

## Research Article

# Nutrient Compositions and Antioxidant Capacity of E'jiao Gao

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### Abstract

#### Background

Aging is a major public health issue and is considered to be associated with oxidative stress. Additionally, foods are basic for nutrients, survival and growth of organisms, and are a good source for antioxidants. E'jiao Gao (EJG, made up of E'jiao, walnut, semen sesame nigrum and rice wine) is a well-known traditional food in China, however, little nutritional and bioactive information is available. Therefore, in the present study, nutritional compositions of EJG, including protein, amino acid, vitamins, fatty acid and mineral contents, were investigated. Furthermore, 55 female aging ICR mice were used to evaluate the anti-aging activity of EJG in vivo, and their antioxidant enzyme activities, including SOD, GPx and CAT, and MDA contents were determined.

#### Results

EJG is abundant in protein, amino acids, vitamin E, essential fatty acids (linoleic acid and linolenic acid), mineral elements (calcium, potassium, magnesium,) and other biological nutrients (selenium, zinc and iron). After administrated with EJG, the activities of SOD, GPx and CAT were remarkably improved ( $P < 0.05$ ) and the content of MDA remarkably decreased ( $P < 0.05$ ).

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### Conclusion

Overall, EJG is a functional food for supplementation of protein, vitamin E, amino acid or calcium, and is a potential food for promoting the activities of endogenous antioxidant enzyme to fight against aging. Therefore, these data are essential theoretical basis for the development of the traditional food of EJG and useful for encouraging people to pay attention to the nutritional composition and bioactivities of food they consume.

**Keywords:** Anti-ageing; Antioxidant; E'jiao Gao (EJG); Nutritional value

### Introduction

Aging is a major public health issue, and the elderly population will be expected to grow from 17.4% to nearly 30% worldwide by 2060 [1]. In the process of aging, the risk of many chronic diseases, such as cancer, Parkinson's and cardiovascular diseases increase [2]. Numerous studies indicate that nutrients, food components and whole diets can influence cognitive and ageing [1]. Nutrients are basic for survival and growth of organisms. However, people usually consume foods that are available or palatable. In addition, with the development of food processing technology, nutritional composition of food can be largely affected by the processing. Therefore, it is urgent to produce foods that are palatable, nutritional and capable to fight against aging process.

Colla corii asini (E'jiao), a solid glue prepared from donkey (*Equus asinus*) skin, has been considered as a health-care food and used as a traditional Chinese medicine in life-nourishing and clinical antianemic therapy for more than 2000 years [3]. E'jiao is mainly composed of amino acids, low molecular weight hydrolyzates of collagen proteins, polysaccharides, volatile substances and inorganic substances [4]. It has attracted great attention due to its biological activities in hematopoiesis, anti-inflammatory, antitumor, anti-ageing, improving immunity, etc [5-8].

E'jiao Gao (EJG), one of the popular edible forms of E'jiao, is made up of E'jiao, walnut, semen sesame nigrum and rice wine. The walnut is a high nutritional food containing high contents of protein, essential fatty acids, polyphenols and to copherols, which has been reported to reduce risk of cancer, coronary heart disease and to improve antioxidant as well as antiproliferative activity [9,10]. Semen sesame nigrum not only benefits in nourishing the liver and anti-ageing, but also in anti-wrinkling, keeping the balance of nerves and beautifying [11]. Rice wine is popular in China and is not only for drinking but also for medical use in traditional Chinese medicine all over the country. Rice wine has been claimed to have physiological functions in preventing aging, cancer and cardiovascular diseases [12].

Free radicals are one of the reasons that associated with aging. In organisms, there are two antioxidant defense systems, antioxidant enzymes and non-enzymatic antioxidant compounds, preventing organisms from damages of free radicals [2]. However, oxidative stress occurs when antioxidant defense systems is too weak to scavenge the

excessive free radicals, which is harmful for organisms. It has been reported to suppress the aging process through enhancing antioxidant activity, scavenging free radicals, and modulating expression of aging-related gene [5].

Therefore, the aim of the present study is to investigate the nutritional composition of EJG and evaluate the effects of EJG on antioxidant enzymes in aged mice

## Materials and Methods

### Materials and chemicals

EJG was provided by Shandong Dong-E-E-Jiao Co., Ltd (Dong'e, China). The measurement kits of Total Superoxide Dismutase (T-SOD), Glutathione Peroxidase (GPx), Catalase (CAT), Malondialdehyde (MDA) and protein quantization were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The other solvents and chemicals used in the experiments were of analytical or High-Performance Liquid Chromatography (HPLC) grade.

