

Food Supplement 20170307-EGR May Increase the Number of Mesenchymal Stem Cells and the Effect of Mitochondrial Protection

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Abstract A few refer to food supplements that can increase the number of mesenchymal stem cells and protect the mitochondria. We measured the amount of mesenchymal stem cells and the activity of the mitochondria in the plant extracts of the culture medium to evaluate the antiaging effect of the food extract of the plant extract. The samples were evaluated for human fat in mesenchymal stem cells, centrifuged to isolate the number of granulocytes, mesenchymal stem cells and increase mitochondrial ATP, and reduce free radical leakage, all associated with aging markers. Our results show that the number of mesenchymal stem cells increased by 39%, the energy of the mitochondrial ATP increased significantly and decreased free radical leakage. This suggests that our plant extracts in food supplements may help revive with antiaging.

Keywords: food supplement, stem cell, mitochondria, ATP

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1. Introduction

The present study is directed to a plant extract composition, in particular a composition for promoting stem cell proliferation. A stem cell is a kind of undifferentiated primary cell in vivo, which can be replicated and renewed for a long time, and has the ability to differentiate into mature cells of a special type and function. In general, the source of human stem cells can be divided into embryonic stem cells (ES), adult stem cells (ASC), and induced pluripotent stem cells (iPS) [1]. Embryonic stem cells are derived from infertile embryos which remain after fertilization, pregnancy termination of embryonic primordial germ cells or from cell fusion; adult stem cell lines are derived from mature individual organs or tissues, by trypsin and other enzyme treatment of isolated Cells; and induced pluripotent stem cells are the skin cells to genetically engineered into a similar embryonic stem cells. Since embryonic stem cells must be obtained from the embryo, ethically still controversial, induced pluripotent stem cells may cause mutations, leading to carcinogenic and immunogenicity of the increased risk. So they are nowadays commonly used in medical stem cells, adult stem cells. In addition to hematopoietic stem cells, mesenchymal stem cells we are most familiar with, are neural stem cells, epidermal stem cells, skeletal muscle stem cells, adipose-derived stem cells, pancreatic stem cells, corneal stem cells, liver stem

cells and intestinal epithelial stem cells. These are also adult stem cells. Adult stem cells have many advantages, including: (1) They exists in various tissues and organs of the human body, these is a wide range of sources, and they do not involve ethical issues; (2) under normal circumstances, adult stem cells are in a quiescent state, in pathological conditions, (3) They can repair also replace the sick or old cells [2,3,4], tissues or organs.

There for the adult stem cells are considered to have unlimited potential for regenerative medicine, and how to promoting the proliferation of adult stem cells effect rely was one of the important issues of the industry and academia. In view of the above, the present invention provides a plant extract composition comprising of a red wine polyphenol, a green tea polyphenol and Emblica [5,6,7,8,9], can be used to promote stem cell proliferation and have the function of protecting and / or promoting mitochondrial functions [10,11].

2. Materials and Methods

2.1. Cell Source

ADSC cultures from donors were used in the study. All cell cultures were retrieved from a cell bank where they were stored at passage 6 (P6). ADSCs were isolated from human subcutaneous adipose tissue in accordance with (IRB) after informed consent. Primary ADSCs cultures were cultivated in 5% autologous serum in hypoxic

conditions (5% O₂) and prepared for storage (5×10⁶ cells per ml) as described elsewhere.

2.2. Cell Culture

After thawing, cells were counted and seeded on 75 cm² tissue culture flasks (~2×10⁵ cells per flask) in a KMSF medium containing 10% fetal bovine serum (FBS), 5mg Bovine pituitary extract, 2.5µg Recombinant epidermal growth factor, 2ml 0.5M N-acetyl-L-cysteine and 2ml 0.08M L-ascorbic acid. ADSC was cultured in a humidified atmosphere at +37°C, 5% CO₂. The medium was replaced every third day. For weeks Passaging continued maintaining the 1:5 split ratio until confluency could not be reached.

2.3. Cell Proliferation Assay

To determine the proliferation of composition on the ADSC cells, 2×10³ cells in single cell suspensions were seeded in individual wells of 96-well plates and incubated for 24 h at 37°C prior to exposure to composition at the indicated concentrations for 24 or 48h. Alamar blue solution was added to each well followed by incubation for 4 h at 37°C prior to removing the culture medium. Cell proliferation was determined by measuring the absorbance at 530/595nm. The cell proliferation for each group was calculated as a percentage of that of the control group.

