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# Integrative effects of an oral supplement mixture containing melon, yeast extract, and licorice on skin pigmentation reduction: Insights from a zebrafish model and a randomized double-blind controlled pilot human trial

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**ABSTRACT**

**Introduction** Intrinsic aging (e.g., genetic and metabolic changes) and extrinsic factors (e.g., sun exposure and environmental pollution) contribute to skin pigmentation and aged facial appearance. Plant extracts, known for their antioxidant properties, may influence skin pigmentation both directly and indirectly through pigment metabolism in the liver, potentially mitigating the effects of aging.

**Objective** This study investigates the effects of a mixture of melon, yeast extract, and licorice (Mix) oral supplement on liver metabolism and skin pigmentation.

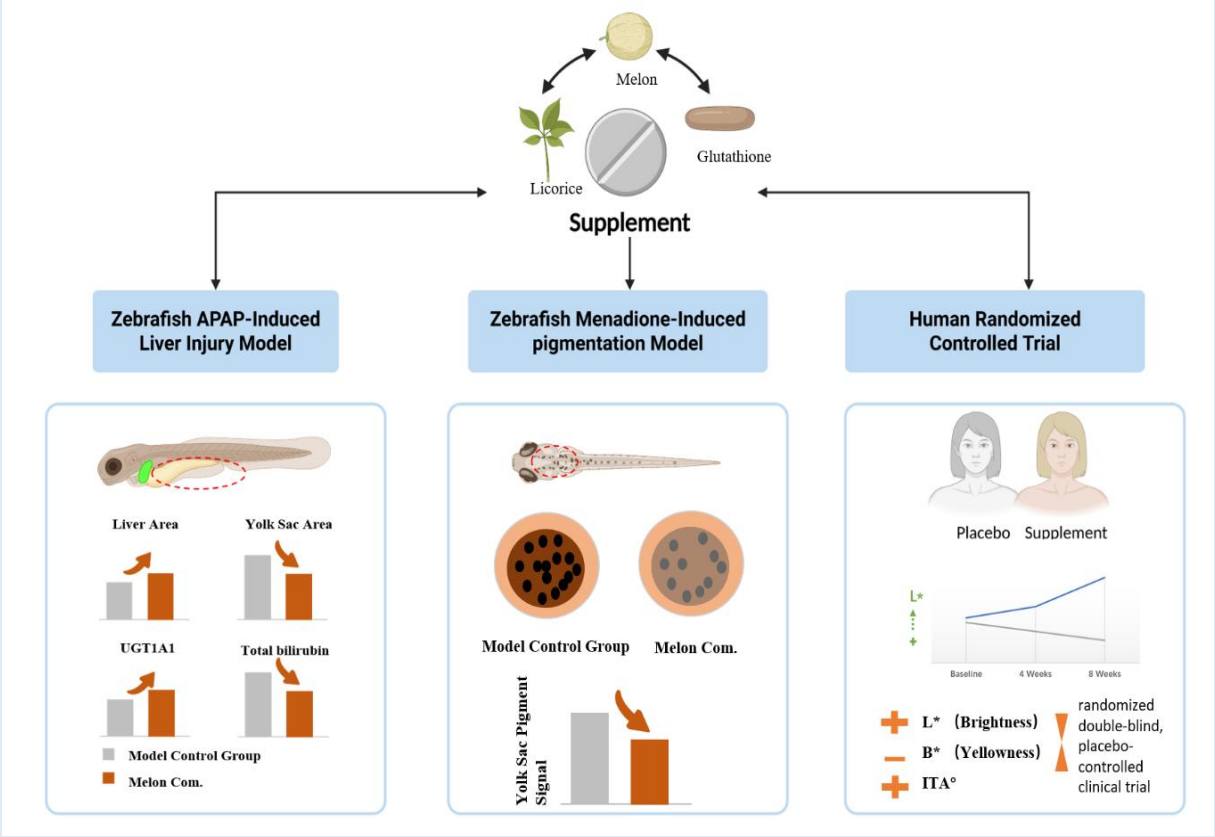
**Methods** A zebrafish model of chemical liver injury was established using acetaminophen (APAP). Liver and yolk sac areas were analyzed, and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) activity and total bilirubin content were measured to assess the supplement's protective effects. Additionally, a zebrafish pigmentation model was induced with menadione, and yolk sac pigmentation intensity was analyzed. A pilot randomized, double-blind, placebo-controlled clinical trial was conducted with 22 healthy female volunteers randomly assigned to either the experimental or placebo

group. Participants consumed 700 mg of the mixture (Mix) daily for 8 weeks. Skin brightness (L*), yellowness (b*), individual typology angle (ITA°), and melanin index (MI) were measured using a skin colorimeter (CL 400), melanin meter (MX 18), and VISIA 7 at baseline, 4 weeks, and 8 weeks.

**Results** In the zebrafish liver injury model, the mixture group（Mix Group）showed a significant increase in liver surface area, a decrease in yolk sac area, increased UGT1A1 activity, and reduced total bilirubin levels compared to the model control group. In the zebrafish pigmentation model, yolk sac pigmentation intensity was lower in the mixture group（Mix Group）. The pilot human trial demonstrated that participants taking the mixture (Mix) experienced significant improvements in skin brightness (L*) and ITA°, along with reductions in skin yellowness (b*) and melanin index (MI) after 4 and 8 weeks.

**Conclusion** An oral supplement comprising bioactive compounds from melon, yeast extract, and licorice effectively reduce skin pigmentation and supports liver pigment metabolism. This formulation, with its demonstrated functional food properties, suggests potential as an anti-aging intervention.

**Keywords**: Skin pigmentation; Liver; Oral supplements; Plant extracts; Antioxidants; Zebrafish




**Graphical Abstract:** Integrative effects of an oral supplement mixture containing melon, yeast extract, and licorice on skin pigmentation reduction

## INTRODUCTION

As a barrier between the human body and the environment, the skin is exposed to ultraviolet rays, pollution, and other harmful factors in the environment for a long time and is vulnerable to a variety of adverse effects. Intrinsic aging (such as genetic and metabolic changes) and extrinsic aging (such as sun exposure and environmental pollution) not only cause skin problems such as pigmentation, dryness, and wrinkles, but may also affect the health of internal organs in a similar way [1-6]. For example, the dysfunction of the liver, the body's main metabolic organ, can lead to skin pigmentation changes, jaundice, and other problems [7-9]. Therefore, maintaining the health of skin and internal organs has become an important subject of modern health management.

The primary approach to improve skin pigmentation is to reduce melanin deposition. Melanin, produced by melanocytes in the basal layer of the epidermis, determines skin color and is transferred to keratinocytes. Ultraviolet (UV) exposure stimulates melanocytes to produce more melanin. Effective skin pigmentation strategies focus on inhibiting melanin synthesis, accelerating its degradation, or blocking its transfer to keratinocytes [10].

