Antioxidant potential of *Centaurium erythraea in vitro*

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1. Introduction

Oxidative stress is caused by the imbalance between the free radicals production and their removal through the antioxidant protective system, and leads to damaging of cellular proteins, lipids and nucleic acids and cellular death. Aside from cells' endogenous antioxidant defence system, exogenous compounds taken by food and beverages also have a big influence in response to oxidative stress. [1].

Since ancient times *Centaurium erythraea* Rafn (CE) has been used as a dietary supplement. It is proven that the extract of the above-ground parts of CE posesses antioxidant ability [2]. The main goal of this study was to examine protective effect of CE methanol extract against hydrogen peroxide (H_2O_2) induced oxidative stress in *in vitro* conditions by measuring its antioxidant potential.

2. Research methods

Methanol extracts of CE was prepared from the above-ground parts of plant. Chemical composition of CE extract was evaluated by determination of total phenolic and total flavonoid contents. The ability of the extract to scavenge H_2O_2 was estimated by the decrease in absorbance of a H_2O_2 solution at 230 nm. The effect of CE extract on survival of Rin5F pancreatic beta cells treated with LD_{50} dose of H_2O_2 for 3 hours was examined by the MTT viability assay. Catalase (CAT) activity in Rin5F cell treated with LD_{50} dose of H_2O_2 with/without CE extract was determined by the rate of H_2O_2 decomposition, while protein level of CAT was estimated by Western blot.

3. Results

The total phenolic (TPC) and total flavonoid (TFC) content of CE methanol extract and its ability to scavenge H_2O_2 increased in concentration-dependent manner (Table 1.). Linear regression and correlation analyses showed that H_2O_2 scavenging strongly and significantly correlates to TPC ($r = 0.9997$; $p = 0.0003$) and TFC ($r = 0.9884$; $p = 0.0116$). Excepting lowest used concentration of CE extract, the viability of H_2O_2-treated Rin5F cells increased with CE extract concentrations (Table 2.). To determine effect of CE extract on the activity and protein level of the main H_2O_2 eliminating enzyme CAT, extract was used in concentration of 0.2 mg/ml. CE extract reduced activity of CAT in H_2O_2-treated Rin5F cells (Fig.1A). CAT protein level was increased in H_2O_2-treated cells, while CE extract slightly but not significantly reduced CAT protein level (Fig.1B).

CE (mg/mL)	0.05	0.1	0.2	0.5
TPC (µg GAE/mL)	1.20	2.02	3.15	9.67
TFC (µg QE/mL)	3.57	5.85	14.10	31.49
H_2O_2 scavenging (%)	17.23	20.57	27.89	62.49

Table 1 – Total phenolic (TPC), total flavonoid (TFC) content and H_2O_2 scavenging activity of CE extract

CE (mg/mL)	0	0.05	0.1	0.2	0.5
% of survival	53.12	53.75	58.57	62.32	70.61

Table 2 – Effect of CE extract on viability of Rin5F pancreatic beta cells treated with 75 µM H_2O_2

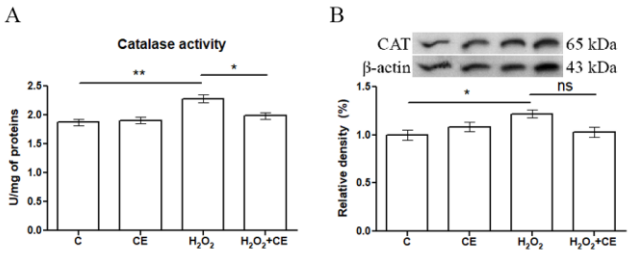

Figure 1 – Effect of CE extract on catalase (CAT) activity and protein level in H_2O_2-treated Rin5F cells

4. Conclusion

The results in this study show that CE extract protects Rin5F cells from H_2O_2-induced oxidative stress. Centaury extract possesses significant antioxidant potential which can be explained by the presence of phenols and flavonoids. Since oxidative stress is responsible for development of diabetes [3], here presented protective effect of CE extract supports traditional use of this plant in diabetes treatment and provides possibility to examine the mechanism of antioxidant action.

5. Literature

[1] Šiler, B., et al. "Centauries as underestimated food additives: Antioxidant and antimicrobial potential." *Food chemistry* 147 (2014): 367-376.

[2] Valentão, P., et al. "Methoxylated xanthones in the quality control of small centaury (Centaurium erythraea) flowering tops." *Journal of agricultural and food chemistry* 50.3 (2002): 460-463.

[3] Maiese, K., et al. "Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus." *Current neurovascular research* 4 (2007): 63–71.