2.4. Measurement of Cellular Oxygen Consumption Rate (OCR)

ADSC's were plated at 8×10⁴ cells/well in gelatin-coated XF 24-well cell culture microplates (Seahorse Bioscience), ADSC's were incubated in a pre-warmed unbuffered DMEM medium (DMEM containing 2 mM GlutaMAX, 1 mM sodium pyruvate, 1.85 g l⁻¹ NaCl and 25 mM glucose) for 1 h. The oxygen consumption rate was measured by the XF24 extracellular flux analyser (Seahorse Biosciences). Mitochondrial biogenesis was profiled by injecting perturbation drugs, 0.5 µM oligomycin, 2 µM FCCP and 0.5 µM antimycin A, in succession. OCR was determined by a plotting the oxygen tension and acidification of the medium in the chamber as a function of time and normalized by protein concentration (picomoles per minute per milligram), respectively.

3. Results

In a 96 well plate dish, 2,000 adipose mesenchymal stem cells were added per Culture medium well. After 24 hours of culture, the medium was replaced with a polyphenol extract (red polyphenol, green tea polyphenol, apple polyphenol), a two-in-one extraction composition (red polyphenol + green tea polyphenol, phyllanthus emblica + red wine Phenols, Phyllanthus emblica + green tea polyphenols) or a triple extraction composition (red polyphenol + green tea polyphenol + Phyllanthus emblica) for 24 or 48 hours. Then, the culture medium was replaced with a culture solution containing 10% Alamar blue, and the fluorescence value was measured at a wavelength of 530/595 nm after culturing for 3-4 hours.

3.1. Effects of Single Polyphenols on Adipose Mesenchymal Stem Cell Proliferation

Please refer to Figure 1 for a comparison of the concentrations of the polyphenols-free medium (Figure 1A), green tea polyphenols (Figure 1B) or apple polyphenols (Figure 1C) can promote the proliferation of adipose mesenchymal stem cells, but only 5-13%.

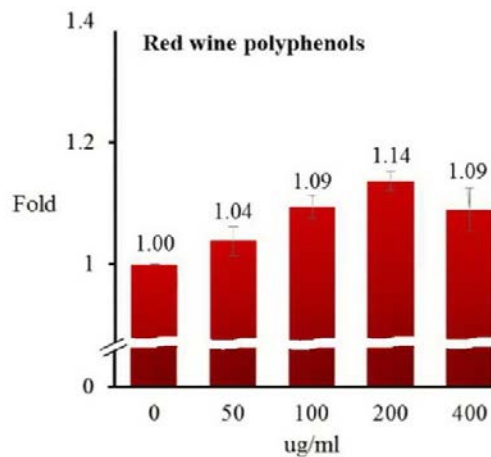


Figure 1A. Compared with the medium without polyphenols, the polyphenols with different concentrations could promote the proliferation of adipose mesenchymal stem cells, but the effect was little, only 5-13%.

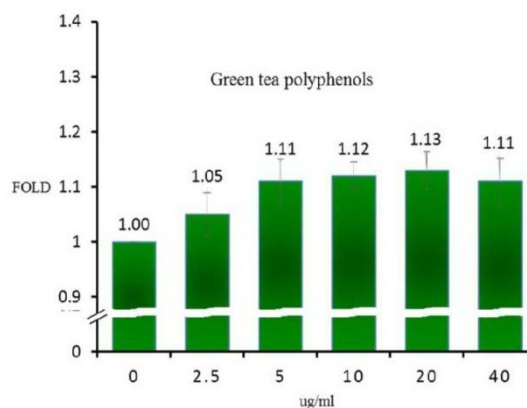


Figure 1B. Compared with the non-polyphenol-based culture medium, the green tea polyphenols could promote the proliferation of adipose mesenchymal stem cells, but the effect was not good, only 5-13%.