Moreover, studies suggest that a person's complexion and facial features can reflect the health of internal organs [4, 11]. Traditional Chinese medicine links liver dysfunction to qi stagnation and blood stasis, resulting in a yellow complexion. Modern research supports this view, showing that liver dysfunction can lead to skin, nail, and hair changes, including pigmentation shifts [7, 12-13]. For instance, bilirubin, a yellow pigment derived from the breakdown of hemoglobin, accumulates in the skin during liver dysfunction, leading to a yellowish appearance [14-15]. Studies have shown that elevated bilirubin levels cause this skin color change [16, 17], indicating that maintaining liver health could positively impact skin color.

In the cosmetics and nutritional sectors, natural skin whitening agents are increasingly favored by consumers. Bioactive compounds such as flavonoids, terpenoids, polysaccharides, and coumarin derivatives are recognized for their antioxidant and anti-inflammatory properties, with growing evidence of pigmentation of their role in preventing pigmentation [18-20].

In recent years, nutritional interventions, particularly antioxidants derived from plant extracts, have received a lot of attention for their potential to improve the health of the body's internal organs and external skin [21-26].

Natural plant extracts such as melon concentrate superoxide dismutase (SOD), glutathione from yeast extract, and licorice extract have demonstrated potent antioxidant properties, making them promising for liver function maintenance and skin whitening. Critically, the three active components exert complementary yet distinct roles. SOD, an antioxidant enzyme, neutralizes superoxide radicals, reducing oxidative stress. Cantaloupe melon (Cucumis melon L.) extract, rich in SOD, exhibits significant antioxidant and anti-inflammatory effects, with studies showing it can enhance hepatic detoxification by upregulating UGT1A1 activity and bilirubin conjugation [27-28].

Glutathione (GSH), a tripeptide found in yeast extracts, acts as a pan-tissue antioxidant, scavenging reactive oxygen species (ROS) in both liver and skin to prevent oxidative stress-induced pigmentation [29][30].

Licorice, derived from the root of Glycyrrhiza glabra, is known for its strong antioxidant and anti-inflammatory properties. Studies have shown that licorice can reduce oxidative stress, inhibit tyrosinase activity, and block melanin synthesis via the PI3K/Akt/MAPK signaling pathway [21]. Topical formulations containing licorice also show whitening effects [30].

Given these findings, we hypothesize that an oral formulation combining these bioactive compounds (SOD-rich melon extract, glutathione, and licorice extract)

could provide synergistic benefits for liver protection and pigmentation improvement, aligning with the concept of a multi-targeted nutraceutical approach.

This study aimed to investigate the hepatoprotective and pigmentation-reducing effects of an oral supplement containing a mixture of melon, yeast extract, and licorice. Using a zebrafish model, we assessed its safety and efficacy. Additionally, we conducted a pilot randomized, double-blind, placebo-controlled clinical trial to evaluate its effects on reducing pigmentation in volunteers. The results provide evidence supporting the use of natural compound extracts for skin health.

## MATERIALS AND METHODS

**Investigational product:** The mixture (Mix) was provided by Sirio Pharma Co., Ltd (China).

For the zebrafish experiments, the mixture was prepared as a stock solution by dissolving it in standard dilution water (reverse osmosis water purified via a Milli-Q system). This stock solution was then serially diluted with the same standard dilution water to achieve the desired final test concentrations directly in the 6-well plates housing the zebrafish larvae. The exposure volume in each well was 3 mL.

The human daily dose was determined using a holistic approach that integrated the following factors: (i) a conversion formula from zebrafish to human dosage based on the invention patent of the Chinese National Intellectual Property Administration (Patent No.: CN 113496071), which provided a preliminary reference, the formula for converting zebrafish dosage to human dosage is as follows:

Zebrafish (mg/L) = (Human (g/day) × 1000) / 6.

(ii) the established safe and effective doses of the core active ingredients (SOD-rich melon extract, glutathione from yeast extract, and licorice extract) reported in published human supplementation literature[31-37]; and (iii) the preliminary efficacy and broad safety profile observed across a range of concentrations in our zebrafish models; and (iv) the formulation feasibility and compliance considerations for a dietary supplement. This integrated strategy ensured the selected dose was biologically relevant, safe, and grounded in existing scientific evidence.

The formulation of the experimental group is 700 mg per tablet and contains the following: melon (Cucumis melon L.) powder 25mg, yeast extract 12.5mg (containing 12% glutathione), licorice powder 50mg, sorbitol, isomalt, microcrystalline cellulose, citric acid, apple grapefruit powder, magnesium stearate, food flavoring, and edible compound colorant. The formulation of the placebo is 700 mg per tablet and lacks melon powder, yeast extract, and licorice powder, while all other ingredients are identical to the product. Subjects were instructed to take two tablets daily with water after breakfast for 8 weeks. All products used in this study were provided in identical packaging.

**Maintenance of brood zebrafish:** Wild-type AB zebrafish and tyr-/- melanin allele mutants were obtained from GeneBio Co., Ltd. (China). A breeding population of healthy adult zebrafish with consistent fertilization rates was maintained for egg collection. The fish were housed in a recirculating system (Aquaneering, Inc., USA) with a 14-hour light and 10-hour dark cycle. Water quality and system hygiene were monitored daily, including checks of temperature, pH, and conductivity. Zebrafish were kept at 28 °C in prepared fish water, made by dissolving 200 mg of instant ocean salt per liter of reverse osmosis water, giving a conductivity of 450–550 µS/cm, pH 6.5–8.5, and hardness of 50–100 mg/L $CaCO_3$. Feeding was carried out twice a day with brine shrimp, in the morning (8:00–9:00) and in the afternoon (16:00–17:00).

Broodstock fish were supplied by the breeding facility of Hunter Biological Technology Co., Ltd. (China). *2.3.*

**Determination of maximum tolerated concentration (MTC):** To determine the maximum tolerated concentration (MTC) of the mixture (Mix), 3 days post-fertilization (3 dpf) wild-type AB zebrafish larvae were randomly selected and placed in a 6-well plate (Hangzhou Bioland Biotechnology Co., Ltd., China) with 30 larvae per well. Each was treated well with different concentrations of the mixture (as shown in Table 1), and a normal control group was set up simultaneously. Each well had a volume of 3 mL. After 24 hours of treatment at 28°C, the MTC of the mixture for zebrafish larvae was measured.

**Establishment of a chemical liver injury model in zebrafish by using APAP (Acetaminophen):** To observe liver phenotypes and establish a chemical liver injury model, 3 days post-fertilization (dpf) wild-type AB strain zebrafish larvae were randomly selected and distributed into 6-well plates, with each well containing 30 larvae. The larvae were treated with sample solutions at concentrations specified in Figure 1a, and a positive control group was treated with 3.20 μg/mL N-acetyl-L-cysteine (NAC) (Shanghai Aladdin Biochemical Technology, China). A normal control group and a model control group were also set up, with each well having a capacity of 3 mL.

Except for the normal control group, all experimental groups were exposed to 8 mM APAP (Shanghai Aladdin Biochemical Technology, China) to induce liver injury. Specifically, 10 zebrafish larvae from each group were immersed in 3 mL APAP or APAP and positive control drug NAC or APAP and different concentrations of the mixture for 48 hours at 28°C, and then the subsequent experiments were carried out [38-39].

