5 were significantly reduced with the G1, and the observed levels were not different from G3. It should be noted that the levels of γ-tocopherol were very low. The discoveries showed that in G3, G4 and G5 where oxidative stress was induced almost complete depletion of γ-tocopherol had happened. Any positive influence of the treatment with TFGN on the level of γ-tocopherol
Figure 2. (a) BGL in G1-G5 at the start of the study. (b) BGL in G1-G5 at the end of the induction period. ***P<0.001, compared to G1. There was no statistically significant difference between G4 or G5, compared to G3.

Figure 3. Body weight in G1-G5. ***P<0.001; *P<0.05; compared to G1. There were no significant differences between G4 or G5, compared to G3.
Figure 4. The levels of MDA–TBA complex in G1-G5. ### P<0.001, ## P<0.01, # P<0.05, compared to G3.

Figure 5. The levels of α-tocopherol plasma in G1-G5. **P<0.01, *P<0.05, compared to G1. There was no significant difference between G4 or G5, compared to G3.

Figure 6. The levels of γ-tocopherol plasma in G1-G5; ***P<0.001, **P<0.01, compared to G1. There was no significant difference between G1 compared to G3.
under the condition of oxidative stress could not be observed. From Figure 7, the level of Q<sub>9</sub> in G4 or G5 was different from the G1 but not from the G3. The diabetic animals in G3 and G4 displayed a significant decrease at this phase of the experiment. This might be an after effect of its depletion as a factor in the antioxidant defence against the induced oxidative stress, which was not equalized by upregulation of its synthesis in this case. There was no significant difference between G4 or G5 on the Q<sub>9</sub> level compared to G3. Thus, the dynamics of the up-regulation and depletion of this molecule are needed to further investigate. For detecting potential toxic effects of TFGN, the animals in G2 received 10× the treatment dose (500 mg/kg). The animals in G2 were not injected with streptozotocin. With regard to the level of BGL, MDA-TBA complex, the fat-soluble antioxidants and body weight measured, no significant differences were observed between G1 and G2.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interest.

**ACKNOWLEDGEMENTS**

We thank the scientific research project of the Inner Mongolia Autonomous Region Universities in China (NJZZ14182).

**REFERENCES**