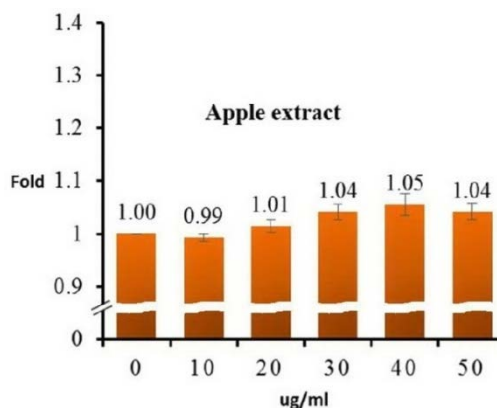


Figure 1C. Compared with the non-polyphenol-based culture medium, the effect of different concentration of apple polyphenols on the growth of mesenchymal stem cells was not significant, but only 5-13%.

3.2. The effect of 2 - in - 1 Extraction Composition on Adipose Mesenchymal Stem Cell Proliferation

Figure 2A the red polyphenol and green tea polyphenols we added to the medium culture of adipose mesenchymal stem cells at the same time.

The results show that only the adipose mesenchymal stem cells could enhance the proliferation rate of 14%, and compared to a single polyphenols, with no Multiplication effect. Furthermore, the combination of phyllanthus emblica with red polyphenols (Figure 2B) or green tea polyphenols (Figure 2C) did not improve the growth rate of adipose mesenchymal stem cells.

3.3. The Effect of Three - in - one Extraction Composition on Adipose Mesenchymal Stem Cell Proliferation

Referring to Figure 3, the present invention is compared to a medium in which no extract is added The plant extract composition of the invention can effectively promote the proliferation of mesenchymal stem cells. In addition, when the concentration of phyllanthus emblica, red wine polyphenol and green tea polyphenol was 10-100µg / ml, 10-100µg / ml and 1-10µg / ml, the number of adipose mesenchymal stem cells could be increased effectively. The fat mesenchymal stem cell proliferation could increase by a rate of 39%.

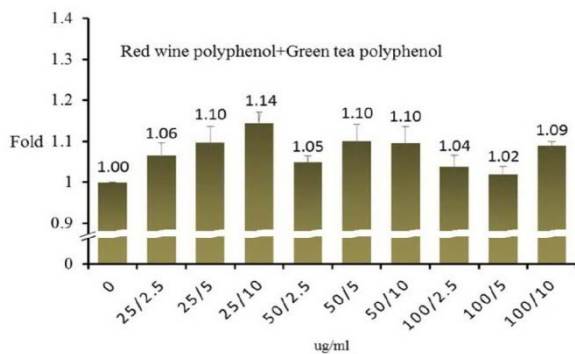


Figure 2A. The results showed that only the interstitial stem cells increased the proliferation rate of 14%, and compared with the single polyphenols, there was no effect of adding the polyphenols and the green tea polyphenols to the culture medium.

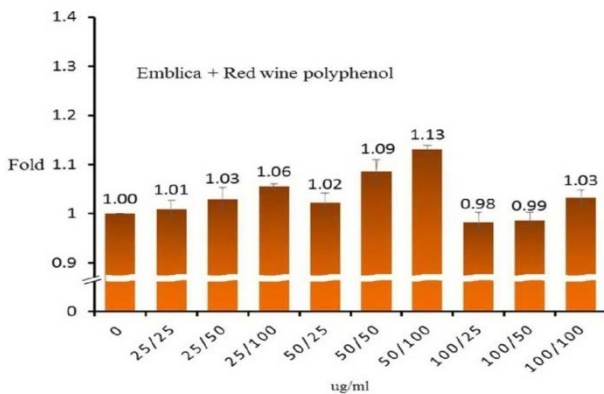


Figure 2B. Foliage and red wine with a combination of polyphenols, can't further enhance the growth rate of interstitial mesenchymal stem cells

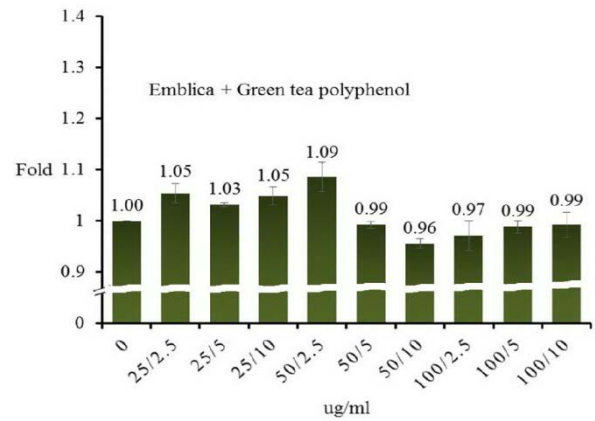


Figure 2C. The combination of Phyllanthus emblica and green tea polyphenol, can't further enhance the growth rate of interstitial mesenchymal stem cells