**Establishment of a pigmentation model in melanin allele mutant zebrafish by using menadione:** To create a pigmentation model, 3 days post-fertilization (dpf) melanin allele mutant zebrafish larvae (albino, tyr-/-) were randomly selected and distributed into 6-well plates, with each well containing 30 larvae. The larvae were treated with aqueous solutions of the mixture (concentrations specified in Figure 3a) and a positive control group was treated with 62.5 μg/mL NAC (Shanghai Aladdin Biochemical Technology, China). A normal control group and a model control group were also set up, with each well having a capacity of 3 mL [40].

After treatment with NAC or different concentrations of the mixture at 28°C for 3 hours, all experimental groups except for the normal control group were exposed to an aqueous solution of menadione (Shanghai Aladdin Biochemical Technology, China) at a concentration of 2.25 μM to induce pigmentation. The exposure of menadione with NAC or the mixture was then continued at 28°C for 24 hours before the subsequent experiments.

**Morphological assessment of liver area and yolk sac area**: Ten zebrafish were randomly selected from each group and photographed under a dissecting microscope (SZX7, OLYMPUS, Japan). The examination of liver and yolk sac area change of zebrafish was analyzed with NIS-Elements D 3.20 advanced image processing software (SZX7, OLYMPUS, Japan).

**Determination of Uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) activity:** Zebrafish samples were collected from each group and the activity of UGT1A1 was detected according to the instructions of Zebrafish UGT1A1 ELISA kit (Shanghai Enzyme-linked Biotechnology, China). The effects of the

samples on the liver were evaluated based on the statistical analysis results.

**Determination of Total bilirubin content:** Zebrafish samples were collected from each group, and the total bilirubin content was detected by following the protocols of chemical oxidation method of the total bilirubin (TBIL) content detection kit (Shanghai Enzyme-linked Biotechnology, China).

**Measurement of pigment signal intensity:** Ten zebrafish were randomly selected from each group and photographed under a dissecting microscope (SZX7, OLYMPUS, Japan). NIS-Elements D 3.20 advanced image processing software (SZX7, OLYMPUS, Japan) was used to analyze and collect data. The zebrafish yolk sac pigment signal intensity was analyzed, and the statistical analysis results of this indicator were used to evaluate the effect of the samples on skin pigmentation.

### Clinical test

**Subject:** This study was a pilot randomized, double-blind, placebo-controlled trial with an 8-week supplementation period. This pilot study was designed as a preliminary observational investigation to evaluate the initial efficacy and safety of the supplement. Twenty-two subjects were recruited, and eleven subjects were randomized to each treatment. Adult female volunteers were enrolled in this study.

After screening, subjects were randomized to the placebo group or the mixture group (Mix group).

Study population criteria: Subjects were healthy adults aged 20–45 years with a skin individual type angle (ITA° value) <45. In addition, subjects were nonsmokers with body mass index of 20–34 kg/m2. Subjects agreed to cooperate with the test, maintain a regular lifestyle during the test period, and refrain from using cosmetics, medications, or health products that could affect the results. Subjects were able to understand the study procedures and were instructed to sign a written informed consent form.

Subjects were excluded if they had a history of, or current, skin diseases that could affect the assessment of the test results; a history of severe allergies; were pregnant, breastfeeding, or planning to become pregnant during the testing period; had severe cardiac, hepatic, or renal dysfunction, or severe immunodeficiency; had a history of psychiatric disorders, severe endocrine diseases, or were taking oral contraceptives. Additionally, subjects were excluded if they were outdoor workers; had participated in drug clinical trials or other trials within the last month, had systematically taken medications that could affect the test results within the last week; had taken or applied cosmetic products that may affect the test results within the last two weeks; were unable to cooperate with the study; or were deemed unsuitable for participation in this study by the principal investigator/researcher.

**Ethical approval:** The studies involving human participants were reviewed and approved by the Shanghai Clinical Research Ethics Committee and the ethics approval number was SECCR2024-79-01.

2.10.3. Skin color was measured using a CL 400 Skin-Colorimeter probe (Courage+ Khazaka Electronic GmbH, Germany), which records data in the CIELAB color system (L*, a*, b*). The CIELAB model, developed by the International Commission on Illumination (CIE), describes colors with three coordinates: L* indicates lightness (0 = black, 100 = white), a* represents the red–green axis (−90 = green, +90 = red), and b* represents the yellow–blue axis (−90 = blue, +90 = yellow).

From these values, the **Individual Typology Angle (ITA°)** was calculated to describe skin tone. Higher ITA°

values correspond to lighter skin. Variations in **L*** values reflected changes in brightness, while shifts in **ITA°** values were used to monitor skin whitening effects.

**Melanin Content:** The melanin content in the skin was measured using the Mexameter® MX 18 probe (Courage+ Khazaka Electronic GmbH, Germany), which operates based on the principles of light absorption and reflection. The probe emits light at three specific wavelengths onto the skin surface, and the amount of light reflected back is measured by the receiver, allowing for the quantification of melanin content in the skin. The melanin index (MI value) was recorded at the test site, with three measurements taken in each test area, and the average value was calculated for analysis.

**Calculation of Derived Indicators:** To evaluate efficacy, the rate of improvement for each parameter was determined for every participant by comparing post-intervention values with baseline values using the formula:

**(Post−intervention−Baseline)/Baseline×100(Post-intervention − Baseline) / Baseline × 100%(Post−intervention−Baseline)/Baseline×100**

**VISIA Facial Skin Analysis:** Facial images were obtained using the VISIA 7 Skin Analysis System (Canfield Scientific, Inc., USA). Photographs were captured under standardized conditions and analyzed with Canfield software. Written photo consent was obtained from all participants.

Throughout the study, the only variable considered in the images was the condition of the skin. To ensure consistency, all non-skin elements (e.g., jewelry, makeup, clothing) were excluded from the image frame across sessions. Lighting, framing, exposure, and magnification were kept constant from baseline to follow-up.

**Statistical Analysis:** For the zebrafish experiments, results were expressed as mean ± standard error of the mean (SEM). Analyses were performed using SPSS software (version 26.0). Comparisons were made using Student's t-test and one-way ANOVA, with significance set at $P < 0.05$.

For the clinical trial, statistical analysis was conducted with GraphPad Prism (version 8.0). Normality of data was tested using the Shapiro–Wilk test. If normally distributed, paired t-tests (within-group) and unpaired t-tests (between-groups) were applied; for non-normal data, nonparametric alternatives were used. A P-value < 0.05 was considered statistically significant.

## RESULTS

**The mixture obtained a broad safety tolerance in zebrafish:** Before the experiment, the safety tolerance of different concentrations (125, 250, 500, 1000 and 2000 μg/mL) of the mixture on the mortality of zebrafish was assessed (Table 1). The development of zebrafish was not significantly retarded, even at the highest concentration of 2000 μg/mL, but the phenotype was more severe compared to the control group, suggesting potential toxicity at this higher dose. The death rate was 0% across all the concentration groups. Therefore, we suggested zebrafish developed normally when the concentration was less than 1000 μg/mL.

Based on the results of pre-tested concentrations of our products in vitro, 30, 62.5 and 250 μg/mL were selected as the experimental concentration gradients.

