

The Effect of Stevioside on Some Oxidative Stress Parameters in the Brain of Chicken Embryos

¹Parisa Sadighara, ¹Mahdi Jahanbakhsh, ²Ahmad Nazaktabar and ³Tahereh Mohajerfar

¹Department of Environmental Health, Food Safety Division,

Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

²Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

³Academic Center of Education, Culture and Research (ACECR), Tehran, Iran

Abstract: Stevioside has become well known for its sweetness in food industries. The present study was designed to investigate the possible effects of Stevioside on oxidative stress in the brain tissue of chicken embryos. *Methods:* one hundred fertile eggs were randomly divided into four groups group (1) as control (Without injection), group (2) received 10 ppm stevioside, group (3) received 100 ppm stevioside, group (4) received 1000 ppm stevioside. Stevioside were injected at the day 4 of incubation of chickens. The experiment was terminated on day 20 of incubation. Then, the embryos were decapitated and brains were collected to evaluate oxidative stress. *Result:* The level of lipid oxidation was significantly different in high doses. Changes in The ferric reducing/antioxidant power assay (FRAP) assay were significant in groups. The changes in level of glutathione (GSH) and cupric ion reducing assay (cupric) were not observed between groups. *Conclusion:* Stevioside may induce some alterations in oxidative parameters.

Key words: Stevioside • Oxidative Stress • Brain

INTRODUCTION

Plants are natural source of producing large number of compounds with precise selectivity [1]. Since the middle of the 19th century, different classes of bioactive chemical constituents have been isolated from plants [2]. Stevioside, are extracts of the leaves of *Stevia rebaudiana* and purified. It is approximately 200 to 300 times sweeter than sucrose. Some food and beverage products currently use stevia as food additives. Brain tissue is rich in polyunsaturated fatty acids (PUFAs). These PUFAs are especially vulnerable to oxidation. Furthermore, some areas of the brain have high iron content. Iron catalyst the process of lipid peroxidation. It can both initiate and amplify lipid peroxidation [3]. The brain consumes about 20% of the oxygen utilized by the body but constitutes only 2% of the body weight. Consequently, reactive oxygen species (ROS) are continuously generated during oxidative metabolism in the brain in high rate [4]. Therefore, this organ is susceptible to oxidative damages.

The purpose of this study was to consider oxidative biomarkers on brain in chicken embryo model induced by Stevioside.

MATERIALS AND METHODS

Study Design: Stevioside with 95.9% purity, product date: Jan 2014 and expiration date: Jan 2017 was supplied by stevia industrial association.

One hundred fertile eggs were obtained from a broiler breeder farm (Ross 308 strain). All eggs with mean weight of 63±1 g were divided to four groups and received different amounts of stevioside by injection in chorioallantoic membrane.

The groups were included: 1) control group (Without injection), 2) group received 10 ppm stevioside, 3) group received 100 ppm stevioside, 4) group received 1000 ppm stevioside.

The eggs were incubated at 37.5 °C and %65 Relative Humidity. On 3rd day of incubation, eggs were candled, clear eggs and dead embryos were removed from

examination. In the 4th day of incubation, the experimental groups received stevioside into the chorioallantoic membrane with 0.2 ml of mentioned doses. To avoid contamination, all injections were carried out in a clean room and all the equipments were sterilized. The injection site was sealed with paraffin and the eggs were returned into the hatchery and kept at a temperature of 37 °C until they hatch. Experiment was terminated on day 20 of Incubation. Then the embryos were decapitated and the embryo brains were collected for biochemical examination.

Measurement of Oxidative Stress Parameters

Measurement of Lipid Peroxidation: The formation of thiobarbituric acid in organ samples was assessed for the measurement of lipid peroxidation according to an original method Sicinska *et al.* [5]. Briefly, the supernatant of the tissue homogenate was mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was measured at 532 nm. The values were expressed in nmolesmalodialdehyde, using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of Total GSH Groups Assay: The glutathione content was applied according to the previous method Gibson *et al.* [6]. The liver was rinsed three times with PBS. The supernatant of the liver homogenate mixed with 20% trichloroacetic acid. Samples were centrifuged. The supernatant was mixed with 4 vol of Tris. Then, 1mM DTNB was added to the sample and incubated for 30 minutes. The absorbance was read at 412 nm.

The Ferric Reducing/antioxidant Power (FRAP): The total antioxidant capacity was determined by the ferric reducing antioxidant power. Briefly, the stocks solutions included 300 Mm acetate buffer 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM FeCl₃.6H₂O solution were prepared. The fresh working solution (FRAP reagent) was prepared by mixing acetate fuffer, TPTZ solution and FeCl₃.6H₂O solution. The samples and deionized water were mixed with 3 mL of the FRAP reagent and allow to react for 5 min in the dark. The changes in absorbance at 593 nm are related to the total reducing power of antioxidants of tissues [7].

Determination of Cupric Ion Reducing Assay (Cupric):

The cupric ion reducing capacity assay measures the cupric reducing capacity. The samples were mixed with

solutions of CuCl₂, neocuproine reagent in ammonium acetate buffer. The resulting absorbance at 450 nm is recorded either directly after incubation at 50 degrees C for 20 min [8].