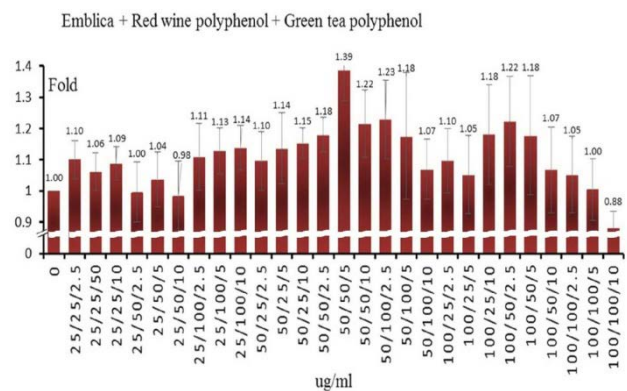


Figure 3. The culture medium containing the plant extract composition of the present invention can effectively promote the proliferation of mesenchymal stem cells as compared with the medium without adding any extract. In addition, when the concentration of polyphenols, red wine polyphenols, green tea polyphenols were 10-100µg / ml, 10-100µg / ml, 1-10µg / ml, the number of mesenchymal stem cells can be effectively increased, the best Can increase the proliferation rate of interstitial mesenchymal stem cells by 39%

3.4. Effects of Three - in - One Extraction Complex on Mitochondrial Function

The plant extract composition of the present invention is added to adipose mesenchymal stem cells for cultivation.

After being cultured for 24 hours, the medium was replaced with 200 mM hydrogen peroxide (H₂O₂) for 30 minutes. Then, the cells were washed, and the intra-cellular mitochondrial function was analyzed with a Seahorse XF[®]24 Extracellular Flux Analyzer. The cells without the addition of the plant extract of the present invention and which were not treated with 200 mM hydrogen peroxide were used as the control group without addition of the plant extract of the present invention but with 200 mM hydrogen peroxide-treated Cells.

Please refer to Figure 4, compared with the control group, to the control group cells of mitochondrial function.

(Figure 4B) The energy per unit of line body energy (Figure 4F), and energy consumption for oxygen consumption (Figure 4D), energy for ATP production (Figure 4 (b)), and the free radical leakage from the control cells (Figure 4C) were also higher than in the control cells. However, the plant extract compositions of the present invention at different concentrations can be effectively protected.

The cytoplasmic function of the cells was not destroyed by the hydrogen peroxide, and compared to the control group, When the concentration of phyllanthus emblica, red polyphenol and green tea polyphenol in the compositions of the present invention were 50µg/ml, 50µg/ml and 5 µg/ml, respectively the base energy of cytoskeletal cells increased by 1.13 fold when the concentration of the composition was 50µg/ml, 50µg/ml and 5 µg / ml, respectively (Figure 4A) (Figure 4B), the energy produced by ATP increased by a factor of 1.6 times (Figure 4D), and the energy used to cope with the stress was increased by 2.7times (Figure 4E). The energy per unit of linear body increased by 1.3 times (Figure 4F), and the energy efficiency of oxygen consumption increased by 1.4 times (Figure 4G). In addition, the occurrence of free radical leakage (Figure 4C) was also reduced by 44.3%.

