

Vitamin C impact on genistein-induced cell death in prostate cancer



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ABSTRACT

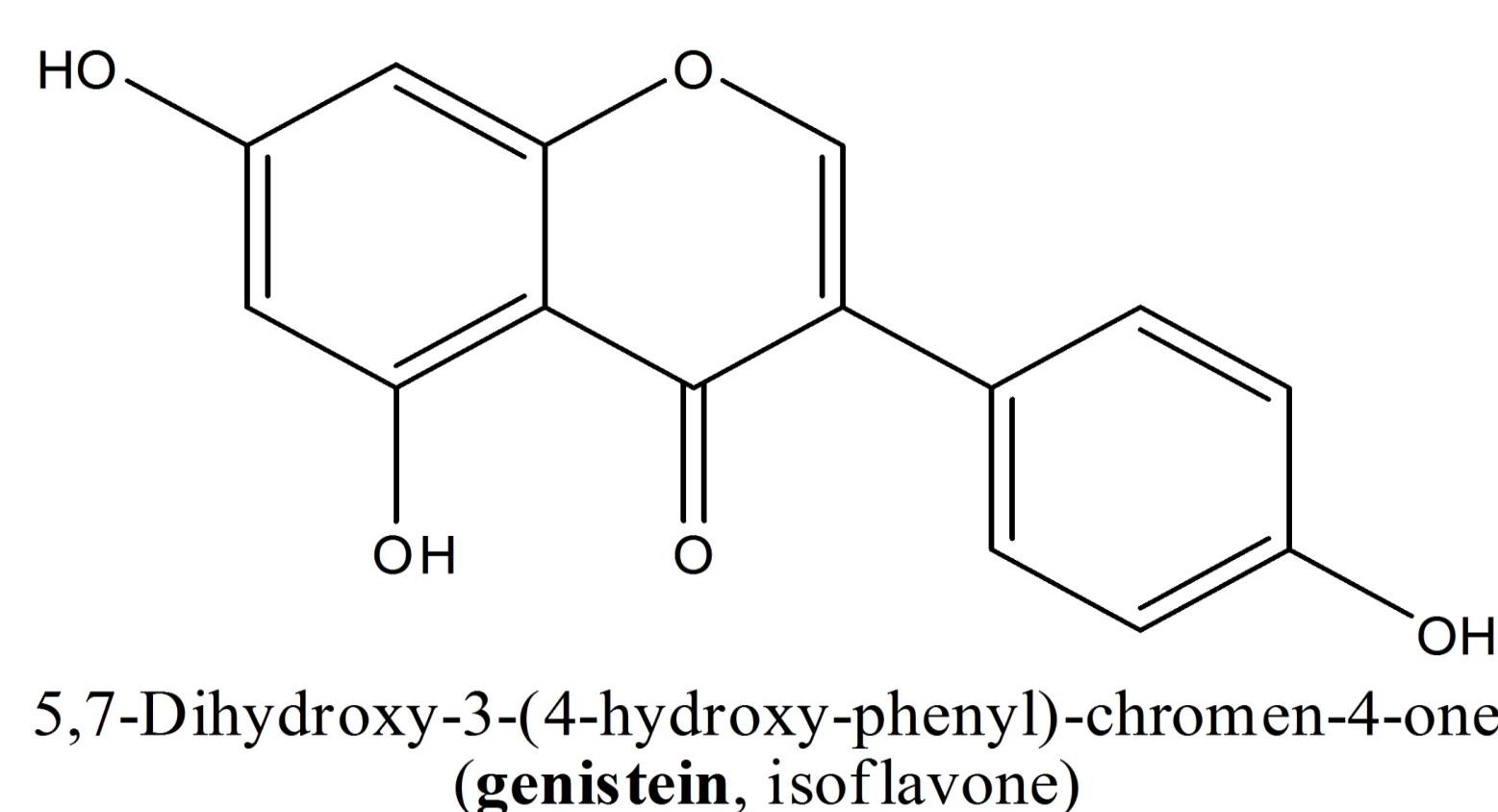
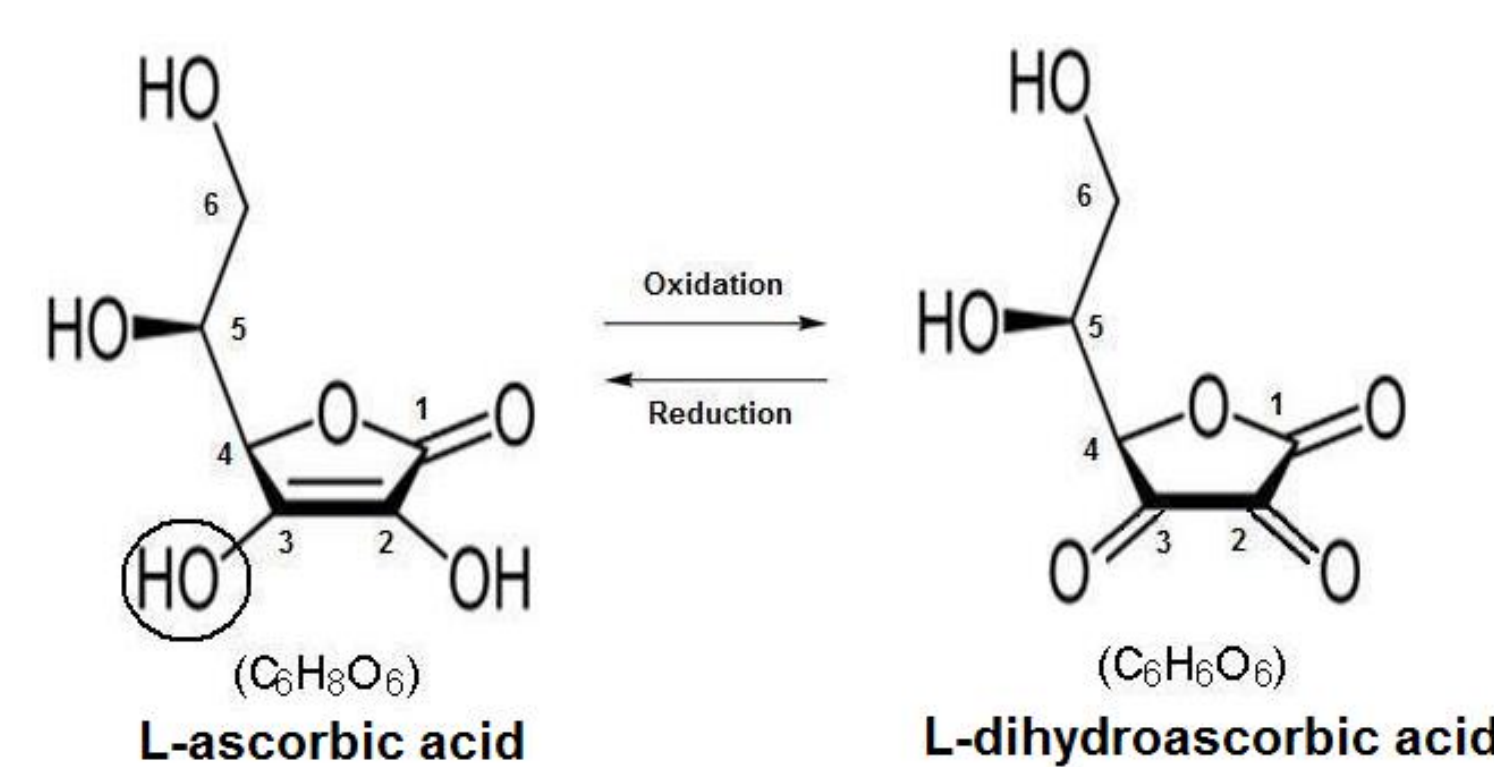
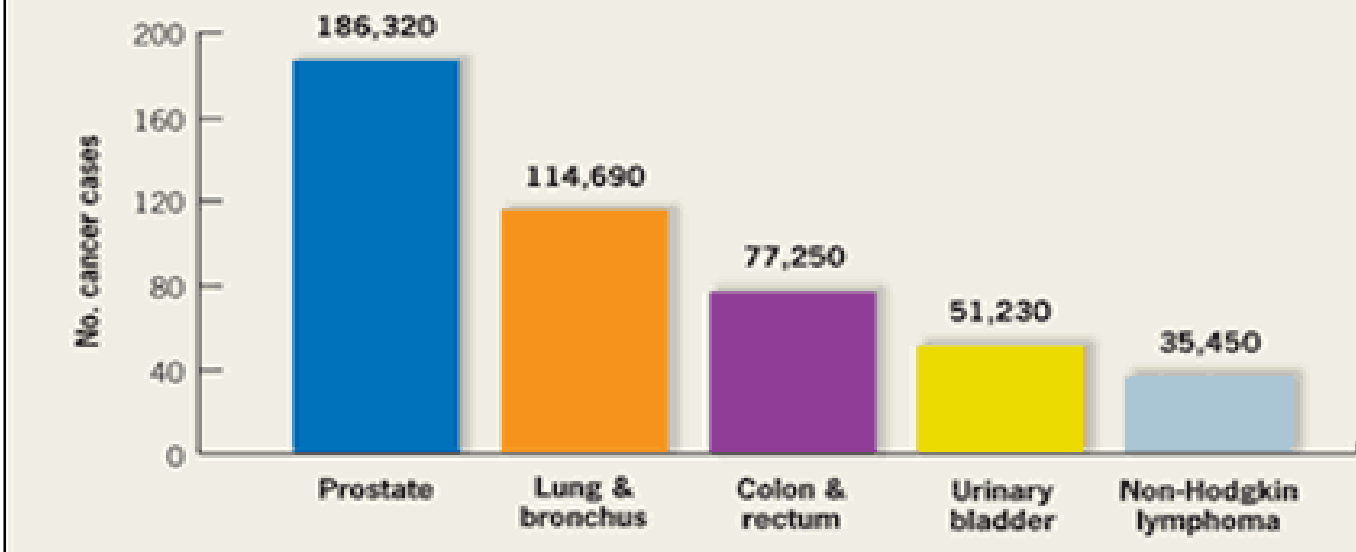
Prostate cancer is the second leading cause of cancer-related death in America. An estimated 220,800 new cases and 27,540 cancer-related deaths are expected in 2015. Reactive Oxygen Species (ROS) can promote cancer cell proliferation when they reach elevated levels. Vitamin C (Vit C) is a water-soluble antioxidant, capable of inhibiting the formation of ROS. Genistein (Gn), an isoflavone found in plants, also possesses the ability to inhibit ROS formation. The purpose of this study was to investigate the impact of vitamin C on genistein-induced apoptosis in LNCaP cells and the potential pathways involved, using cell-based assays including: MTT assay to determine the effect of the various treatments (Gn, Vit C and Gn+Vit C combination) on LNCaP; Nitroblue tetrazolium assay (NBT) to assess treatment-induced intracellular ROS levels; Fluorescence microscopy to determine the mode of treatment-induced cell death. Briefly, LNCaP cells were exposed to varying concentrations of genistein (Gn10-70 μ M) and vitamin C (C10-70 μ M) as single treatments, and Gn-VitC combination. For Gn-VitC combination regimen, the IC₅₀ (40 μ M) of the Vit C (previously determined), was used with each concentration of the genistein. Post-treatment effects on the cells were assessed after 48 hr using the assays listed above.

