Pharmacological actions of Danggui Buxue Tang (DBT) on Oxygen-Glucose Deprivation and Reoxygenation (OGD/R)-insulted mouse brain endothelial (bEnd.3) cells

Karry Yuen-Ching Cheung[1,3], Hei-Yin Wong[2,3], Bun Tsoi[1,3], Sai-Wang Seto [1,3,4]

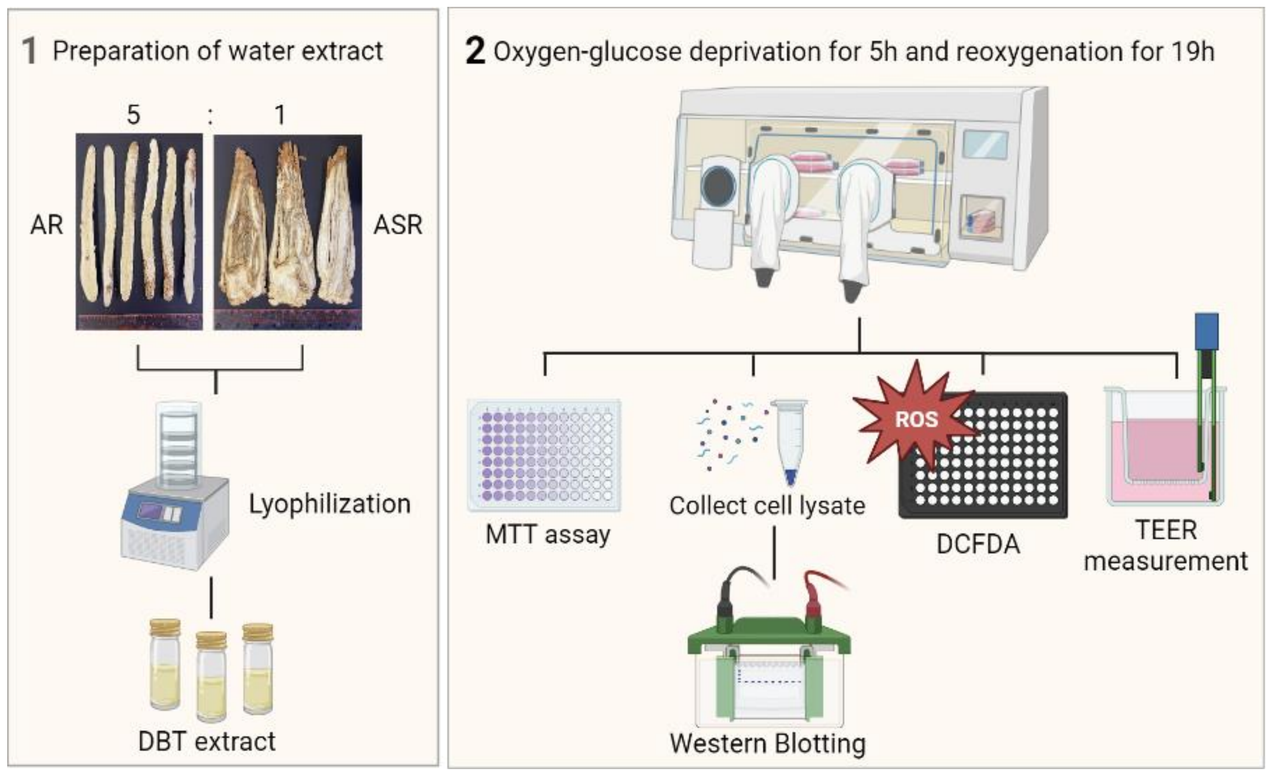
[1] Department of Food Science and Nutrition, The Hong Kong Polytechnic University, Hong Kong SAR, China, [2] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong SAR, China, [3] Research Centre for Chinese Medicine Innovation, The Hong Kong Polytechnic University, Hong Kong SAR, China, [4] NICM Health Research Institute, Western Sydney University, Penrith, NSW, 2751, Australia

BACKGROUND

Menopause is the permanent cessation of menses and the significant reduction in oestrogen is often considered as the major risk factor for vascular dementia (VaD) in menopausal women. VaD is characterized by cerebral hypoperfusion and disruption of the blood-brain barrier (BBB). Currently, hormone replacement therapy (HRT) is the only treatment to alleviate distressing menopausal symptoms. However, large body of evidence showed that chronic HRT could increase the risk of various chronic diseases. Hence, alternative therapy that is safe and effective is urgently needed.

Danggui Buxue Tang (DBT) is an ancient Chinese herbal decoction consisting of 2 herbs, Astragali Radix (Huangqi) and Angelicae Sinensis Radix (Danggui). It has been shown to possess estrogenic and osteogenic effects and thus a popular herbal formula among menopausal women in China and other Asian that DBT could increase proliferation, migration and enhance tight junction molecules expression in OGD/R-insulted bcountries. However, its pharmacological effects on cerebral endothelial cells have not been fully elucidated. We hypothesized that DBT could increase proliferation, enhance tight junction molecules expression, promote BBB protection and reduce ROS release in OGD/R-insulted bEnd.3 cells.