### Determination of main nutrients

Moisture, proteins, fat and ash in the samples were analyzed according to the recommended methods of AOAC [13]. Moisture content was determined using a direct drying method (the fresh sample was dried in an oven at 105°C until constant weight. Crude protein and crude fat contents were determined using a Kjeldahl and Soxhlet methods, respectively [13]. Ash was determined by incineration at 600 ±15°C in a Muffle furnace for 48h [13].

### Determination of detailed composition

**Analysis of fatty acids analysis:** Fatty acids were analyzed according to [14,15]. The oil obtained by soxhlet extraction was saponified with 8mL of 2% NaOH at 80°C until the oil droplets disappeared and methylated with 7mL of 15% boron trifluoride methanol solution at 80°C for 2min. After cooling, the n-heptane and saturated sodium chloride solution were added to obtain phase separation. The analysis was performed by a gas chromatographer (Agilent 7890A, Agilent Technologies Co. Ltd., USA) equipped with a flame ionization detector and a column (60m×0.25mm i.d.×0.25µm<sub>d<sub>i</sub></sub>) after the upper phase was recovered and filtered with 0.2µm Nylon filter from Milipore in a vial. The column temperature program was set as follows: 130°C(1min); to 170°C at 6.5°C/min; to 215°C(12min) at 2.75°C/min; to 230°C(3min) at 4°C/min. 1.0µL of sample was injected and fatty acids were identified by comparing the relative retention times with standards.

**Analysis of amino acids:** Amino acids were determined according to [16]. The sample was hydrolyzed by 6M HCl at 110°C for 22h under nitrogen gas. The hydrolyzed mixture was filtered to remove residue and the filtrate was dried under vacuum. Before being analyzed in amino acid analyzer, the dried filtrate was redissolved in sodium citrate buffer (pH 2.2) and filtered with 0.2µm millipore filter.

**Analysis of elements:** Mineral analysis, including heavy metal elements (Mn), macro-elements (K, Ca, Mg) and trace elements (Fe, Zn, Se, F, I), was determined according to the Chinese National Standards (CNS). The sample was ashed at 550°C for 4 to 5h using a muffle furnace. After cooling, the sample was digested with HNO<sub>3</sub> and then diluted with deionized water to 25mL. A blank was treated in the same way. The concentration was determined by Flame Atomic Absorption

Spectrometry (FAAS) on the ICE 3500 Thermo Scientific or Inductively Coupled Plasma-Mass Spectrometry (ICP MS) on the ICP-MS X2 Thermo Scientific.

**Analysis of vitamins:** The contents of vitamin C and vitamin E were determined using a liquid chromatography method with an Agilent 1100 Auto Analyzer [17].

### Analysis of antioxidant activity

**Animals and treatments:** In the present experiments, 10 adult female ICR mice (20~23g, 40~42 days old) and 55 aged female ICR mice (35~45g, 8months old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animals were housed in barrier lab in Institute of Basic Research, Zhejiang Academy of Traditional Chinese Medicine, 25±2°C, 50%-60% humidity and 12h daylight cycle with water and food available. After a week of adjustable feeding, the aged mice were divided into 5 groups (n=11 for each group) and the adult mice were regarded as the young control group. The details were as follows:

High-dose EJG group (HE): administered EJG (4.29gkg<sup>-1</sup>b.w., i.g) for 60 days

Medium-dose group (ME): administered EJG (1.43gkg<sup>-1</sup>b.w., i.g) for 60 days

Low-dose EJG group (LE): administered EJG (0.71gkg<sup>-1</sup>b.w., i.g) for 60 days

Vitamin E control group (VE): administered VE (72mgkg<sup>-1</sup>b.w., i.g) for 60 days

Model control group (MC): administered normal saline (20mlkg<sup>-1</sup>b.w., i.g) for 60 days

Young control group (YC): administered normal saline (20mlkg<sup>-1</sup>b.w., i.g) for 60 days

**Enzymatic endogenous antioxidant system:** During the experiment, the mice were weighed once a week and sacrificed after treatment for 60days. The serum was obtained from the collected blood after centrifugation using eye enucleation method. The liver and brain tissue were collected immediately, weighed and homogenized at 4°C with normal saline to the concentration of 10% for biochemical assay. MDA is an end-product of lipid peroxidation during oxidative stress and is frequently used as an indicator of lipid peroxidation. In addition, the contents of SOD, CAT and GPx largely represent the antioxidant capacity. Therefore, the contents of MDA and the activities of SOD, CAT and GPx in the serum, liver and brain tissues were measured using the commercial kits (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China).

### Statistical analysis

The results were expressed as mean values±Standard Deviations (SD). Statistical analysis was performed by one-way Analysis of Variance (ANOVA) using SPSS.18.0 software. A P<0.05 was indicative of significant difference.