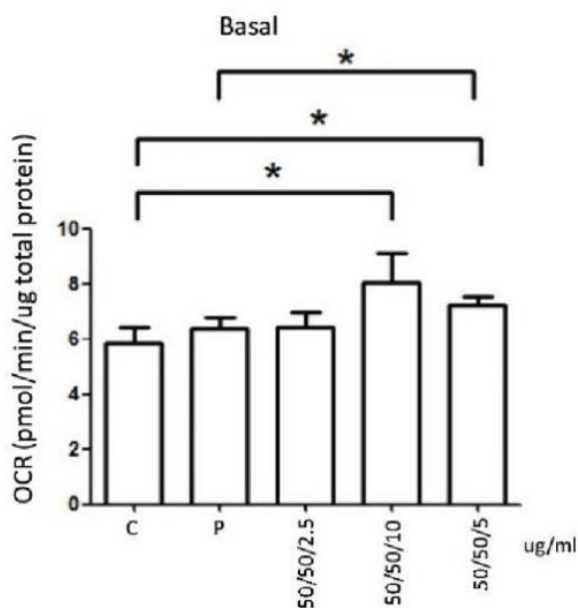


Figure 4A. The comparison between the control group, and when the plant of the invention is extracted

The base energy of cytoskeletal cells increased by 1.13 folds when the concentration of the composition was 50 µg / ml, 50 µg / ml and 5 µg / ml, respectively.

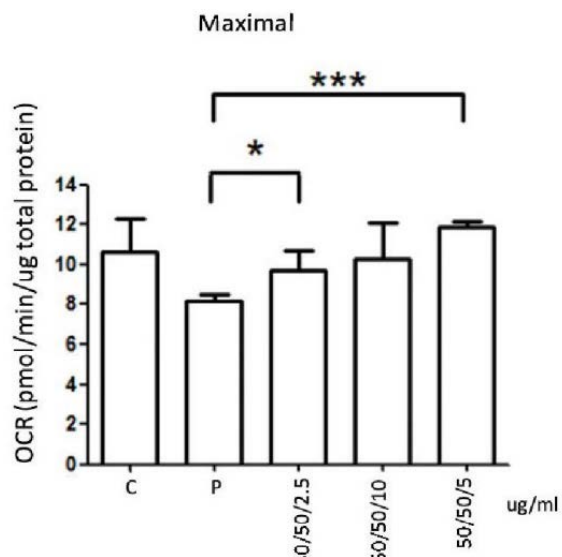


Figure 4B. Limit energy increased by 1.46 times.

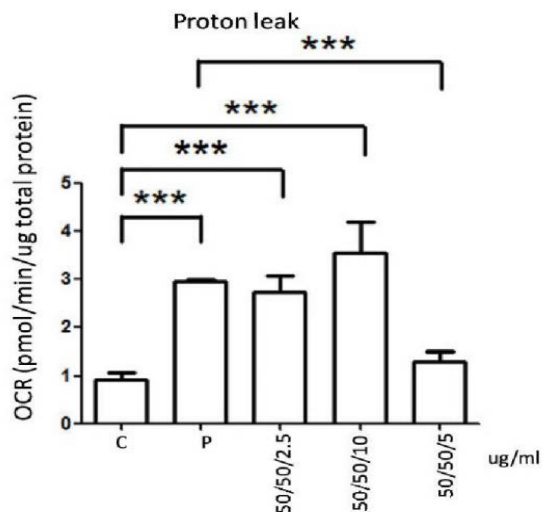


Figure 4C. In addition, the occurrence of free radical leakage was also reduced by 44.3%.

