ACB Bamboo Isoflavones PF
Cell Proliferation Assay (MTT)

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Abstract

The purpose of this study was to determine whether or not ACB Bamboo Isoflavones PF is capable of increasing cellular proliferation by conducting an MTT assay.

Today MTT assays are one of the most accepted and reliable methods used to examine cell proliferation. In this assay the tetrazolium MTT (3-(4,5-deimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), a water-soluble yellow dye, is reduced to formazan, a water-insoluble purple dye. This reduction occurs in the mitochondria of metabolically active cells. A spectrophotometer is then used to quantify the absorbance of the formazan at a wavelength between 500 and 600 nm.

Since the conversion of the yellow dye into the purple dye only occurs in live cells, this conversion is directly correlated to an increase in cellular proliferation. The increase can be quantified by measuring the absorbance of the sample as darker substances are understood to exhibit a higher absorbance.

MTT assays are also commonly used as in-vitro indicators of toxicity. This is because if the absorbance results are lower in the variable than the control that is an indication that the substance affected the viability of the cells, and therefore it should be considered as toxic.

Materials

- MTT solution: 5mg/ml MTT in PBS. (Solution was filter sterilized after adding MTT)
  - Storage temperature: 4°C.
- MTT solvent: 4nM HCL, 0.1% Nondet P-40 (NP40) in isopropanol.
- Tested materials:
  - ACB Bamboo Isoflavones PF (0.1%, 0.5% & 1.0%)
  - FGM

Method

1. A T-25 flask was trypsinized.
2. 5 ml of complete media were added to the trypsinized cells.
3. Cells were centrifuged in a sterile 15 ml falcon tube at 500 rpm in a swinging bucked rotor (~400 x g) for 5 minutes.
4. Media was removed and cells were suspended to 1ml with complete media.
5. Cells per ml were counted aseptically.
6. Complete media was used to dilute cells to 75,000 cells per ml.
7. 100 µl of cells were added into each well.
8. Wells were incubated overnight.

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9. Cells were treated with 30 ng/ml of the tested materials.
10. 20 µl of 5 mg/ml MTT solution were added to each well aseptically.
    - One set of wells with MTT without cells was used as control.
11. Wells were incubated for 3.5 hours at 37°C in culture hood.
12. All media was carefully removed.
13. 150 µl of MTT solvent were added.
14. Wells were covered with tinfoil.
15. An orbital shaker was used to agitate wells for 15 minutes.
16. A reference filter of 620 nm was used to read the absorbance at 590 nm.

Results

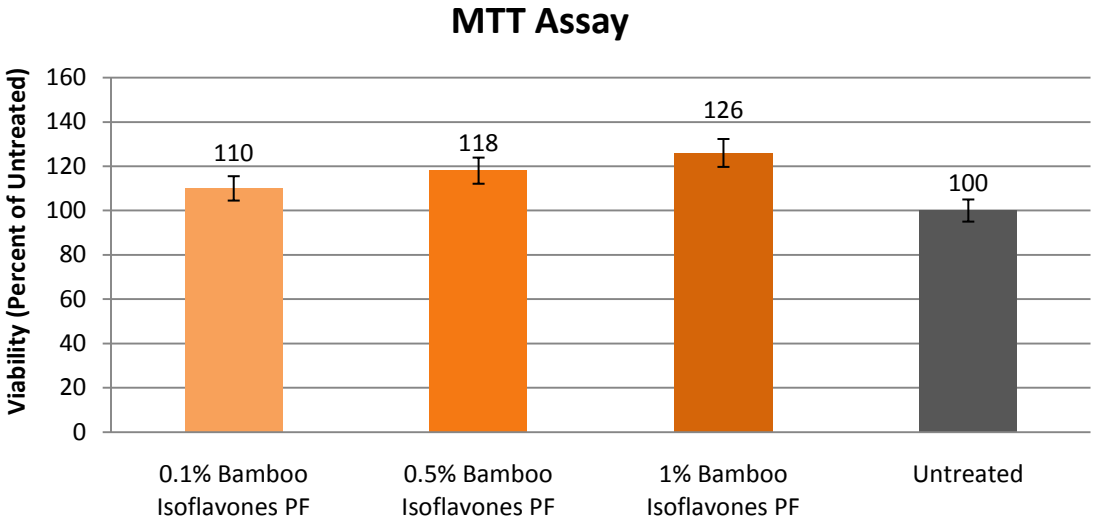

Figure 1. Fibroblast Proliferation Results

Discussion

As shown in figure 1, ACB Bamboo Isoflavones PF exhibited an increase in fibroblast proliferation. However, as the concentration increased, the viability also increased, for this reason we can cocnlude that the cellular proliferation properties of ACB Bamboo Isoflavones PF are dose dependant.

Based on our results, we can confirm that ACB Bamboo Isoflavones PF in addition to providing excellent slip properties and antioxidant benefits, it is also an ideal ingredient to use in cosmetic applications where an increase in cellular proliferation is desired, to help provide a younger, healthier looking and more radiant complexion.