## Results

### Chemical composition

Proximate compositions of the studied EJG were presented in table 1. The contents of ash, moisture and protein in EJG were

determined. Moisture is one of the most important components of food, affecting physical, textural characteristics and self stability of food products. Lower moisture content of food products means higher nutrients and typically has longer shelf life [18]. The moisture content in EJG was found to be 11.3g/100g.

The content of ash (3.4g/100g) in EJG was higher than many fruits, such as pineapple (2.38g/100g DW), breadfruit (2.8-3.3g/100g DW), peach palm (1.2-2.2g/100g DW) and cashew fruit (2.66 g/100g DW) [19].

The content of protein in EJG was 20.90%, and was mainly composed of 20 amino acids, including 8 essential amino acids, 10 non-essential amino acids and 2 conditionally essential amino acids. 10g EJG contributes to more than 4% of the RDI. The ratio of essential amino acids to non-essential amino acids was 0.44, a little bit lower than the reference value of 0.6 recommended by the Food and Agriculture Organization (FAO) [21]. Lysine is the first limiting amino acid in EJG when compared to the RDI. However, EJG is a relatively good source of cysteine, which has the potent antioxidant activity against linoleate oxidation and superoxide due to its higher reactivity of the thiol toward the radical species [22]. In addition, EJG is abundant in glutamic, glycine and arginine, contenting 2287mg/100g, 2307mg/100g and 1.33mg/100g, respectively.

Vitamin E content of the EJG was 6.525mg/100g, and Vitamin C was 1.100mg/100g. Per 10g of the EJG represents 4.35% of RDI in vitamin E. Vitamin E is a lipophilic antioxidant that can increase the proliferation and survival of fibroblasts and elevate the production of collagen, which finally can protect skin from UVB-Irradiation [23].

Linolenic acid and linoleic acid are two essential fatty acids for human beings. In our study, the contents of linolenic acid and linoleic acid were 1.35g/100g and 10.42g/100g, respectively. Lack of essential fatty acids would cause slow growth as well as dermatitis, and supplementing linoleic acid and linolenic acid can prevent these symptoms [24]. What's more, linoleic acid and linolenic acid have certain effects on the prevention of cardiovascular diseases, hypertension and rheumatoid arthritis, asthma and allergy [25].

It implicated that supplementation of EJG could be good for promoting health. Nine kinds of minerals of in EJG were analyzed in the present study. The results showed that calcium was the most abundant (454.09mg/100g) among these minerals. In terms of RDI per 10g, the EJG presents 34.93%. Ca, K and Mg are essential for the energy metabolism, bone development and protein synthesis in humans [26]. She was also found in EJG (0.013mg/100g), which was an essential nutrient element in humans due to its possible roles in oxidative damage, chronic diseases and inflammatory [27].

### Weight gain and organ index

The effects of EJG on the weight gain and organ indexes were shown in table 2 and figure 1. After administrated with EJG, the weight gain between groups EJG (HE, ME and LE) and group MC showed insignificant difference ( $P>0.05$ ); liver indexes in the group ME and group VE were significantly higher than that of group MC, while insignificant difference was found in the brain ( $P>0.05$ ).

Constituents	Per 100g	RDI	%CS
Moisture (g)	9.47		
Lipids (g)	29.00	78 <sup>a</sup>	3.72
Protein (g)	20.90	50 <sup>a</sup>	4.18
Ash (g)	3.40		
Total dietary fiber (g)	2.96	28 <sup>a</sup>	1.06
Linoleic acid (g)	10.42		
Linolenic acid (g)	1.35		
<b>Amino acids (mg)</b>	<b>Per 100g</b>	<b>RDI</b>	<b>%CS</b>
Essential			
Isoleucine	387	1400 <sup>b</sup>	2.76
Lysine	484	2100 <sup>b</sup>	2.30
Methionine	196	700 <sup>b</sup>	2.80
Leucine	778	2730 <sup>b</sup>	2.85
Phenylalanine	789	1750 <sup>b</sup>	4.51
Tryptophan	71	280 <sup>b</sup>	2.54
Threonine	464	1050 <sup>b</sup>	4.42
Valine	546	1820 <sup>b</sup>	3.00
Non-essential			
Arginine	1330		
Aspartic acid	1109		
Alanine	1.157		
Cysteine	146	280 <sup>b</sup>	5.21
Glycine	2307		
Glutamic acid	2287		
Histidine	274	700 <sup>b</sup>	3.91
Proline	1474		
Serine	676		
Tyrosine	394		
<b>Vitamins (mg)</b>	<b>Per 100g</b>	<b>RDI</b>	<b>%CS</b>
Vitamin E	6.525	15 <sup>a</sup>	4.35
Vitamin C	1.100	90 <sup>a</sup>	0.12
<b>Minerals (mg)</b>	<b>Per 100g</b>	<b>RDI</b>	<b>%CS</b>
Ca	454.09	1300 <sup>a</sup>	3.49
P	295.00	1250 <sup>a</sup>	2.36
K	283.00		
Mg	157.95	420 <sup>a</sup>	3.76
Mn	1.59	2.3 <sup>a</sup>	6.91
Zn	2.05	11 <sup>a</sup>	1.86
Fe	3.24	18 <sup>a</sup>	1.80
Se	0.013	0.055 <sup>a</sup>	2.36
I	0.004	0.150 <sup>a</sup>	0.27