**Table 1.** Effects of the mixture (Mix) on embryonic mortality of zebrafish (n = 30).

| Group | Concentration (μg/mL) | Number of Deaths (Tail) | Mortality Rate (%) | Phenotype |
|---|---|---|---|---|
| Control Group | - | 0 | 0 | No significant abnormalities |
| Mix Group | 125 | 0 | 0 | Similar to control group |
| Mix Group | 250 | 0 | 0 | Similar to control group |
| Mix Group | 500 | 0 | 0 | Similar to control group |
| Mix Group | 1000 | 0 | 0 | Similar to control group |
| Mix Group | 2000 | 0 | 0 | More severe than control |

**Protective effects of The mixture on liver in Zebrafish:** Zebrafish share a genetic similarity of up to 87% with humans, making them highly applicable to human studies[42]. Zebrafish have been widely used in studying drug-induced liver injury (DILI). Their transparent larvae allow dynamic monitoring of liver damage through imaging techniques, which have been employed to investigate the hepatotoxicity of various exogenous compounds, such as acetaminophen (APAP)[43] .

Liver size serves as a critical indicator of hepatic health and functional status. Changes in liver size can indicate the liver's response to toxic substances or its regenerative processes. In APAP-induced liver injury models of zebrafish, variations in liver size can serve as markers for liver damage and regeneration [44] , and the yolk sac provides nutrition during early zebrafish embryo and larval development, and its size can reflect nutrient utilization and metabolic status. Delayed absorption of the yolk sac might indicate metabolic issues or toxic responses, which could be used to study hepatoprotective effects [45].

In our study, APAP was used to investigate the mixture (Mix) on the liver protection of larval zebrafish. The results showed Mix exhibited improved effects with respect to the increase of liver size and yolk sac area at 30 μg/mL, 62.5 μg/mL, 250 μg/mL groups (Table 2). The effects of Mix were detected in two-dimensional images of the liver and yolk sac (Figure 1a). Notably, at 62.5 μg/mL, liver volume was the largest and yolk sac area the smallest (Figure 1b-1c), comparable to the normal control and NAC positive control group. However, the mixture groups didn't exhibit dose-independent effect on the increase of liver area and yolk absorption delay.

These results indicated that the mixture (Mix) promotes liver regeneration and normal metabolic/developmental functions in zebrafish.

**Table 2.** Experimental results of liver phenotype change evaluation. (n = 10).

| Group | Concentration (μg/mL) | Liver Area (pixels, mean ± SE) | Yolk Sac Area (pixels, mean ± SE) |
|---|---|---|---|
| Normal Control Group | - | 29788 ± 851*** | 19461 ± 3829*** |
| Model Control Group | - | 19851 ± 1672 | 76781 ± 7673 |
| Positive Control Group | 3.20 | 29602±1149*** | 32183 ± 5255*** |
| Mix Group | 30.0 | 26845 ± 1005** | 27831 ± 3520*** |
| Mix Group | 62.5 | 28905 ± 1098*** | 14284 ± 2045*** |
| Mix Group | 250 | 28720 ± 769*** | 31895 ± 7719** |

The data are presented as the means ± SEM (n=10), ** $p < 0.01$, ***$p < 0.001$ vs the Model Control group.

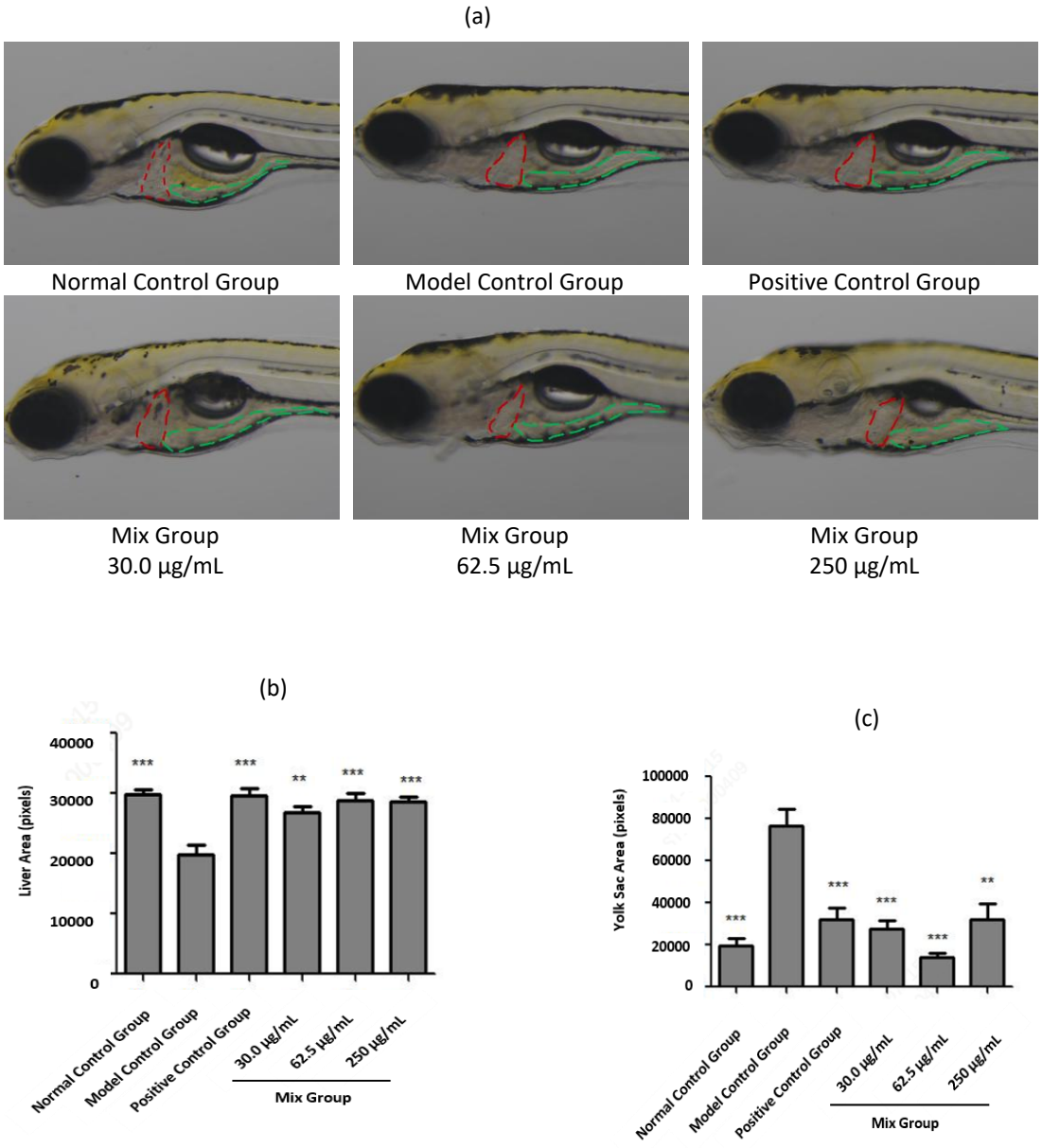



**Figure 1.** Effects of the mixture (Mix) on liver phenotype of zebrafish larvae. (a) 2-dimensional whole-mount images of liver (outlined with red dotted lines) and yolk sac area (outlined with green dotted lines). (b) Quantification of liver area. (c) Quantification of yolk sac area . The data are presented as the means ± SEM (n=10), ** $p < 0.01$, ***$p < 0.001$ vs the Model Control group.