Statistical Analysis: The evaluation was made by comparing groups. The difference more than 95 % ($p=0.05$) was considered significant. The data values are presented as mean±SD

RESULTS AND DISCUSSION

The present study was designed to investigate the role of oxidative stress to assess neurotoxicity of stevia sweetener. The oxidative stress parameter includes FRAP, GSH, MDA and cupric assay after the exposure to stevioside was measured and results are shown in Table 1. Results of the present study showed that exposure to high concentration of Stevioside causes excessive levels of ROS. The level of lipid oxidation was significantly different between control with group 3 ($p=0.02$) also group 1 and group 2 ($p=0.03$), group 1 and group 3 ($p=0.006$). Changes in FRAP assay were significant in groups. The difference between control compare to group 2 ($p=0.02$) and 3 ($p=0.004$). The difference between 10 ppm compare to 100 ppm ($p=0.001$) and 1000 ($p=0.002$).

The level of GSH decrease in high doses (Table 1) but these changes were not statistically significant.

The polyunsaturated fatty acid is susceptible to free radical attack. PUFA peroxidations can be self-propagating, their high concentrations in brain make this organ especially vulnerable to free radical damage [9]. The pathway of catalytic iron can be induced by two different iron -dependent mechanisms. In the first mechanism, iron serves as a reagent for the Fenton reaction, forming HO•. HO• induce lipid peroxidation. In the second mechanism, iron forms iron-oxygen complexes, such as perferryl ion or ferrylion [3].

Presence of antioxidants has an important role on the prevention oxidative changes. Therefore, antioxidants can lower the occurrence of oxidative stress. The level of lipid peroxidation was increased. It is possible that enzymatic antioxidant is unable to protect the lipid oxidation. The brain has low antioxidant enzymes [3]. The level of lipid peroxidation suggests that stevioside could induce oxidative stress in high doses. Oxidative stress has been implicated in the progression of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis [10].

Table 1: Level of oxidative stress parameters

	Level of lipid peroxidation (nmol/ 0.5 g tissue)	GSH $\mu\text{mol}/ 0.5 \text{ g tissue}$	ferric reducing capacity mmol/0.5 g tissue	cupric assay nm
Control	0.33 $\pm$ 0.1	0.13 $\pm$ 0.09	1.9 $\pm$ 0.3	0.22 $\pm$ 0.03
Group 1(10ppm)	0.31 $\pm$ 0.1	0.13 $\pm$ 0.04	2.18 $\pm$ 0.36	0.19 $\pm$ 0.07
Group2(100 ppm)	0.41 $\pm$ 0.09	0.10 $\pm$ 0.03	1.2 $\pm$ 0.25	0.22 $\pm$ 0.04
Group3(1000ppm)	0.51 $\pm$ 0.14	0.11 $\pm$ 0.03	1.2 $\pm$ 0.29	0.22 $\pm$ 0.1

Collectively, it is concluded that some oxidative stress biomarkers of brain might be change in high doses of stevioside. Further toxicity tests should examine by different doses for finding margin of safety.

REFERENCES

1. Usman, R., A. Khan, S. Gul, A. Rauf and N. Muhammad, 2012. Preliminary Anti-Oxidant Profile of Selected Medicinal Plants of Pakistan. Middle-East Journal of Medicinal Plants Research, 1: 24-27.
2. Rauf, A., M. Qaisar, G. Uddin, S. Akhtar and N. Muhammad, 2012. Antioxidant Profile and Phytochemical Screening of *E. helioscopia*. Middle-East Journal of Medicinal Plants Research, 1: 19-23.
3. Schafer, F., S. Qian and G. Buettner, 2000. Iron and free radical oxidations in cell membranes. Cell Mol Biol, 46: 657-662.
4. Dringen, R., 2000. Metabolism and functions of glutathione in brain. Progress in Neurobiology, 62: 649-671.
5. Sicinska, P., B. Bukowska, J. Michalowicz and W. Duda, 2006. Damage of cell membrane and ant-oxidative system in human erythrocytes incubated with microcystin-LR *in vitro*. Toxicon, 47: 387-397.
6. Gibson, X.A., A. Shartava and J. McIntyre, 1998. The efficacy of reducing agents or antioxidants in blocking the formation of dense cells and irreversibly sickled cells *in vitro*. Blood, 91: 4373-4378.
7. Morales, G. and A. Paredes, 2014. Antioxidant activities of *Lampaya medicinalis* extracts and their main chemical constituents. Complementary and Alternative Medicine, 14: 259-271.
8. Apak, R., K. Güçlü, M. Özyürek and S. Celik, 2008. Mechanism of antioxidant capacity assays and the CUPRAC (Cupric ion reducing antioxidant capacity) assay. MicrochimicaActa, 160: 413-419.
9. VanRollins, M., R. Woltjer, H. Yin, J. Morrow and T. Montine, 2008. F₂-Dihomo-isoprostanes arise from free radical attack on adrenic acid. J Lipid Res., 49(5): 995-1005.
10. Kevin, J.B., C.L. Masters and A.L. Bush, 2004. Neurodegenerative diseases and oxidative stress Nature Reviews Drug Discovery, 3: 205-214.