**Table 1:** Chemical composition of E'jiao Gao (EJG). The contribution of per 10g to the Recommended Daily Intake (RDI) is Expressed in Percentage (%CS).

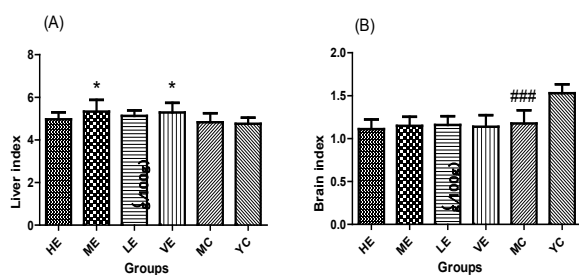
**Note:** <sup>a</sup>Dietary reference intakes for fat, fiber, protein, vitamin C, vitamin E, calcium, phosphorus, magnesium, manganese, zinc, iron, selenium and iodine. Considering a body weight of 70Kg.

<sup>b</sup>WHO/FAO/UNU Report (2007). Considering a body weight of 70Kg [20].

Groups	n	0 days	30 days	60 days
HE	11	42.8±3.17	41.3±2.75	43.1±4.17
ME	11	42.6±1.90	41.6±3.06	42.2±3.68
LE	11	42.6±1.80	41.5±2.48	41.7±3.38
VE	11	41.7±3.35	44.2±4.02	43.9±4.66
MC	11	41.1±4.07***	43.0±4.77***	43.3±4.87***
YC	10	25.8±0.66	27.2±1.28	29.7±0.88

**Table 2:** Effects of E'jiao Gao (EJG) on the weight gain of experimental mice.

**Note:** \*\*\*compare to group YC, P<0.001. Each value was expressed as mean±SD. HE: 4.29gkg<sup>-1</sup>b.w., E'jiao Gao; ME: 1.43gkg<sup>-1</sup>b.w., E'jiao Gao; LE: 0.71gkg<sup>-1</sup>b.w., E'jiao Gao; VE: 0.72mgkg<sup>-1</sup>b.w., Vitamin E; MC: 20mLkg<sup>-1</sup>b.w., normal saline; YC: 20mLkg<sup>-1</sup>b.w., normal saline.

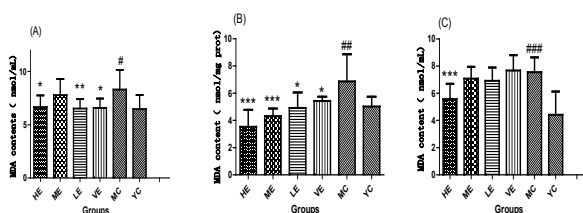


**Figure 1:** Liver index (A) and Brain index (B) of all groups.

**Note:** \* Compared to group MC, P<0.05, P<0.01 or P<0.001; # compared to group YC, P<0.05, P<0.01 or P<0.001. Each value was expressed as mean±SD. HE: 4.29gkg<sup>-1</sup>b.w., E'jiao Gao; ME: 1.43gkg<sup>-1</sup>b.w., E'jiao Gao; LE: 0.71gkg<sup>-1</sup>b.w., E'jiao Gao; VE: 0.72mgkg<sup>-1</sup>b.w., Vitamin E; MC: 20mLkg<sup>-1</sup>b.w., normal saline; YC: 20mLkg<sup>-1</sup>b.w., normal saline.

### Effect of EJG on the MDA content in aged mice

As shown in figure 2, the MDA contents of the group MC in the serum, liver and brain were much higher than that of the group YC (P<0.05, P<0.01 or P<0.001). In liver, the MDA content remarkably decreased after being supplemented with EJG, compared to the group MC (P<0.05 or P<0.01). Same results occurred in the serum, except that unremarkable was found in the group ME. However, as for brain, a statistically significant difference was only observed in the group HE (P<0.001). In addition, group VE had insignificant difference to EJG groups.

