NEUROPROTECTIVE EFFECTS OF EDIBLE BIRD'S NEST AGAINST HYDROGEN PEROXIDE INDUCED OXIDATIVE STRESS IN HUMAN SH-SY5Y CELL

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Introduction

Aging is a slow and gradual biological process, associated with multiple physiological and pathological changes including redox reaction. In brain senescence, ROS starts to accumulate in neurons before clinically evident signs and symptoms of the disease can be detected [1].When ROS accumulate, oxidative damage is normally prevented by induction of protective factors like antioxidants, which may not be effective if the insult is too overwhelming. In such cases apoptotic mechanisms set in to remove neurons deemed unsalvageable [2]. SH-SY5Y cell line is widely used for studying *in vitro* neuronal cell. Hydrogen peroxide (H_2O_2) played the role of reactive oxygen species which induced redox reaction and apoptosis in various cells including neurons [2]. Edible bird's nest (EBN) is the nest of swiftlets and is constructed with salivary glue, which is a cementing substance. Due to its nutritious and medicinal properties, EBN has been deemed a precious food tonic in Chinese community ever since the Tang (608-907AD) dynasties [3]. Lactoferrin (LF) and ovotransferrin (OVF) are glycoprotein and family members of transferrin. It was also found that EBNs share a 77 KDa protein that has properties similar to transferrin protein [4]. Therefore, this study was to determine the concentration of lactoferrin and ovotransferrin; further it examined the potential effectiveness and related mechanisms of EBN extraction used for anti-aging in SH-SY5Y cells.

Materials and Methods

Water-soluble protein was obtained from house white Edible Bird's Nest from Malaysia following method as previously reported [5]. Total protein of EBN water extract was determined by using the Bradford method protein assay kit (modified Bradford) with bovine serum albumin (BSA) as the standard, reading at 595nm in Synergy H1 Hybrid Multi-Mode Microplate Reader. LF and OVF concentration in EBN water extract were detected using Chicken Lactoferrin Elisa kit and Chicken Ovotransferrin Elisa Kit, Biosource (San Diego, California, USA). SH-SY5Y cells were seeded into 96-well culture plates at density of 2×10^5cells/mL and, after two days, cells were differentiated with retinoic acid (10 μM) for 7 days prior to treatment. The cells were treated with EBNE (1ug/ml-1mg/ml), LF (1ug/ml and 5ug/ml) and OV(1ug/ml and 10ug/ml) individually for 24 h and followed without and with H_2O_2 for 2 h to detect the toxicity and neuroprotective ability by MTT method. Similar procedure was used to harvest cells, then absorbance at 490nm and 480/530nm was recorded to detect SOD and ROS ELISA assay by Synergy H1 Hybrid Multi-Mode Microplate Reader. Finally, Annexin V-FITC and Propidium

Iodide Double-Staining Assay to detect apoptosis via flow cytometry. All the data was conducted (analysed?) by one-way ANOVA test using Statistical Package for Social Science (SPSS) version 20(SPSS Inc., Chicago, IL). $p< 0.05$ was considered as statistically significant difference.