MATERIALS AND METHODS



RESULTS

1. DBT promoted cell proliferation and suppressed OGD/R-induced cell death

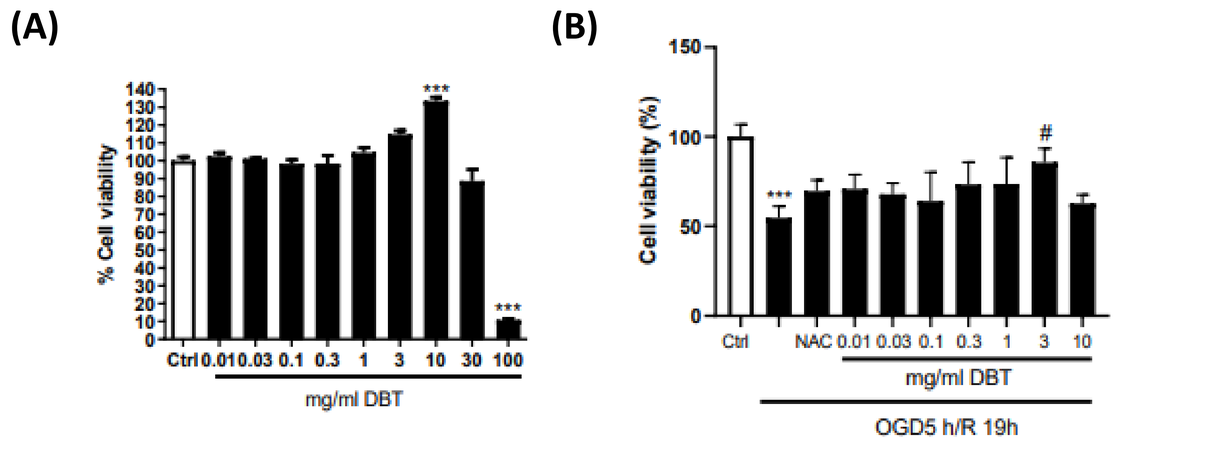


Figure 1. Examination of DBT on bEnd.3 cell viability under (A) normoxic (n=3) and (B) exposed to OGD for 5 hours, followed by 19-hour reoxygenation (n=3) by MTT assay. ***p < 0.0001 versus control and #p < 0.01 versus OGD/R model

2. DBT increased junctional protein (ZO-1, Claudin-5) expression

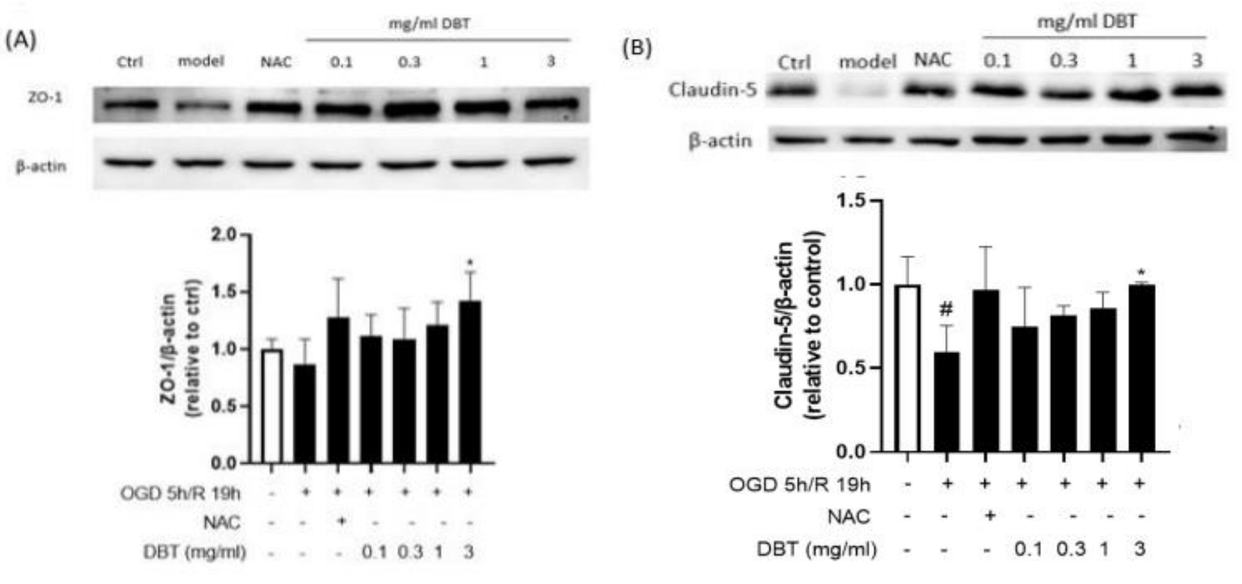


Figure 2. DBT showed restoration of (A) ZO-1 (n=3), (B) Claudin-5 (n=3) after OGD for 5 hours, followed by 19-hour reoxygenation. *p < 0.01 versus OGD/R model and #p < 0.01 versus control