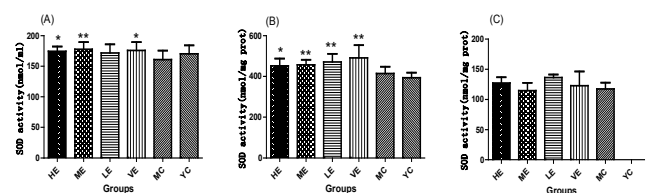


**Figure 2:** MDA contents of all groups in Serum (A), Liver (B) and Brain (C).

**Note:** \* Compared to group MC, P<0.05, P<0.01 or P<0.001; # compared to group YC, P<0.05, P<0.01 or P<0.001. Each value was expressed as mean±SD. HE: 4.29gkg<sup>-1</sup>b.w., E'jiao Gao; ME: 1.43gkg<sup>-1</sup>b.w., E'jiao Gao; LE: 0.71gkg<sup>-1</sup>b.w., E'jiao Gao; VE: 0.72mgkg<sup>-1</sup>b.w., Vitamin E; MC: 20mLkg<sup>-1</sup>b.w., normal saline; YC: 20mLkg<sup>-1</sup>b.w., normal saline.

### Effect of EJG on SOD activity in aged mice

As shown in figure 3, the SOD activity of group MC was lower than that in the group YC, but no statistically significant difference was found (P>0.05). The SOD activities in both groups EJG (group HE, ME and LE) and group VE were remarkably increased in the serum and liver, compared to the group MC (P<0.05 or P<0.01). However, in brain neither EJG groups nor group VE could significantly improve SOD activities. The effects of EJG and VE on SOD activity were insignificantly different in serum, liver or brain.

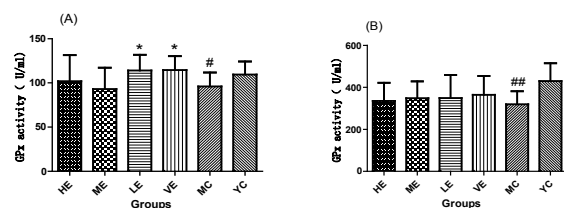


**Figure 3:** SOD activities of all groups in Serum (A), Liver (B) and Brain (C).

**Note:** \* Compared to group MC, P<0.05, P<0.01 or P<0.001; # compared to group YC, P<0.05, P<0.01 or P<0.001. Each value was expressed as mean±SD. HE: 4.29gkg<sup>-1</sup>b.w., E'jiao Gao; ME: 1.43gkg<sup>-1</sup>b.w., E'jiao Gao; LE: 0.71gkg<sup>-1</sup>b.w., E'jiao Gao; VE: 0.72mgkg<sup>-1</sup>b.w., Vitamin E; MC: 20mLkg<sup>-1</sup>b.w., normal saline; YC: 20mLkg<sup>-1</sup>b.w., normal saline.

### Effect of EJG on GPx in aged mice

As shown in figure 4, a remarkable decrease in group MC was found when compared to group YC (P<0.05 or P<0.01). In serum, EJG and VE supplementation can improve the GPx activity, except group ME. As for liver, neither EJG groups nor group VE found significant difference.



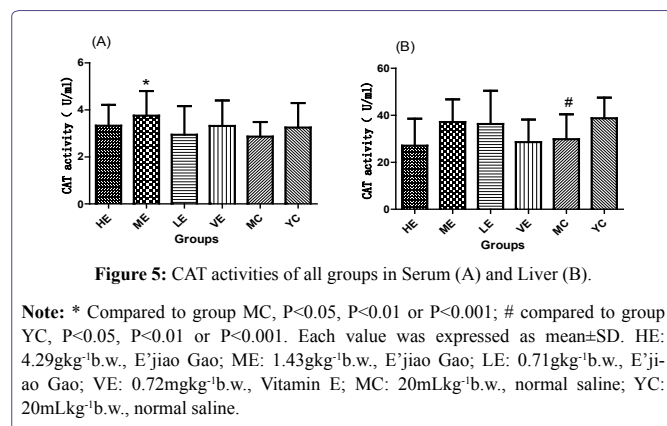
**Figure 4:** GPx activities of all groups in Serum (A) and Liver (B).

**Note:** \* Compared to group MC, P<0.05, P<0.01 or P<0.001; # compared to group YC, P<0.05, P<0.01 or P<0.001. Each value was expressed as mean±SD. HE: 4.29gkg<sup>-1</sup>b.w., E'jiao Gao; ME: 1.43gkg<sup>-1</sup>b.w., E'jiao Gao; LE: 0.71gkg<sup>-1</sup>b.w., E'jiao Gao; VE: 0.72mgkg<sup>-1</sup>b.w., Vitamin E; MC: 20mLkg<sup>-1</sup>b.w., normal saline; YC: 20mLkg<sup>-1</sup>b.w., normal saline.