Results and Discussion

The soluble water protein detect LF and OVF concentration by chicken source ELISA assay; the results are not shown. We hypothesized that LF and OVF may be the important biomarkers in EBN identification because the physiological functions; on the other hands, we need more evidence to confirm the function mechanism in EBN. First of all, the toxicity of EBN, LF and OVF were detected by MTT, as shown in Fig.1A, EBN(1μg/mL-1mg/ml), LF(1μg/mL and 5 μg/mL) and OVF(1μg/mL and 10μg/mL) displayed above 80% viability on SH-SY5Y cells, and there is no significant differences compared to control group($P>0.05$). As reported in our previous paper [6] and shown in Fig.1B, SH-SY5Y cells exposure to 250 μM $H_2O_2$for 2h resulted in approximately 50% cell cytotoxicity comparing to control cells ($p< 0.01$). As shown on Fig.1B, 1mg/ml EBN, 5μg/ml LF and 10μg/ml OVF improved cell viability better than other concentrations due to its higher amounts- wrong choice of words of antioxidants. Therefore, for our subsequent experiments, 1mg/ml EBN, 5μg/ml LF and 10μg/ml OV were used. The relative activity of SOD in SH-SY5Y cells exposed to 250mM H2O2 2 h decreased to 69.6% comparing to control group. Pretreatment with EBN, LF and OVF for 24h recovered the SOD activity to 90.7%, 87.8% and 80.9% (Fig. 2A). The disruption in the balance between ROS and the endogenous antioxidant systems contributes to oxidative damage of cellular macromolecules ultimately leading to cell death in the special region of brain [44]. ROS kit was used to examine intracellular hydrogen peroxide/hydroxyl radical. However, the increase of intracellular hydrogen peroxide/hydroxyl radical was reduced by EBN, LF and OVF treatment. The increase of hydrogen peroxide/hydroxyl radical was almost completely reversed by incubation with EBN (Fig. 2B), followed by LF and OVF treatment. Treatment of cells with 250uM H_2O_2 significantly increased the percentage of early apoptosis (R6) and late apoptosis (R4) to 23.61 ± 0.64% and 27.01 ± 1.85% from 3.23 ± 0.29% to 2.11 ± 0.29% of the control value, respectively (Fig. 5, $P< 0.01$ and 0.001). Control group showed 80.05 ± 2.29% of viable cells (R5) and 1.01 ± 0.09% of late necrosis cells (R3), while the H_2O_2 group expressed only 48.1 ± 1.29% and 0.48 ± 0.02% respectively(Fig.3, $P<0.01$). However, pretreatment of EBN, LF and OVF showed 87.56 ± 1.23%, 78.35 ± 1.09% and 65.62 ± 0.89% of viable cell that were significant increased viable cells compared H_2O_2 group(Fig.3, $P<0.01$).

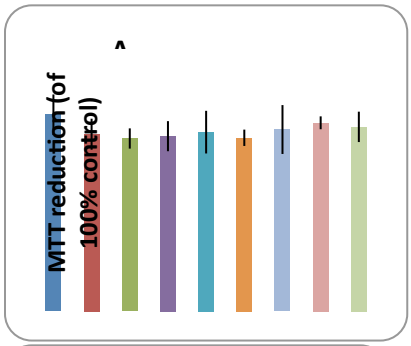
Conclusions

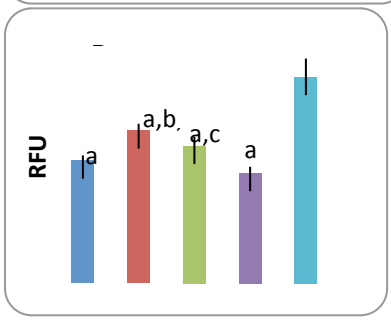
In summary, the present study demonstrated that EBN protects SH-SY5Y cells against H_2O_2-induced cytotoxicity and cell oxidative stress. The neuroprotective effects of EBN and its biomarkers may be related to its antioxidant and anti-apoptotic properties as EBN, LF and OVF can attenuate H2O2 induced oxidants and inhibited early apoptosis. This study may offer a new strategy for treatment of progressive neurodegenerative diseases such as Alzheimer.

Reference

1) Gandhi S, Abramov AY: Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev 2012,2012:11 pages. Article ID 428010.