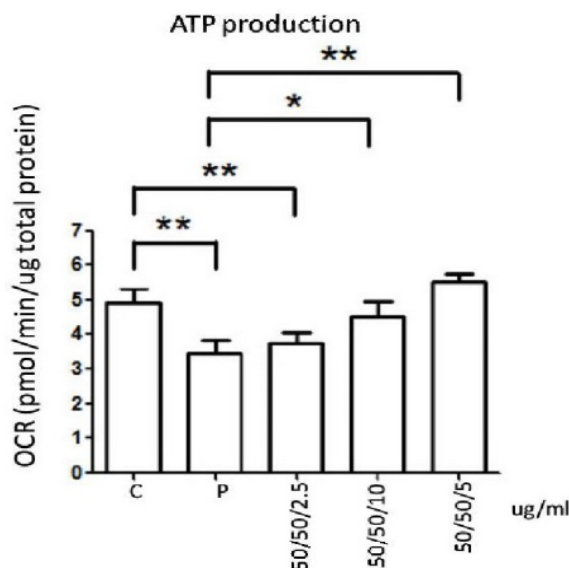


Figure 4D. Resulting in an increase of 1.6 times the ATP energy

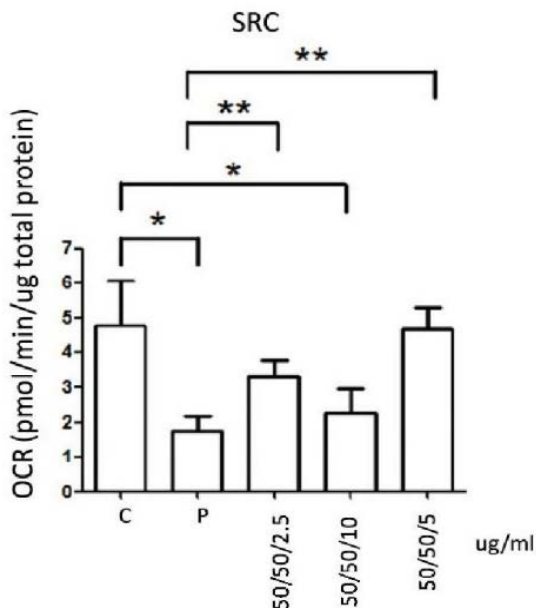


Figure 4E. The pre-stored energy for stress increases by 2.7 times.

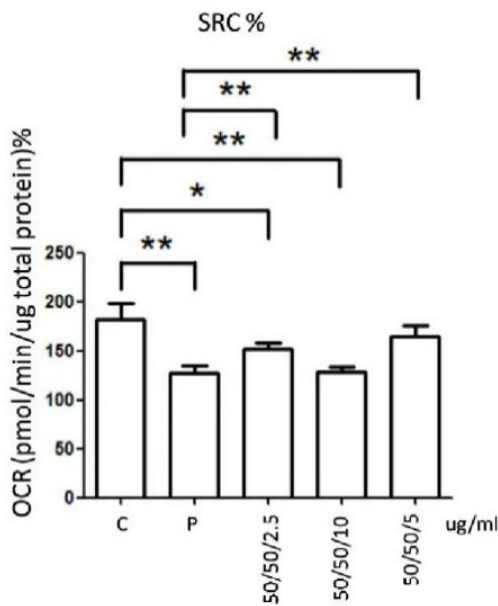


Figure 4F. The energy per unit of mitochondria increased by 1.3 times

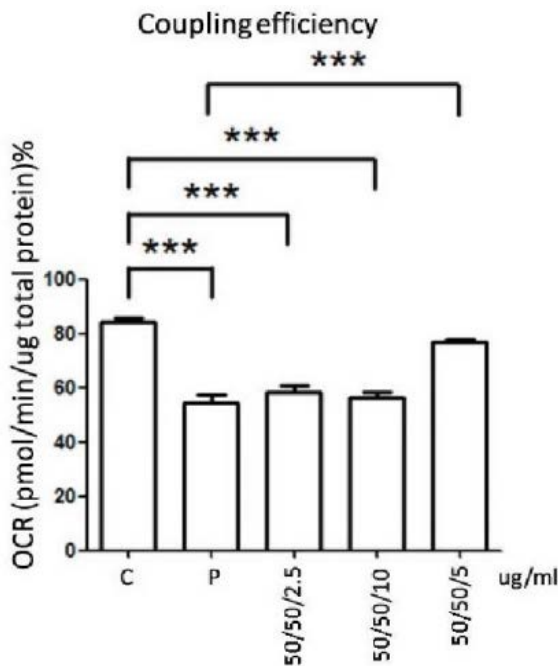


Figure 4G. Oxygen production efficiency of the media also increased by 1.4 times

3.5. Effects of Three - in - One Extractive Composition on Anaerobic Respiration of Cells

Please refer to Figure 5, the control group of cells of mitochondrial function by hydrogen peroxide damage,

The oxygen consumption of the inflamed reaction was higher than that of the control group, but the addition of the plant extract composition of the present invention containing 50 µg / ml of Phyllanthus Emblica, 50 µg / ml of red polyphenol and 5 µg / ml of green tea polyphenol reduced the inflammatory reaction oxygen consumption rate by 34%, showing that the plant extract combination of the present invention effects the of protecting mitochondrial function.