### Effect of EJG on CAT activity in aged mice

As shown in figure 5, the CAT activity in group MC was remarkably lower than the group YC in the liver (P<0.01) but not in the serum (P>0.05). Administrated with EJG can significantly improve the CAT activity in the serum, compared to group MC (P<0.05). As for liver, the same situation was not found in EJG groups or in group VE (P>0.05).





## Discussions

Nutrients are basic for survival and growth of organisms. There are two kinds of nutrients: macro-nutrients and micro-nutrients. Macro-nutrients, including carbohydrates, fiber, fats, protein and water [28], is an energy provider for metabolism of an organism, while micro-nutrients, including at least iron, vitamins, cobalt, copper, manganese, chromium, iodine, selenium, zinc, and molybdenum take part in building and repairing of tissue and regulating body processes. Organisms obtain nutrients from environments and their growth and health conditions are largely influenced by what they eat. A poor diet, especially a Western pattern diet, is associated with many diseases, such as obesity, metabolic syndrome, cardiovascular disease, diabetes and even deficiency disease of blindness [29-32].

Food intake is greatly influenced by availability, the processing and palatability of food. However, with the development of food processing technology, the nutritional components in food can be largely affected by the processing. Nowadays, nutritional value becomes an important factor in people's choice of food. E'jiao is a snack that is mixed with walnut, semen sesame nigrum and rice wine, which is delicious and nutritional and is popular with women in China.

Collagen is the most abundant protein in mammals [33], which can be found in many fibrous tissues, and is believed to be the main active component in E'jiao. It is widely used to nourish the blood. A previous study isolated two collagen-derived peptides from E'jiao and confirmed exhibition of strong hematopoietic activity by elevated CFU-E (colony-forming units-erythroid) and CFU-GM (colony-forming units granulocyte-monocyte) of mouse bone marrow cells [6]. Collagen is important for women, which is not only because of its ability to treat gynecologic diseases but also its importance in delaying natural ageing process by tightening and smoothing the skin internally [34]. Nowadays, many people use the cosmetic products of collagen, such as creams and lotions, to keep skin health. However, these products can only treat some lines and smaller wrinkles on the surface, and to do some substantial effect was considered to take oral applications [35]. From nutrients analysis, protein was a dominated component in E'jiao and it was mainly from E'jiao. It is known that E'jiao contains about 80% collagen; therefore E'jiao can be a good supplementation of collagen to fight against aging.

E'jiao contains many essential or non-essential amino acids that have various biological functions. For example, glycine has been considered an important inhibitory neurotransmitter in brain stem and

medulla [36]. Hydroxyproline is an important component of the synthesis of the connective tissue collagen. The deficiency of L-arginine is frequently associated with aging, and L-Arginine supplementation can improve endothelial function and decreased systolic blood pressure by normalizing plasma L-arginine and L-arginine/L-ornithine ratio [37].

Recently, many studies suggest that vitamins, minerals and botanicals are natural ingredients to improve antioxidant activities and anti-ageing. In the present study, Vitamin E was extremely abundant in E'jiao, which suggested that E'jiao was a potential antioxidant. Vitamin E is considered to be a good peroxy radical scavenger and has been developed as a commercial antioxidant, protecting tissues from damage of free radicals, protecting lipids and preventing the oxidation of polyunsaturated fatty acids [30]. In addition, many macro-elements and trace elements of mineral that are beneficial for humans have been found in E'jiao. Among these detected mineral elements, selenium is an essential nutrient for its important antioxidant functions and neuroprotective actions [38]. Human usually obtain natural selenium from dietary, such as meat, eggs and vegetables [39]. E'jiao contains about 0.013mg/100g selenium, which would be another choice for humans.

Reactive oxygen species or free radicals, including superoxide anion, hydroxyl radical, nitric oxide and hydrogen peroxide, are unstable and reactive. In general, they play an important role in signal transduction, leukocyte adhesion, gene transcription and many other critical cellular reactions. However, though the excess of free radicals can be detoxified by cell-produced antioxidants, such as catalases, peroxidases and superoxide dismutase, the oxidative stress will happen with aging or other pathological conditions. Oxidative stress can damage many critical molecules, increase DNA mutations and cause cell death [40]. Supplementation with antioxidants can decrease cellular damage from excess reactive oxygen species.