2) Namiko Suematsu, Miki Hosoda, Ko Fujimori. Protective effects of quercetin against hydrogen peroxide-induced apoptosis in human neuronal SH-SY5Y cells. Neuroscience Letters 504 (2011) 223– 227
3) Koon, L. C., & Cranbrook, Earl of (2002). Swiftlets of Borneo – Builders of edible nests (pp. 1–171). Sabah, Malaysia: Natural History Publication (Borneo) SDN., B.H.D.
4) Massimo F. Marcone. Characterization of the edible bird's nest the "Caviar of the East". Food Research International 38 (2005) 1125–1134.
5) Yajun Wu, Ying Chen, Bin Wang, Liqun Bai, Wu ri han, Yiqiang Ge, Fei Yuan. Application of SYBRgreen PCR and 2DGE methods to authenticate edible bird's nest food. Food Research International 43 (2010) 2020–2026
6) Norsharina Ismail, Maznah Ismail , Siti Farhana Fathy , Siti Nor Asma Musa, Mustapha Umar Imam, Jhi Biau Foo and Shahid Iqbal. Neuroprotective Effects of Germinated Brown Rice against Hydrogen Peroxide Induced Cell Death in Human SH-SY5Y Cells. Int. J. Mol. Sci. 2012,13, 9692-9708; doi:10.3390/ijms13089692





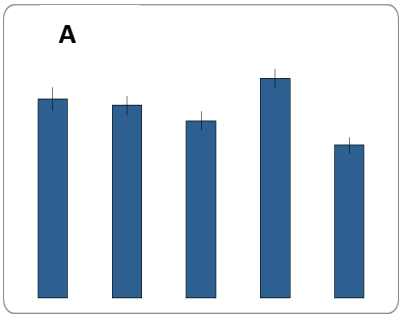


Fig 1. (A) Neurotoxicity effects of EBN, LF/ OVF on SH-SY5Y cell viability. Cells were incubated with EBN(1 µg/mL-1mg/ml), LF(5µg/mL/1 µg/mL) and OVF(10µg/mL1µg/mL) for 24 h; (B) EBN, LF and OVF pretreated SH-SY5Y with different concentration for 24h then with H_2O_2(250 µM) for an additional 2 h. Results are presented as the mean ± SD in triplicates. *p< 0.01 *vs* H_2O_2, #p<

Fig. 2 Expression levels of SOD and ROS in SH-SY5Y cells, following treatment with1mg/ml EBN water extract, 5µg/mL LF, 10µg/mL OVF and subsequent treatment with or without 250µMH_2O_2. A, SOD expression; B, ROS expression. Results are presented as the mean ± SD in triplicates. [a]p< 0.01 *vs* H_2O_2, [b]p< 0.01*vs* control, [c]p< 0.01 *vs* EBN.

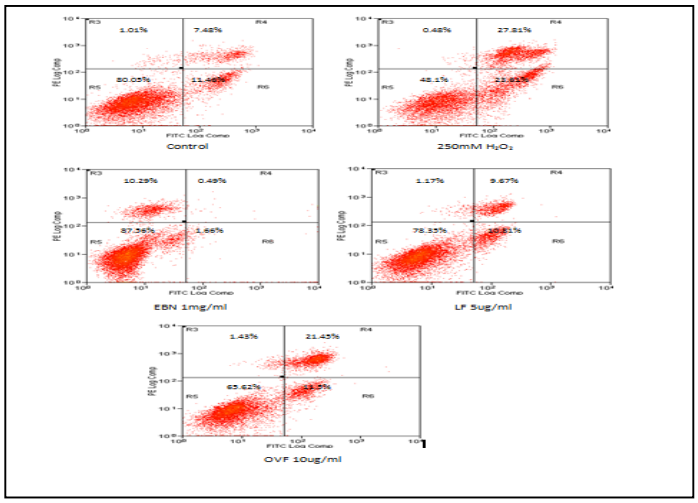


Figure3. Flow cytometry determination of apoptosis on SH-SY5Y cells with or without pretreatment after exposure to 250 µM H_2O_2 by Annexin V-FITC and PI staining assay. Control, untreated SH-SY5Y cells; SH-SY5Y cells treated with 250 µM $H_2O_2$for 24 h; exposure of SH-SY5Y cells to 250 µM H2O2over 24 h in the presence or absence of 24 h pre-treated 1mg/ml EBN, 5µg/mL LF and 10 µg/mL OVF. R3: late necrosis; R4: late apoptosis/early necrosis; R5: viable cells; R6: early apoptosis. Results are presented the means ± SD in triplicates.