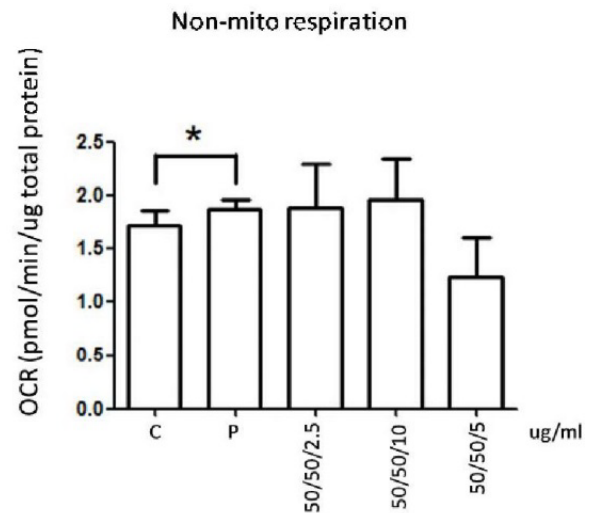


Figure 5. A 34% reduction in the oxygen consumption rate of the inflammatory reaction was shown to show that the plant extract combination of the present invention has the function of protecting the granules

4. Discussion

Phyllanthus emblica extract is usually extracted from leaves for polyphenols as its use. The extract is widely used in anti-inflammatory mechanisms. In some anti-inflammatory experiments that indicate the extent and drug-related. And we also found that these studies are based on its extract as a material and did not show which of the ingredients caused the role, but to polyphenols as a research basis.

Plants are the main source of antioxidants and play a protective role in oxidative stress in biology [1,2].

Therefore, in our study, the antioxidant cascade effect of different plant extracts increased the antioxidant capacity. In addition to the addition of green tea and red wine extract, green tea in many of the literature mainly to EGCG as the main extract and direct use of green tea as the experiment has a significant antioxidant effect [3].

Our study uses green tea extract as one of the ingredients to confirm the efficacy of green tea extract. Red wine is fermented by red grapes as alcohols, and some studies have shown that their antioxidant capacity [4]. The largest study of red wine is the use of its resveratrol antioxidant agent to show anti-inflammatory ability.[5] Tannins show different concentrations in plant foods, and the concentrations are relatively high in green tea and red wine. This experiment provides a new mechanism for the evidence to understand the effects of tannins. They also reveal the underlying pharmacological activity of green tea and red wine-induced biological activity [6].

Based on the study of the above three plant extracts, our study was based on the control of the effects of different plant extracts on the effects of mesenchymal stem cells on the energy of the mitochondrial energy. Food supplements 20170307-EGR is made from Phyllanthus emblica extract, green tea extract, and red wine extract. Our study shows that the food supplement 20170307-EGR increases 39% of the mesenchymal stem cells while also enhancing the activity of the mitochondria and reducing free radical attack on the mitochondria. We can't prove that these

antioxidants prevent the electron transport in the process of particle energy transfer or the formation of an antioxidant wall of these antioxidants to protect the free radicals on the grain of the body attack.

However, we have experimentally confirmed that the mitochondria are protected by this plant complex, and that the more reasonable explanation is that when the mitochondria are protected and energized, these energies are the motivations that increase the number of mesenchymal stem cells.

Our research object is the separation of mesenchymal stem cells from human body fat and centrifugal separation of the mitochondria, our study clearly pointed out that do not add any animal raw materials to avoid interference with human cells. This is our research methods and generally different focus.

5. Conclusion

The antioxidant aim is to reduce the free radicals on the body tissue attack caused by injury or disease. Researchers used a single plant extracts to do antioxidant test, and the compound of plant extracts and when comparing it to the composite plant extracts, it was found that the antioxidant capacity increase significantly [7].

Our experiments clearly show that the free radicals involved in the energy production of the cells are effectively neutralized by many of the free radicals of our complexed plant extracts, and this action defends part of the free attack on body tissues, and also We also significantly enhanced the intracellular activity of mitochondria, and also increased the number of stem cells [8,9,10].

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Compliance with Ethical Standards

Competing interests Material support this study was provided by the Taiwan mitochondrion Ltd. .None of the sponsors (list companies) had any role in the design or execution of this study. None of the authors have any financial interest in any of the products or devices mentioned in this manuscript or any competing financial interests.

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