After administrated with E'jiao, the activities of endogenous antioxidant enzymes in aging mice (SOD, GPx, and CAT) were remarkably improved and the content of MDA was decreased, when compared to the model control group. SOD is the first line of antioxidant-defense systems, the best characterized, to catalyze the dismutation of O<sub>2</sub><sup>-</sup> into non-toxic of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> [41]. GPx protects the organism from oxidative damage mainly by reducing lipid hydroperoxides [42]. CAT is also a very important enzyme that catalyzes the hydrogen peroxide into less-reactive gaseous oxygen and water molecules [43]. Therefore, it is essential for the organisms to keep the activity of endogenous antioxidant enzymes. The capability of E'jiao in improving SOD, GPx and CAT activities in aged mice, may be due to the containment of some potential antioxidant nutrients. For example, zinc and manganese are critical cofactors for superoxide dismutase; vitamin E and other vitamins reduce reactive oxygen species [44].

In conclusion, E'jiao is a nutritious snack food, which is abundant in protein, essential amino acids, vitamins, essential fatty acids and minerals. *In vivo* experiments, E'jiao exhibited a good ability to improve the activities of SOD, GPx and CAT in aged mice. In addition, it decreased the content of MDA in aged mice. It indicated that E'jiao was a healthy food, containing potential anti-ageing capacity, for consumption.

## Conflict of Interest

Authors have no conflict of interests.

## Reference

- Vauzour D, Camprubi-Robles M, Miquel-Kergoat S, Andres-Lacueva C, Bánáti D, et al. (2017) Nutrition for the ageing brain: Towards evidence for an optimal diet. *Ageing Res Rev* 35: 222-240.
- Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4: 118-126.
- Tian JS, Zhang X, Liu H, Xiang H, Xing J, et al. (2017) The hematinic effect of Colla corii asini (Ejiao) using 1H-NMR metabolomics coupled with correlation analysis in APH-induced anemic rats. *RSC Advances* 7: 8952-8962.
- Wang D, Ru W, Xu Y, Zhang J, He X, et al. (2014) Chemical constituents and bioactivities of Colla corii asini. *Drug Discov Ther* 8: 201-207.
- Wang D, Liu M, Cao J, Cheng Y, Zhuo C, et al. (2012) Effect of Colla corii asini (E'jiao) on D-galactose induced aging mice. *Biol Pharm Bull* 35: 2128-2132.
- Wu H, Ren C, Yang F, Qin Y, Zhang Y, et al. (2016) Extraction and identification of collagen-derived peptides with hematopoietic activity from Colla Corii Asini. *J Ethnopharmacol* 182: 129-136.
- Song YM, Mao GN, Kang RR, et al. (2011) Effect of Colla corri asini effervescent granules on immune function in mice. *Progress in Veterinary Medicine*.
- Fu-dong Z, Jing-cheng D, Yan C, Jin-yu X, Shan-mei W (2006) The effect of Ejiao on airway inflammation and Th1/Th2 cytokines in serum of asthmatic rats. *Chinese Journal of Experimental Traditional Medical Formulae*.
- Homan M, Fereidoon S (2008) Lipid class compositions, tocopherols and sterols of tree nut oils extracted with different solvents. *Journal of Food Lipids* 15: 81-96.
- Anjum S, Gani A, Ahmad M, Shah A, Masoodi FA, et al (2016) Antioxidant and antiproliferative activity of walnut extract (*Juglans regia L.*) processed by different methods and identification of compounds using GC/MS and LC/MS technique. *Journal of Food Processing and Preservation* 41: 12756.
- Wang BS (1998) Black sesame and beauty. *Chinese cosmetics*.
- Que F, Mao L, Pan X (2006) Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. *Food Research International* 39: 581-587.
- Association of Official Analytical Chemists (1990) Official Methods of Analysis. Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement, Volume 15. Official Methods of Analysis, Rockville, USA.
- Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 226: 497-509.
- Metcalfe LD, Schmitz AA, Pelka JR (1996) Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* 38: 514-515.
- Ishida Y, Fujita T, Asai K (1981) New detection and separation method for amino acids by high-performance liquid chromatography. *J Chromatogr* 204: 143-148.
- Chatzimichalakis PF, Samanidou VF, Papadoyannis IN (2004) Development of a validated liquid chromatography method for the simultaneous determination of eight fat-soluble vitamins in biological fluids after solid-phase extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 805: 289-296.
- Ergun R, Lietha R, Hartel RW (2010) Moisture and shelf life in sugar confections. *Crit Rev Food Sci Nutr* 50: 162-192.
- Leterme P, Buldgen A, Estrada F, Londoño A (2006) Mineral content of tropical fruits and unconventional foods of the Andes and the rain forest of Colombia. *Food Chemistry* 95: 644-652.
- World Health Organization (2007) Protein and amino acid requirements in human nutrition: Report of a joint FAO/WHO/UNU expert consultation. World Health Organization, Geneva, Switzerland.
- Liu Y, Chen D, You Y, Zeng S, Li Y, et al. (2016) Nutritional composition of boletus mushrooms from Southwest China and their antihyperglycemic and antioxidant activities. *Food Chem* 211: 83-91.
- Miura Y, Honda S, Masuda A, Masuda T (2014) Antioxidant activities of cysteine derivatives against lipid oxidation in anhydrous media. *Bioscience Biotechnology and Biochemistry* 78: 1452-1455.
- Kim WS, Kim I, Kim WK, Choi JY, Kim DY, et al. (2016) Mitochondria-targeted vitamin E protects skin from UVB-irradiation. *Biomol Ther (Seoul)* 24: 305-311.
- Burr GO, Burr MM (1930) On the nature and rôle of the fatty acids essential in nutrition. *J Biol Chem* 86: 587-621.
- Weaver B J, Holob B J (1988) Health effects and metabolism of dietary eicosapentaenoic acid. *Prog Food Nutr Sci* 12: 111-150.
- Lai TN, André C, Rogez H, Mignolet E, Nguyen TB, et al. (2015) Nutritional composition and antioxidant properties of the sim fruit (*Rhodomyrtus tomentosa*). *Food Chem* 168: 410-416.
- Yamashita Y, Yabu T, Yamashita M (2010) Discovery of the strong antioxidant selenoneine in tuna and selenium redox metabolism. *World J Biol Chem* 1: 144-150.
- Fuhrman J (2014) *The End of Dieting: How to Live for Life*. HarperCollins, New York, USA.
- Esmailzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, et al. (2007) Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. *Am J Clin Nutr* 85: 910-918.
- Whitney EN, Rolfes SR (2005) *Understanding Nutrition*. Thomson/Wadsworth, California, USA.
- Naja F, Hwalla N, Itani L, Karam S, Sibai AM, et al. (2015) A Western dietary pattern is associated with overweight and obesity in a national sample of Lebanese adolescents (13-19 years): A cross-sectional study. *Br J Nutr* 114: 1909-1919.
- Medina-Remón A, Kirwan R, Lamuela-Raventós RM, Estruch R (2018) Dietary patterns and the risk of obesity, type 2 diabetes mellitus, cardiovascular diseases, asthma, and mental health problems. *Crit Rev Food Sci Nutr* 58: 262-296.
- Di Lullo GA, Sweeney SM, Korkko J, Ala-Kokko L, San Antonio JD (2002) Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. *J Biol Chem* 277: 4223-4231.
- Li Y, He H, Yang L, Li X, Li D, et al. (2016) Therapeutic effect of Colla corii asini on improving anemia and hemoglobin compositions in pregnant women with thalassemia. *Int J Hematol* 104: 559-565.
- Collagen peptides: Beauty from within.
- Hernandes MS, Troncone LR (2009) Glycine as a neurotransmitter in the forebrain: A short review. *J Neural Transm (Vienna)* 116: 1551-1560.
- Moretto J, Guglielmetti AS, Tournier-Nappey M, Martin H, Prigent-Tessier A, et al. (2017) Effects of a chronic l-arginine supplementation on the arginase pathway in aged rats. *Exp Gerontol* 90: 52-60.