**The mixture enhances UGT1A1 activity and reduces bilirubin levels for liver recovery in Zebrafish:** The APAP-elicited hepatic injury was measured by the level of UGT1A1 activity and total bilirubin content with homogenate of whole larvae. UGT1A1 enzyme activity and total bilirubin content are both well-determined hallmarks for assessing liver function. UGT1A1 enzyme activity reflects the function of liver detoxification enzymes, while total bilirubin content directly reflects the liver's ability to metabolize and excrete waste[46, 47].

UGT1A1 enzyme activity was significantly increased by the mixture (Mix) at all concentration groups compared with model control group. The UGT1A1 enzyme activity levels in these groups were comparable to that observed in the normal control and positive

control groups (Table 3, Figure 2a). Additionally, as UGT1A1 is the only metabolizing enzyme for bilirubin, increased UGT1A1 activity indicates that bilirubin can be metabolized more rapidly. Therefore, we also examined the bilirubin content. The level of total bilirubin content showed a significant decrease in all concentration groups of Mix (Table 4, Figure 2b).

These results suggested that the mixture effectively enhanced liver detoxification and waste excretion, highlighting their potential therapeutic value in mitigating liver damage and liver pigmentation metabolism.

**Table 3.** Experimental results of UGT1A1 Activity (n = 3).

| Group | Concentration (μg/mL) | UGT1A1 Activity (U/mgprot, mean ± SE) |
|---|---|---|
| Normal Control Group | - | 564 ± 14.5** |
| Model Control Group | - | 472 ± 7.21 |
| Positive Control Group | 3.20 | 523 ± 9.55* |
| Mix Group | 30.0 | 541 ± 12.2** |
| Mix Group | 62.5 | 548 ± 11.0** |
| Mix Group | 250 | 551 ± 12.6** |

The data are presented as the means ± SEM (n=3),* p < 0.05, ** p < 0.01 vs the Model Control group.

**Table 4.** Experimental results of Total Bilirubin Content (n = 3).

| Group | Concentration (μg/mL) | Total Bilirubin Content (μmol/gprot, mean ± SE) |
|---|---|---|
| Normal Control Group | - | 1.80 ± 0.078** |
| Model Control Group | - | 3.06 ± 0.165 |
| Positive Control Group | 3.20 | 1.71 ± 0.121** |
| Mix Group | 30.0 | 2.12 ± 0.049*** |
| Mix Group | 62.5 | 2.02 ± 0.141*** |
| Mix Group | 250 | 1.70 ± 0.156*** |

The data are presented as the means ± SEM (n=3) ,* p < 0.05, ** p < 0.01, ***p < 0.001 vs the Model Control group.

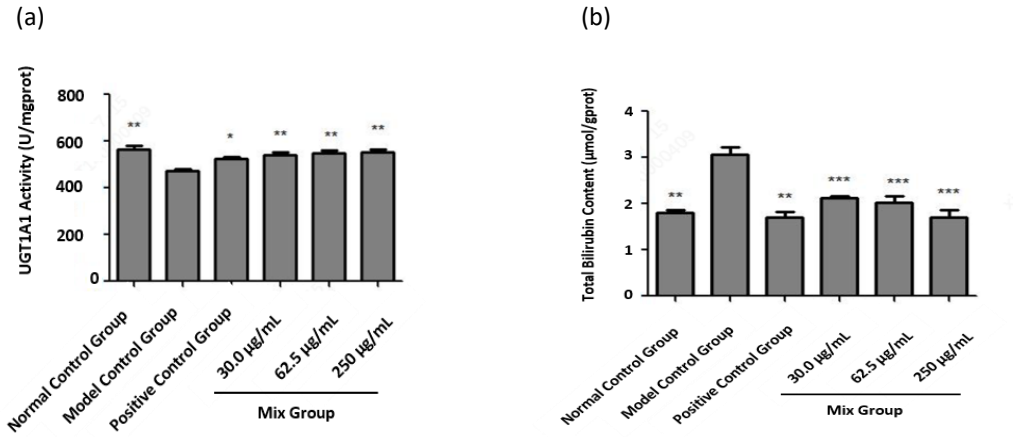



**Figure 2.** Effects of the mixture (Mix) on liver function of zebrafish larvae. (a) Experimental results of UGT1A1 activity. (b) Experimental results of total bilirubin content. The data are presented as the means ± SEM (n=3),* p < 0.05, ** p < 0.01, ***p < 0.001 vs the Model Control group.

**The mixture reduces yolk sac pigmentation in Zebrafish and enhances skin brightness:** Melanin allele mutant zebrafish are genetically modified zebrafish used in research to study pigmentation, melanogenesis, and

related biological processes. Melanin production in zebrafish is primarily regulated by genes involved in the melanin synthesis pathway, such as tyrosinase. Mutations in these genes can lead to pigmentation defects [48-50]. Therefore, we used melanin allele mutant zebrafish to assess the effect of the mixture (Mix) on skin brightness.

Dark skin results from multiple factors. One important factor is the oxidative stress reaction induced by active oxygen free radicals, which leads to pigmentation and dull skin. Menadione was used as the experimental model drug to generate active oxygen free radicals [51-52]. Oxidative stress can lead to protein denaturation and lipid oxidation, ultimately resulting in phenotypic pigmentation. The yolk sac was chosen for observation due to its high lipid and protein content. After treatment with menadione, the phenotype became more obvious, indicating that the model was successfully established.

The results showed that compared with the model control group, the pigment of the zebrafish yolk sac was dramatically reduced in Mix Group (Table 5). Moreover, all Mix groups exhibited better effects than the NAC positive control group.

The effects of the mixture were detected in two-dimensional images of the yolk sac (Figure 3a). Intriguingly, Mix reduced yolk sac pigment levels in a dose-dependent manner, suggesting that high doses of Mix had a stronger skin-lightening effect (Figure 3b).

