Gene	Species	Direction	Sequence
CCT2	Human	F	AAGCCACGAAGGCTGCAA
CCT2	Human	R	TCATCGGAACCATGATCAACTG
CCT7	Human	F	GTGGCATGGACAAGCTTATTGTAG
CCT7	Human	R	CAGAATTGTGGCCCCATCA
GAPDH	Human	F	ACAACTTTGGTATCGTGGAAGG
GAPDH	Human	R	GCCATCACGCCACAGTTTC

Table 1: Species-specific quantitative PCR (qPCR) primers.

Clinical trial design

The clinical study had been approved by Antai-Tian-Sheng Memorial Hospital Institutional Review Board (TSMH-IRB 21-057-A), and the study had been registered on ClinicalTrials.gov Identifier: NCT05191056. Fifty-one (51) adult subjects (30-60 years old) were recruited in this trial between Sep 2021 and Feb 2022. Informed consent was obtained from all subjects before the study at Chia Nan University of Pharmacy and Science. The subjects were divided into a placebo group (n = 27) and a formula beverage group (n = 24). Each subject was informed about intaking a bottle of formula beverage labeled 30ml or a placebo drink daily for 8 weeks and was not allowed to take any other supplement during the intervention period. The exclusion criteria included: i) liver cirrhosis, or chronic renal failure; ii) allergy to cosmetics, drugs, or foods; iii) pregnant and breastfeeding. All subjects were examined blood biochemistry and somatosensory condition through questionnaires at 0, 8 weeks.

Test sample

Formula beverage (MelaGene+™, Melaleuca, China) contained 3% salmon roes, 0.36% brown rice, 0.2% snow fungus, citric acid, water. Placebo beverage of the main ingredient: citric acid, water. Each subject was required to examine blood biochemistry at 0, and 8 weeks.

Statistical analysis

The comparison of measurement results for skin parameters among groups and between groups was analyzed by independent T-test through GraphPad Prism 6, as $P < 0.05$ was considered statistical significance.

Results

Effect of fish egg, brown rice and snow fungus beverage on antioxidant and mitochondrial activity *in vitro.*

Oxidative stress refers to elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA [20]. The formula beverage to treat SH-SY5Y cells and examined mitochondrial activity and ROS. The results showed that formula beverage significantly increased mitochondrial activity of nerve cells by 20.6% compared to control group (Figure 1A). Additionally, hydrogen peroxide (H_2O_2)-induced ROS production. The results showed that H_2O_2 (200 uM) induced ROS expression. The formula beverage significantly decreased ROS generation by 60.3% compared to H_2O_2 group (Figure 1B). These results showed that formula beverage promoted nerve signal transmission and effectively protected nerve cells from oxidative stress *in vitro*.

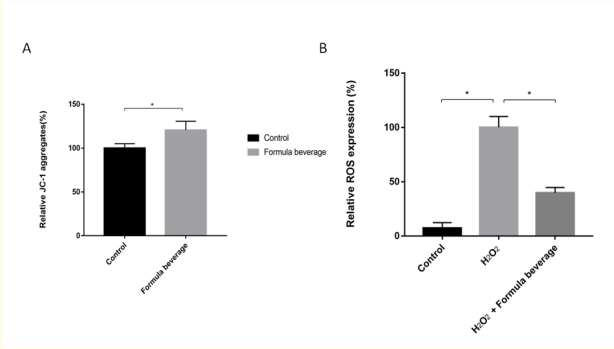

Figure 1: Formula beverage increased the mitochondrial activity and decreased ROS. (A) Formula beverage treated in SH-SY5Y cells for 24 h, then examined mitochondrial activity through JC-1 assay. (B) H_2O_2 (200 μM) induced ROS expression, and formula beverage treated in SH-SY5Y cells for 24 h, and then examined through fluorescence microscopy. *p* values < 0.05 to claim statistical significance. Error bars represent ± standard deviation.