38. Ellwanger JH, Franke SI, Bordin DL, Prá D, Henriques JA (2016) Biological functions of selenium and its potential influence on Parkinson's disease. *An Acad Bras Cienc* 88: 1655-1674.
39. Lemire M, Fillion M, Barbosa F, Guimarães JR, Mergler D (2010) Elevated levels of selenium in the typical diet of Amazonian riverside populations. *Sci Total Environ* 408: 4076-4084.
40. Buehler BA (2012) The free radical theory of aging and antioxidant supplements: A systematic review. *Journal of Evidence-Based Integrative Medicine* 17: 218-220.
41. den Hartog GJ, Haenen GR, Vegt E, van der Vijgh WJ, Bast A (2003) Superoxide dismutase: The balance between prevention and induction of oxidative damage. *Chem Biol Interact* 145: 33-39.
42. Dulcy CP, Singh HK, Preethi J, Rajan KE (2012) Standardized extract of *Bacopa monniera* (BESEB CDRI-08) attenuates contextual associative learning deficits in the aging rat's brain induced by D-galactose. *J Neurosci Res* 90: 2053-2064.
43. Gaetani GF, Ferraris AM, Rolfó M, Mangerini R, Arena S, et al. (1996) Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood* 87: 1595-1599.
44. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. *Nutrition* 18: 872-879.



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