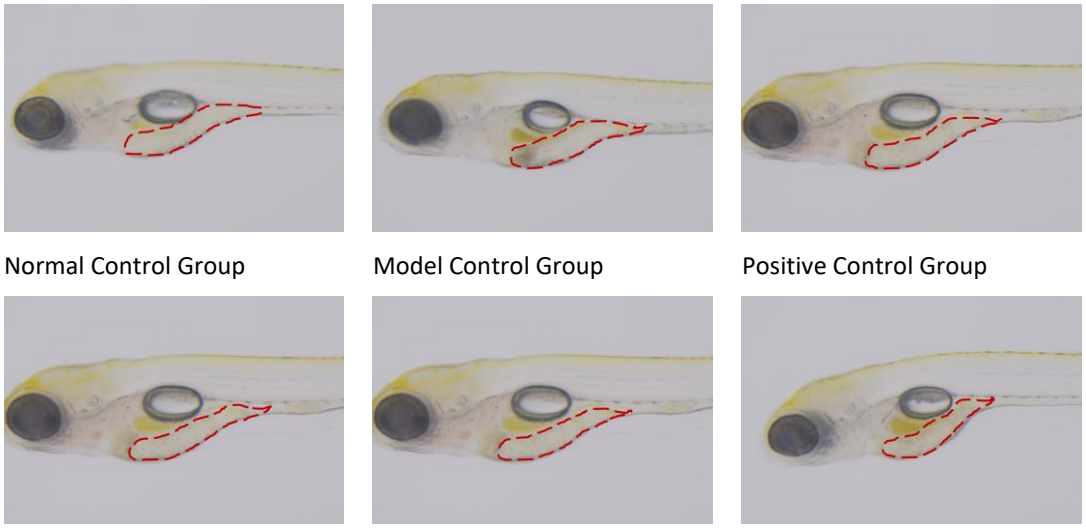
Overall, these results indicated that the mixture effectively reduces yolk sac pigmentation intensity in zebrafish, suggesting its potential utility in improving skin brightness and addressing hyperpigmentation.

**Table 5.** Experimental results of pigment signal intensity of yolk sac of zebrafish treated with the mixture (Mix) (n = 10).

| Group | Concentration (μg/mL) | Yolk Sac Pigment Signal Intensity (pixels, mean ± SE) |
|---|---|---|
| Normal Control Group | - | 20548 ± 1407*** |
| Model Control Group | - | 30000 ± 1928 |
| Positive Control Group | 62.5 | 25038 ± 992* |
| Mix Group | 30.0 | 23138 ± 1183* |
| Mix Group | 62.5 | 22692 ± 1448* |
| Mix Group | 250 | 22299 ± 1986 |

The data are presented as the means ± SEM (n=10),* $p < 0.05$, ***$p < 0.001$ vs the Model Control group.

(a)



Normal Control Group | Model Control Group | Positive Control Group

Mix Group 30.0 μg/mL | Mix Group 62.5 μg/mL | Mix Group 250 μg/mL

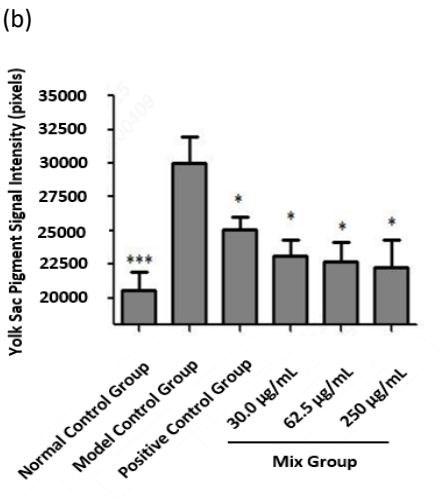



**Figure 3**. Effects of the mixture (Mix) on lighting skin of zebrafish pigmentation model. (a) 2-dimensional whole-mount images of yolk sac pigment signal intensity (outlined with red dotted lines) (b) Quantification of yolk sac pigment signal intensity. The data are presented as the means ± SEM (n=10),* $p < 0.05$, ***$p < 0.001$ vs the Model Control group.

**Effects of the mixture on skin color of subjects:** A total of 22 subjects participated in this pilot trial. During the study, two participants from the placebo group withdrew for personal reasons. Consequently, 20 subjects completed the 8-week trial, with 9 in the placebo group and 11 in the mixture group Mix Group). The collected baseline information of the subjects was described in Table 6. The mean age, weight, height, and body mass index were within normal ranges at baseline, with no significant differences observed between the groups.

The summary of volunteers' skin chromaticity indicators was presented as follows. At baseline, there were no significant differences in skin chromaticity indicators between the mixture group (Mix Group) and the placebo group (data not shown). Compared to the placebo group, the changes in brightness (L*) (Figure 4a) and individual typology angle ITA° (Figure 4e) after 4 and 8 weeks of administration in the mixture group were significantly increased, and the changes in blue-yellow coordinate (b*) (Figure 4c) were significantly decreased.

After 4 weeks and 8 weeks of intervention, the brightness (L*) and ITA° in the mixture group (Mix Group) increased and were significantly higher than their own baseline. The brightness value*） in the mixture group (Mix Group) significantly increased by 0.64 ± 0.39 and 0.98 ± 0.42 after 4 and 8 weeks, with an increase rate of 1.06% and 1.61% (Figure 4b), and the ITA° significantly increased by 2.45 ± 0.92 and 4.16 ± 1.23 after 4 and 8 weeks, with an increase rate of 6.7% and 11.36% (Figure 4f). Meanwhile, the blue-yellow coordinate value（b*） in the mixture group (Mix Group) decreased and was significantly lower than its own baseline. The blue-yellow coordinate value（b*）significantly decreased by 0.44 ± 0.28 and 0.89 ± 0.47 after 4 and 8 weeks, with a decrease rate of 3.08% and 6.15% (Figure 4d). These results indicated a significant improvement in skin brightness and a significant lightening of skin color.

**Table 6.** Volunteer Basic Information

| Index | Mix (n = 11) | Placebo ( n = 9) | P value |
|---|---|---|---|
| Gender: Female | 11 (100%) | 9 (100%) | NA |
| Age (years) | 32.70±7.9 | 36.2±6.1 | 0.29 |
| Weight (kg) | 56±6 | 55±7 | 0.839 |
| Height (m) | 1.59±0.06 | 1.59±0.04 | 0.938 |
| BMI (kg/m2) | 22.0±2.4 | 21.8±2.5 | 0.808 |