Effect of fish egg, brown rice and snow fungus beverage on antioxidant ability and anti-aging on clinical trials

The results showed that formula beverage significantly increased antioxidant-sulfur compounds (f-Thiols) (Figure 2A) and significantly decreased oxidative stress damage marker- High-

sensitivity CRP (HsCRP) compared to placebo group (Figure 2B). Furthermore, we analyzed anti-aging-related genes, chaperonin-containing T-complex, such as CCT2 and CCT7. The results showed that formula beverage significantly increased CCT2 expression by about 3.12 times compared to placebo group, and significantly increased CCT2 expression by about 27 times compared to baseline (week 0) (Figure 3A). In addition, formula beverage significantly increased CCT7 expression by about 2.83 times compared to placebo group, and significantly increased CCT7 expression by about 3.4 times compared to baseline (week 0) (Figure 3B). Finally, we used questionnaires to investigate the somatosensory status of the subjects. After taking formula beverage for 8 weeks, subjects reported the consciously better health, often feel unhappy, unhealthy affects social, feeling emotionally sensitive, often feeling irritable had improved (Figure 4). Taken together, the formula beverages increased antioxidants, decreased oxidative stress, improved depression, and achieved anti-aging goals.

Discussion

Aging is associated with an increased risk of morbidity and mortality. Growing evidence suggests that oxidative stress plays a key role in the aging process and various degenerative diseases [21]. This study was the first to find that the formula beverage with salmon egg with brown rice, snow fungus had anti-aging effects. Result from this work revealed that formula beverage can increase mitochondrial activity and decrease oxidative stress *in vitro*. In clinical trial, formula beverage had antioxidant ability and anti-aging effects.

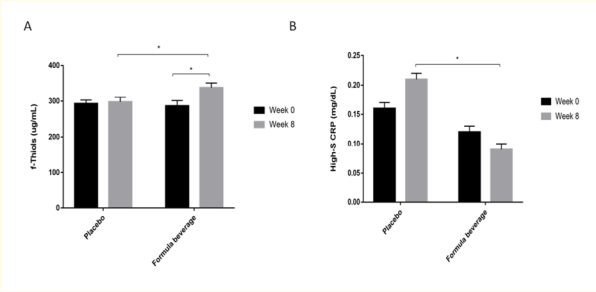

Figure 2: Formula beverage increased antioxidant-sulfur compounds decreased oxidative damage. Subjects took formula beverage for 8 weeks, and then examined (A) f-Thiols, (B) HsCRP in the blood. *p* values < 0.05 to claim statistical significance. Error bars represent ± standard deviation.

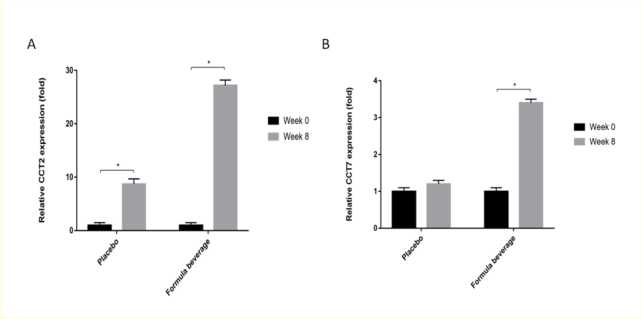

Figure 3: Formula beverage increased anti-aging-related genes. Subjects took formula beverage for 8 weeks, and then examined (A) CCT2, (B) CCT7 in the blood. *p* values < 0.05 to claim statistical significance. Error bars represent ± standard deviation.

