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## Original Article

# Efficacy of protein rich pearl powder on antioxidant status in a randomized placebo-controlled trial

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## ABSTRACT

Pearl is one of the well-known traditional Chinese medicine (TCM) prescribed for treating various skin and bone related disorders due to its abundant proteins and mineral contents. The present investigation focused on antioxidation and life span prolonging effects from different extracts of pearl powder. During *in vitro* studies, various oxidative indices were evaluated, along with lifespan-prolonging effect were checked using wild-type *Caenorhabditis elegans*. For the clinical trial, 20 healthy middle-aged subjects were recruited and separated into 2 groups as experimental and placebo group, who received 3 g of pearl powder/d ( $n = 10$ ) and 3 g of placebo/d ( $n = 10$ ) for 8 weeks, respectively. During the initial, 2nd, 4th, 6th, 8th and 10th weeks the blood samples were collected for biochemical analysis. The protein extract of pearl powder recorded maximum ( $p < 0.05$ ) antioxidant activity (20–68%) as well as efficiently prolonged the life span of *C. elegans* by 18.87%. Pearl powder supplemented subjects showed a substantial increase ( $p < 0.05$ ) in total antioxidant capacity from 0.45 to 0.69 mM, total thiols from 0.23 to 0.29 mM, Glutathione content from 5.89 to 9.19  $\mu\text{M}$ , enzymic antioxidant activity (SOD-1248 to 1308; Gpx-30 to 32; GR-2.4 to 2.9) as well as considerably suppressed the lipid peroxidation products from 4.95 to 3.27  $\mu\text{M}$ . The outcome of both *in-vitro* and *in-vivo* antioxidant activity inferred that protein extract of pearl powder was a potent antioxidant and thereby prolonged the lifespan of *C. elegans*. Hence, pearl powder could be recommended for treating various age-related degenerative disorders.

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## 1. Introduction

The ocean contains an array of bioactive molecules that are actively being researched for their health benefits, one such aquatic material is a pearl. The formation of natural pearl are by the accidental lodging of sand or parasite (irritant) into the body of oyster and layered by nacre to form a pearl [1]. It is made up of various carbonates in minute crystalline form, which has been deposited in concentric layers [2]. Pearl and its grounded pure pearl powder are commonly used in traditional Chinese medicine (TCM) for beauty treatment and various ailments [3]. Moreover, in Taiwan, Pregnant women are highly recommended to intake pearl powder for improving fetal skin beauty [4]. Therefore, numerous pharmaceutical companies (Dragon Herbs, Jing Herbs, Vita Fede) are started to produce various pearl related products especially as a dietary supplement or nutraceuticals to improve health status and for beauty purpose. Pearl contains mainly calcium carbonate and magnesium carbonate, which accounted for 91%, followed by silica, calcium phosphate, aluminum oxide and ferric oxide as well as some trace elements such as sodium, magnesium, manganese, selenium, aluminum, and copper. It also contains essential amino acids such as histidine (His), lysine (Lys), arginine (Arg), valine (Val), threonine (Thr), proline (Pro), methionine (Met), leucine (Leu), phenylalanine (Phe), tryptophan (Trp) and non-essential amino acids such as aspartic acid (Asp), glycine (Gly), alanine (Ala), glutamic acid (Glu), tyrosine (Try), and serine (Ser) [5,6].

Proteins, peptides, and amino acids also contribute to the body antioxidant defense system [7]. Saiga and his coworkers [8] reported that Asp and Glu possess antioxidant properties. In addition, Val, Ile, Leu, Met, Phe, Trp, and Cys belonging to hydrophobic amino acids were also reported to exhibit better free radical quenching ability [9]. Furthermore, the presence of trace elements (calcium, magnesium, selenium) in pearl powder also favors antioxidant activity, by acting as cofactors to antioxidant enzymes [10].

Free radicals can induce lipid peroxidation by causing irreversible damage to cellular macromolecules including membrane lipids, proteins, and nucleic acids [7]. The excessive production of ROS (reactive oxygen species) is an indicator of oxidative stress (imbalance between oxidant and antioxidant) leading to cellular damage and accelerate aging process. The oxidative stress and continuous ROS production also indirectly contribute to the pathophysiology of diabetes, inflammation, neurological disorders, and obesity [11]. Natural antioxidants in the daily diet can bind to unstable free radicals and thereby render a protection from the various degenerative diseases [12,13]. Several animal experiments showed that pearl could exert numerous pharmacological properties like antioxidation, anti-aging, anti-osteoporosis as well as immunomodulatory and wound-healing activities [3,14]. However, the antioxidant effect of natural pearl powder in clinical trials as well as its anti-aging property (*C. elegans* model) has not been evaluated. Hence, the current novel experiment was blueprinted to explore the antioxidation and life span prolonging efficacy in various extracts of pearl powder and protein extract in healthy human subjects and wild type *C. elegans* respectively.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Folin-Ciocalteu phenol reagent, gallic acid, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), sodium hydroxide (NaOH), sodium nitrite ( $\text{NaNO}_2$ ), hydrochloric acid (HCl), ascorbic acid,  $\alpha$ -tocopherol, 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), ferrous chloride ( $\text{FeCl}_3$ ), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), horseradish peroxidase (HRP), potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ), trichloroacetic acid (TCA) were procured from Sigma (St. Louis, MO). Deionized water (dd  $\text{H}_2\text{O}$ ) was prepared using an Ultrapure™ water purification system (Lotun Science Co., Ltd. Taipei, Taiwan).

### 2.2. Pearl powder capsule

Pearl powder (from naturally aqua-cultured pearl) capsule was provided by Jing Wang Nano Technology Corporation, Taiwan. Each capsule (500 mg) contain 2.27% protein, 37% calcium, 0.01% iron, 0.04% zinc and various trace elements with amino acids such as Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Leu, Tyr, Phe, His, Lys, Arg. Placebo capsules contain calcium carbonate, starch and look similar to pearl powder capsules.

### 2.3. Pearl powder extraction

Extraction of pearl protein was carried out by the method of Brandt and Muir [15] and estimated by Lowry [16] method. The Pearl protein profile was determined by using SDS-PAGE analysis. The protein of pearl powder was removed by the method of Hirano [17] to get a non-protein extract of pearl powder. Both protein extract and non-protein extract of pearl powder were stored at  $-20^\circ\text{C}$ . For *in vitro* studies, protein extract, non-protein extract, and whole pearl powder were dissolved in double distilled water, respectively.

### 2.4. In vitro studies

#### 2.4.1. Determination of various oxidative indices

Total antioxidant ability (Trolox equivalent antioxidant capacity; TEAC) was performed by Arnao et al. method [18]. The ferrous ion chelating potential was estimated by the method of Dinis [19]. DPPH radical-scavenging activity was determined based on the method of Shimada et al. [20]. Superoxide anion scavenging effects were done by Robak and Gryglewski method [21].

#### 2.4.2. Lifespan prolonging efficacy in wild-type nematodes

2.4.2.1. *Culturing of C. elegans*. The wild nematode strain was obtained from the National Taiwan University, Taipei, Taiwan and was maintained and cultured at  $20^\circ\text{C}$  on Nematode Growth Medium (NGM) agar plates fed with live bacteria like *Escherichia coli* (food) [22].

2.4.2.2. *Prolonging activity of protein extract of pearl powder*. Age synchronous populations were done as described

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