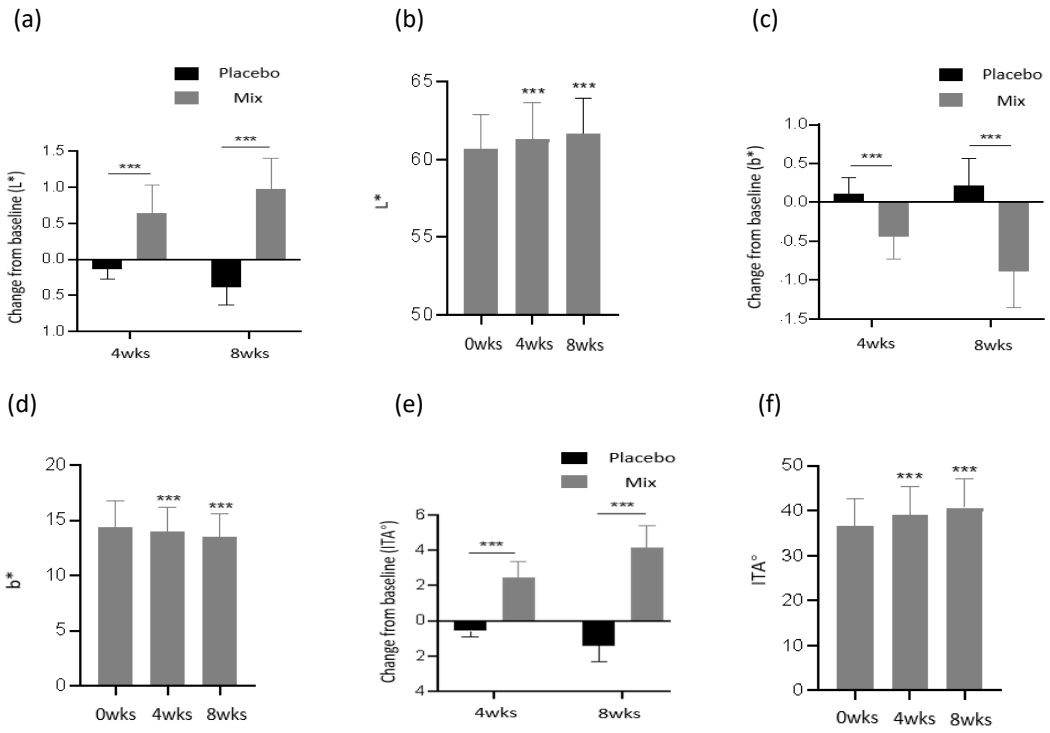
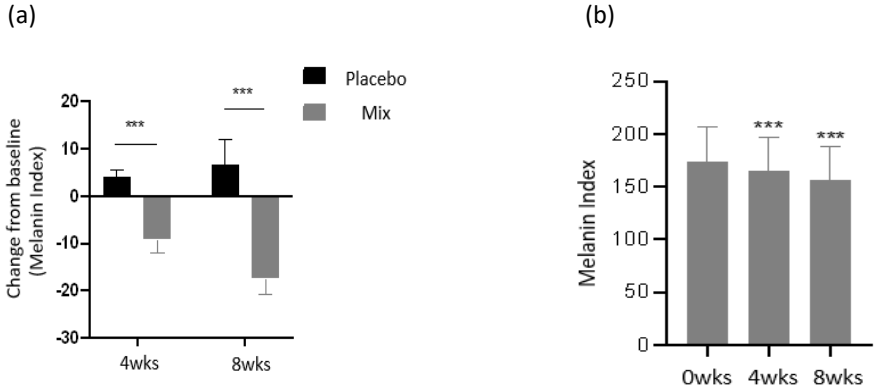



**Figure 4.** Effects of an oral supplement mixture on skin color indexes of volunteers. (a) Changes from baseline of L* in the placebo group and the mixture group (Mix) after 4 and 8 weeks. (b) L* in the mixture group (Mix). (c) Changes from baseline of b* in the placebo group and the mixture group (Mix) after 4 and 8 weeks. (d) b* in the mixture group (Mix). (e) Changes from baseline of ITA° in the placebo group and the mixture group (Mix) after 4 and 8 weeks. (f) ITA° in the mixture group (Mix). The data are presented as the means ± SEM, ***p < 0.001.

**Effects of the mixture on skin pigment content of subjects:** At baseline, there was no significant difference in melanin index (MI) levels between the mixture group and the placebo group (data not shown). Compared to the changes in the placebo group, the MI of the skin in the mixture group significantly decreased after 4 weeks and 8 weeks of administration (Figure 5a). Moreover, compared to its own baseline, the MI in the mixture group significantly decreased by 9.09 ± 2.88 (a reduction rate of 5.21%) after 4 weeks, and by 17.45 ± 3.50 (a reduction rate of 11.12%) after 8 weeks (Figure 5b). Digital photographs of two participants before and after 8 weeks of the mixture intake were illustrated in Figure 5c.

These results showed that treatment with the mixture could significantly improve the pigment deposition, which was consistent with our hypothesis.



(c)

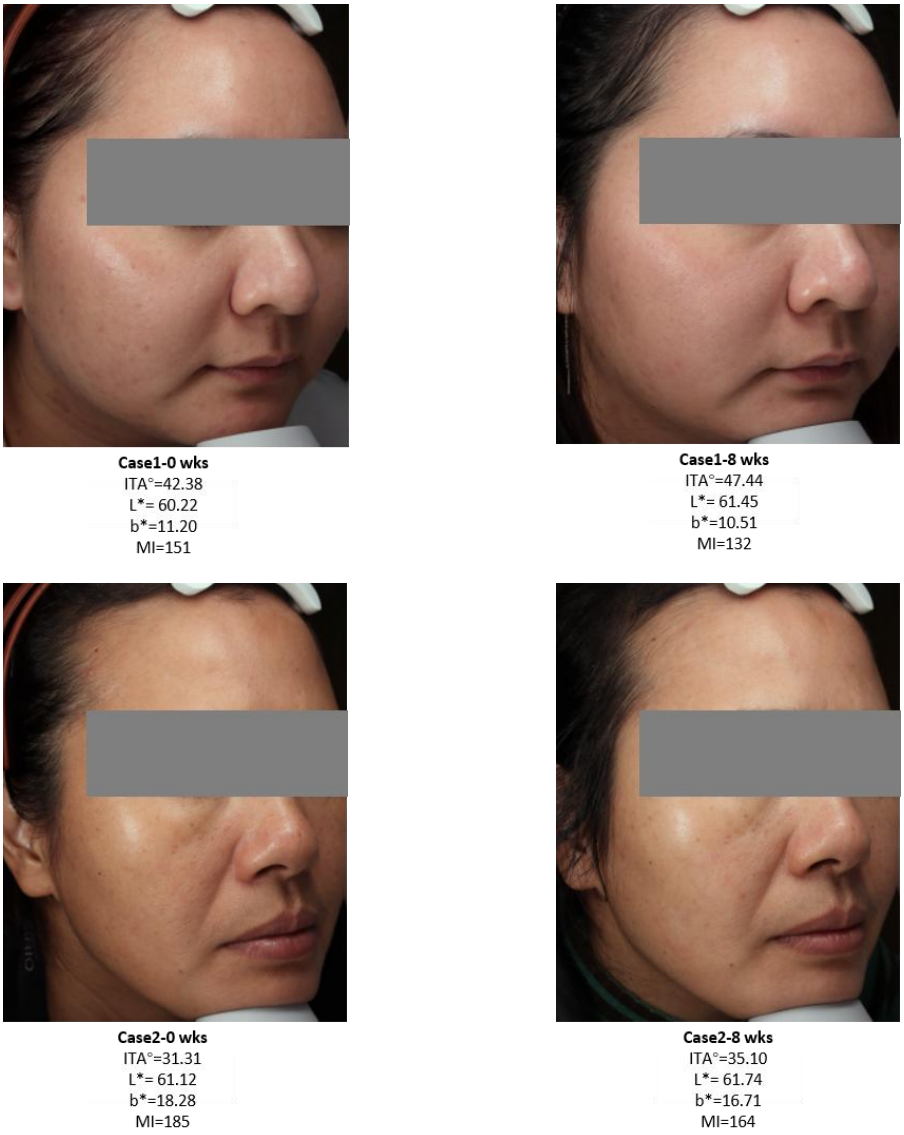



**Figure 5.** Influences of an oral supplement mixture on skin melanin index of volunteers. (a) Changes from baseline of melanin index (MI) in the placebo group and the mixture group (Mix Group) after 4 and 8 weeks. (b) MI in the mixture group (Mix Group). (c) VISIA facial skin analysis images of two volunteers in the mixture group (Mix Group). The data are presented as the means ± SEM,***p < 0.001. Images were acquired under standardized lighting and magnification conditions.