3. DBT restored damaged BBB as shown by increased TEER values

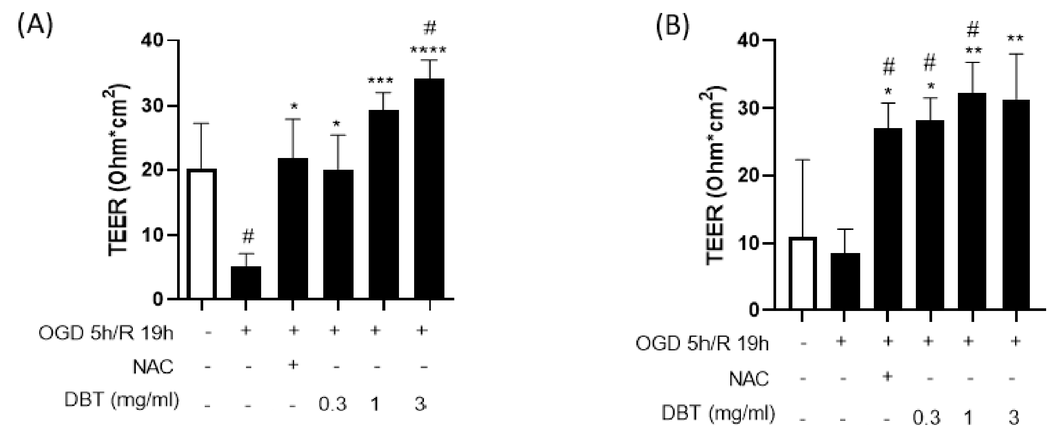


Figure 3. Increased TEER of (A) monoculture (bEnd.3) and (B) co-culture (bEnd.3+N2a) system treated with DBT dose-dependently. (A) Only bEnd.3 cells were seeded on the apical side of the transwell (n=3). # p< 0.05 versus ctrl. *p< 0.05, *** p< 0.001 and **** p< 0.0001 versus OGD/R model. (B) bEnd.3 cells were seeded on the apical side of the transwell while N2a cells were seeded on the basal part (n=3). #p < 0.05 versus ctrl, *p < 0.05, **p < 0.01 versus OGD/R model.

4. DBT suppressed the release of ROS under OGD/R condition

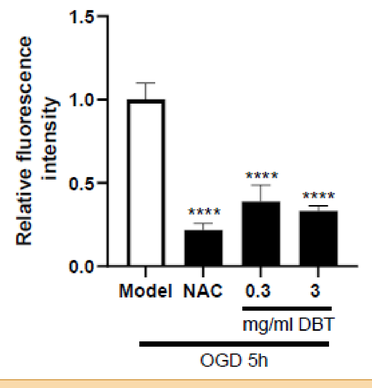


Figure 4. Decreased ROS level under OGD/R injury with administration of 1 mM NAC, 0.3 or 3 mg/ml DBT. DCFDA assay showed that 1 mM NAC and DBT remarkably reduced fluorescence intensity after OGD/R challenge (n=3), meaning that the ROS level is greatly suppressed. ****p< 0.0001 versus model.

5. The elevation of HO-1 could be the pathway that DBT worked on reducing ROS

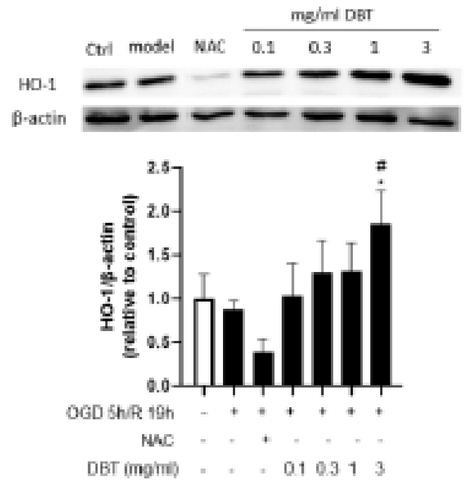


Figure 5. The administration of DBT showed increased HO-1 expression dose-dependently (n=3). Cells were treated with 1 mM N-acetyl cysteine and 0.01-10 mg/ml of DBT in HG DMEM before exposed to OGD for 5 hours, followed by 19-hour reoxygenation. *p < 0.01 versus OGD/R model and #p < 0.01 versus control.

CONCLUSION

Our result showed that **1)** DBT (0.01-10 mg/ml) increased proliferation of bEnd.3 cells in a concentration-dependent manner. **2)** OGD/R markedly reduced cell viability by 60%. DBT (0.01 – 10 mg/ml) suppressed the OGD/R-induced cell death in a concentration-dependent manner. **3)** OGD/R reduced ZO-1 and occludin protein expressions which were normalised by DBT (0.1-3 mg/ml). **4)** The damaged BBB during OGD/R was restored by DBT (0.1-3 mg/ml). **5)** DBT suppressed ROS release under OGD/R condition which the increased HO-1 expression could explain.

ACKNOWLEDGEMENT

This work was supported by fundings from the Department of Applied Biology and Chemical Technology and Research Centre for Chinese Medicine Innovation, The Hong Kong Polytechnic University.