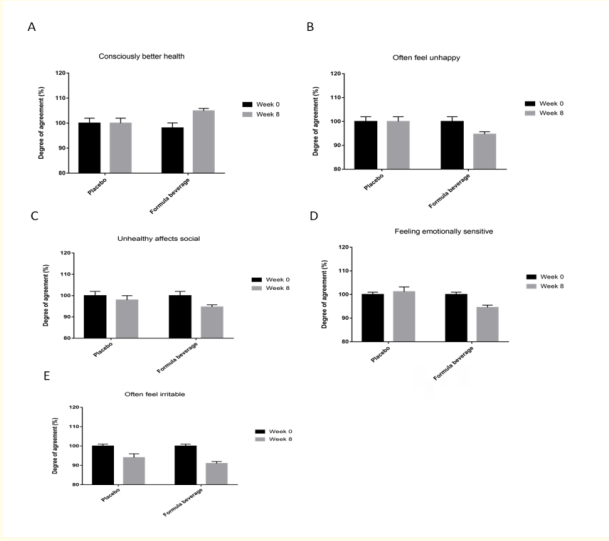

Figure 4: Formula beverage improved depression. (A) Subjects took Formula beverage for 8 weeks, and then filled out the questionnaire for the survey (A) consciously better health, (B) often feel unhappy and (C) unhealthy affects social, (D) feeling emotionally sensitive, (E) often feeling irritable.

Some studies showed that compounds derived from marine and plant organisms for their antioxidant and anti-aging activities [22,23]. Salmon roe is the eggs of salmon, and they are one of the most nutrient-dense foods on earth(ref). Processed by removing the whole egg bag from the fish and soaking it in salt [24]. Salmon egg is a potential source of anti-aging materials because it contains vitamins A, B, D, and K2, zinc, iodine, along with the neuroprotective and omega 3 brain-building fatty acids EPA and DHA [25]. The nutraceuticals derived from salmon egg promoted the expression of collagen type I and decreased ROS [25]. Human dermal fibroblast treated with salmon egg extract increased multiple antioxidative genes, including oxidation resistance 1(OXR1), Thioredoxin reductase 1(TXNRD1), and Peroxiredoxin (PRDX) family genes [25]. Salmon egg rich in PUFA could decrease the inflammatory cytokines, such as interleukin-6 or tumor necrosis factor α, which are inducers of CRP, through cyclooxygenase-2 pathways [26]. The pink colour of salmon comes from its rich levels of a protective antioxidant called astaxanthin., and which synergistically modulates antioxidant f-Thiols [27]. Taking omega-3-rich fish tend to have a lower risk of depression and a more positive affect [28].

Brown rice provides dietary fibre and essential nutrients such as vitamin B, E and some essential phytochemicals such as g-oryzanol, ferulic acid and inocitol [13]. Gamma aminobutyric acid (GABA), commonly produced by germination of brown rice grain [29]. GABA reduces ROS level and restores oxidative redox status [29]. GABA has anti-aging potential by playing roles in energy homeostasis, reducing carbohydrate and lipid level, increasing antioxidative capacity, improved mitochondrial function [30]. Brown rice is also known for its high phenolic content that promotes human health by reducing oxidative damage, such as phenolic acids and flavonoids [14]. Specifically, among phenolic acids, ferulic acid, though also found in brown rice. Ferulic acid decreased oxidative stress by upregulating antioxidant genes (SODs) and anti-apoptotic genes (NF-κB and Bcl-2) and by downregulating pro-apoptotic genes (BAX, and caspase-9) [31,32]. The pre-germinated brown rice (PGBR) increase of serotonin (5-HT) levels in the mouse frontal cortex contributes to the antidepressant-like effects [33].

Snow fungus is a traditional nutritional food in China and is used as a traditional Chinese medicine and dietary supplement [34]. Recent studies have indicated that the medicinal and tonic properties of snow fungus are due to its polysaccharides and can protect human skin fibroblasts from oxidative stress and apoptosis caused by hydrogen peroxide [34]. The purified polysaccharides improved cell viability and mitochondrial function and restored the abnormal expression of apoptosis-related proteins [35]. In addition, snow fungus is naturally rich in vitamin D, and can help lift mood and ward off depression. Consistent with our results, formula beverage included salmon egg with brown rice, snow fungus had anti-aging effects and improved depression.

Conclusion

This study found that combined use of salmon egg with brown rice, snow fungus has antioxidant and anti-aging effects, suggest that these complex formulations are potential therapeutics for anti-oxidative stress and anti-aging.

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