## DISCUSSION

In this study, a novel oral supplement formulation containing bioactive compounds from melon (SOD), yeast extract (glutathione), and licorice was found to promote liver metabolism and reduce pigmentation in both zebrafish and in human subjects. The study found that the mixture demonstrated broad safety tolerance across various concentrations in zebrafish models. Although no acute toxicity was observed in zebrafish or humans, long-term safety and cumulative effects warrant further investigation.

In the APAP-induced liver injury model, the mixture at concentrations of 30, 62.5, and 250 μg/mL exhibited significant protective effects, including liver area increase and yolk sac area reduction, indicating improved liver regeneration and normal metabolic functions. Additionally, the mixture significantly increased UGT1A1 enzyme activity and decreased total bilirubin levels,

demonstrating enhanced liver detoxification and waste excretion capabilities. Similarly, in the menadione-induced oxidative stress model, the mixture at the same concentrations effectively reduced yolk sac pigmentation intensity, suggesting its potential for improving skin brightness and reducing hyperpigmentation.

To further assess its efficacy, we conducted a pilot randomized, double-blind, placebo-controlled clinical trial. Participants received 700 mg of the mixture for eight weeks. The results showed significantly greater improvements in facial skin lightening indicators compared to the placebo. Moreover, the sample group exhibited a significant reduction in melanin index. The mixture was found to be safe and well-tolerated, with no reported side effects.

These results provide empirical evidence for the synergistic effects of melon extract SOD, yeast extract glutathione and licorice in inhibiting skin pigmentation and improving liver pigment metabolism.

Skin tone is influenced by several biochromes that contribute to protection against solar radiation, with melanin being the most important pigment. At present, Researchers have found that lots of ingredients extracted from natural plants are powerful antioxidants. They can reduce the formation of melanin through these mechanisms include: (1) Inhibition of tyrosinase activity: Tyrosinase is the key factor in the synthesis of melanin by melanocytes. Antioxidants can inhibit the synthesis of melanin by binding to tyrosinase non-competitively or competitively, blocking its activity [53]. (2) Reducing melanin precursors: Melanin precursors are mainly dopa and dopaquinone, which are intermediates in the synthesis of melanin and one of the sources of free radicals [54-55]. Antioxidants can reduce the concentration of melanin precursors by removing or neutralizing these free radicals, thereby reducing the production of melanin[56]. (3) Melanin degradation: Antioxidants can reduce skin pigmentation by increasing the metabolic rate of the stratum corneum, promoting the shedding of aging keratinocytes and melanin particles, or by reducing the o-quinone structure of melanin to a phenolic structure, causing the pigment to fade [27-30, 57, 59-62]. Our study demonstrates that this oral supplement, comprising bioactive compounds (melon-derived SOD, glutathione from yeast extract, and licorice extract), effectively reduces skin hyperpigmentation and supports hepatic pigment metabolism [63]. Unlike conventional single target whitening agents, this formulation combines three complementary bioactive compounds that target both hepatic pigment metabolism and cutaneous melanogenesis, offering a systemic approach to skin lightening and embodying the principles of functional food science by providing health benefits beyond basic nutrition. We speculate that maintaining the healthy function of body organs, particularly the liver, can also contribute to skin whitening. In traditional Chinese medicine (TCM), there are multiple sources that discuss the relationship between liver health and changes in facial complexion, particularly the darkening of the face. According to "Chinese Internal Medicine", liver dysfunction can lead to alterations in facial color, especially in conditions such as liver qi stagnation or liver yin deficiency, where the face may appear darker or more sallow. Additionally, the classic TCM book "Bian Que's Heart Book" mentions that liver diseases can cause the complexion to darken, particularly in cases involving jaundice or hepatic congestion. These TCM theories suggest that the liver is intricately linked to the body's overall qi (vital energy) and blood circulation. When the liver's function is impaired, it can result in poor blood flow and qi stagnation, leading to a darker facial complexion. Conditions such as liver qi stagnation, liver blood deficiency, or liver yin deficiency are commonly associated with these symptoms.

With the development of modern medicine, more and more studies have confirmed these theories of traditional Chinese medicine. A review indicates that the

skin serves as a window to our overall health, with many systemic diseases causing various skin changes. Skin abnormalities are quite common among liver disease patients. The most common symptom in these patients is pruritus. Other common features include spider angiomas, palmar erythema, paper currency skin, xanthomas, pigment changes, and nutritional deficiencies. The presence of hyperpigmentation, jaundice, and xanthomas could suggest a potential diagnosis of primary biliary cirrhosis (PBC)[58]. Some patients with chronic liver disease often exhibit muddy grey hyperpigmentation in sun-exposed areas. The pigmentation may be patchy or diffuse and is sometimes more pronounced around the mouth, eyes, and palmar creases [8] .

At the same time, some studies have shown that some metabolites of the liver, such as hemoglobin and bilirubin, are the main chromophores of the skin. Together with melanin, carotene, etc., they can determine the overall appearance of the skin [41, 59]. In a study on the simulation analysis of the effect of blood composition changes on skin color, it was found that not only hemoglobin but also bilirubin in the blood played a role in color development. As the concentration of bilirubin increased, the skin color gradually turned yellow [16].

Recent studies have observed significant differences in facial spectrum and color characteristics among healthy participants and those in a sub-healthy state across different visceral regions such as the heart, liver, spleen, lungs, and kidneys [11]. And the skin, as the largest organ of the human body, often displays early manifestations of visceral diseases. Changes in the skin can be used to diagnose systemic and visceral diseases, providing important diagnostic information [4, 60]. Therefore, we speculate effective organs function such as effective liver function helps regulate the metabolites and chromophores that influence skin color, thereby preventing undesirable pigmentation changes and promoting a healthier, more radiant complexion.

Based on the findings, we conclude that the mixture may promote skin whitening through two primary mechanisms: enhancing liver function and reducing melanin production.

## CONCLUSION

In conclusion, this study demonstrates that the oral supplement, which combines key bioactive compounds (SOD-rich melon extract, glutathione, and licorice), exhibits dual efficacy in liver protection and skin brightening. In zebrafish models, the formulation significantly promoted liver regeneration, enhanced detoxification enzyme activity (UGT1A1), and reduced bilirubin levels, indicating improved hepatic function under APAP-induced injury. Concurrently, it effectively attenuated oxidative stress-mediated pigmentation in melanin-deficient zebrafish, suggesting a direct role in melanogenesis regulation. Clinical trials further validated these findings, with subjects receiving the mixture showing statistically significant increases in skin brightness (L* and ITA° values) and reductions in the blue-yellow coordinate value（b*）and melanin index (MI) after 8 weeks.

While the study highlights the potential of natural antioxidants in addressing age-related skin and organ dysfunction, limitations include the small clinical cohort and short-term observation period. Future research should explore long-term safety, bioavailability optimization, and mechanistic interactions among the components. Overall, this work underscores the promise of integrative nutraceuticals in promoting systemic health and offers a foundation for developing multifunctional supplements targeting both cutaneous and internal aging.

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