The overall data from the result revealed a dose-dependent effect in all the three treatments, and apoptosis as the major mode of cell death and that vitamin C significantly augmented the effects of genistein. The combination treatment showed the most dramatic effect, causing most apoptosis. Details of the overall data implicates ROS in the treatment-induced apoptosis and significant positive impact of vitamin C on genistein treatment: an indication of the potential chemo/phyto-preventive significance of the nutrients. Further studies are in progress.

INTRODUCTION

Prostate Cancer Still No. 1

Prostate cancer remains the most commonly diagnosed malignancy among men in the United States. It accounted for one quarter of the 745,180 estimated new cancers diagnosed in men in 2008, according to the American Cancer Society. The top five cancers in American men are shown here.



HYPOTHESIS

Combine treatment of genistein and vitamin C will cause more cells to die by apoptosis than genistein only.

OBJECTIVES

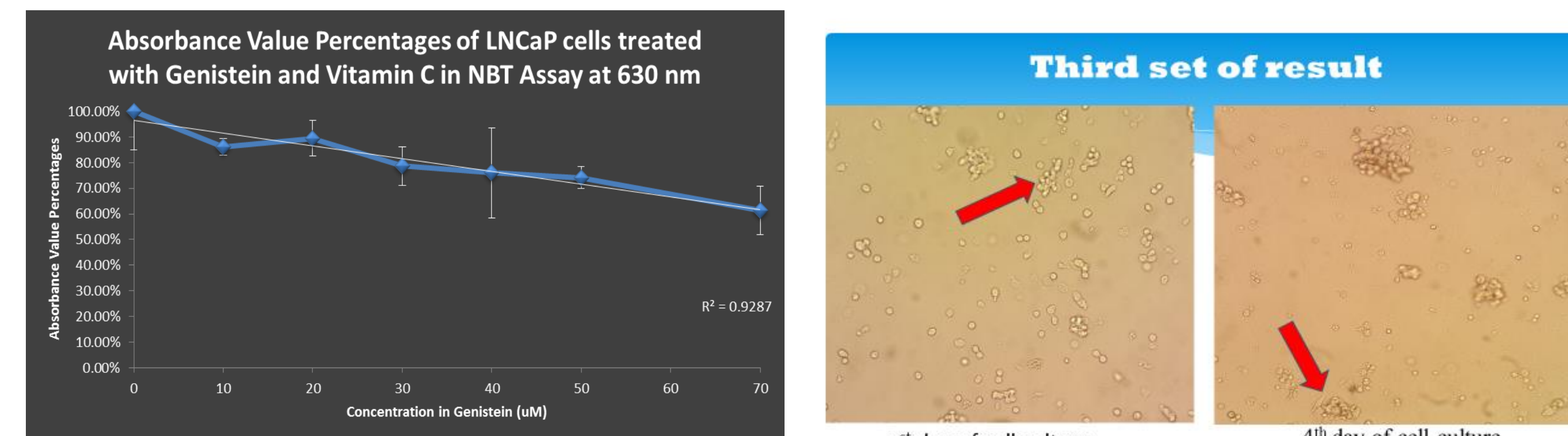
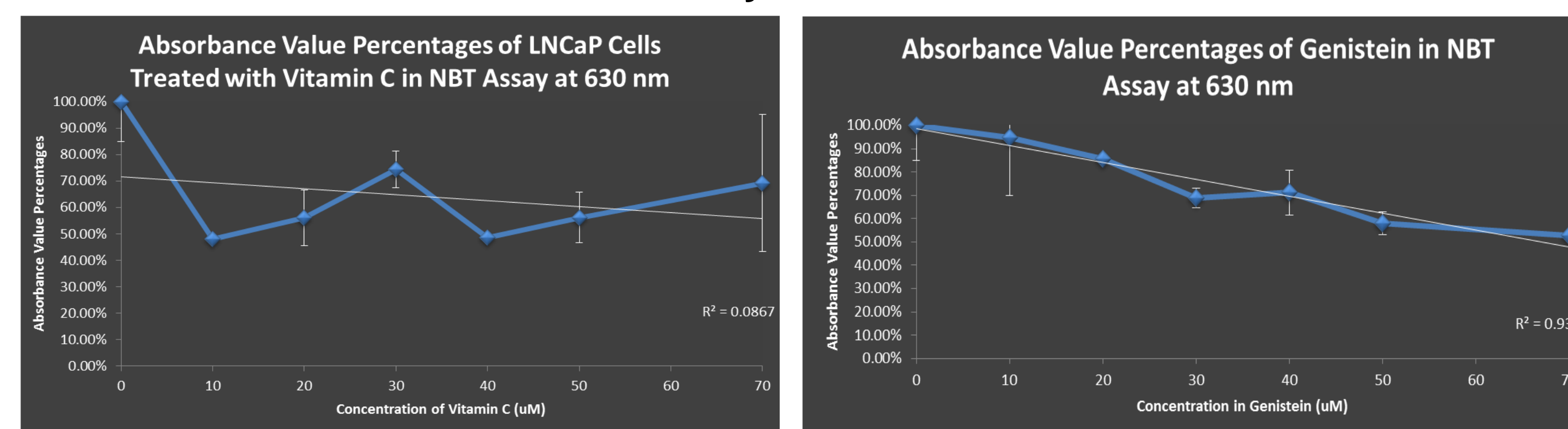
The purpose of this study was to investigate and determine the potential therapeutic additive effect of genistein and vitamin C.

MATERIALS AND METHODS

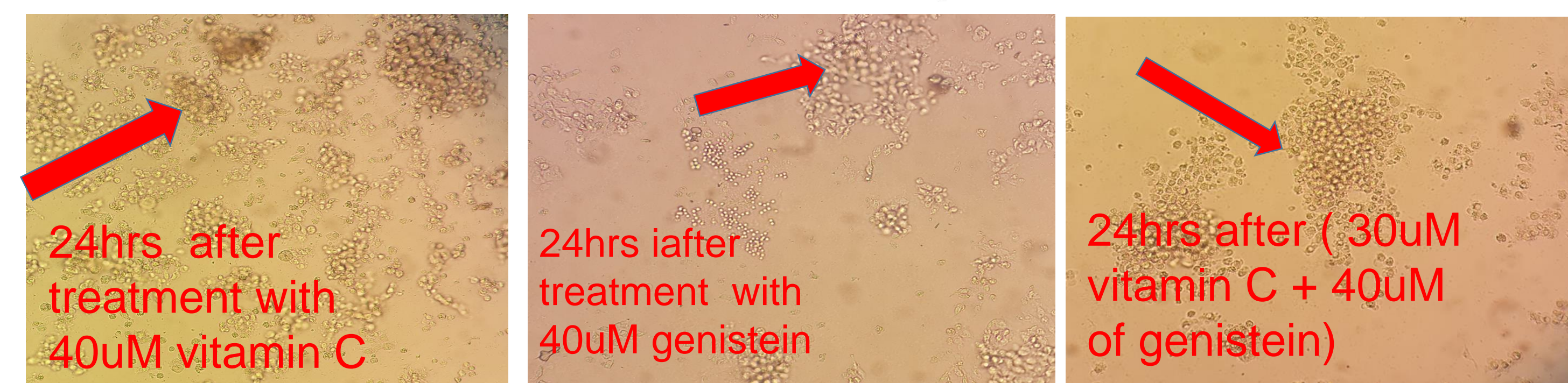
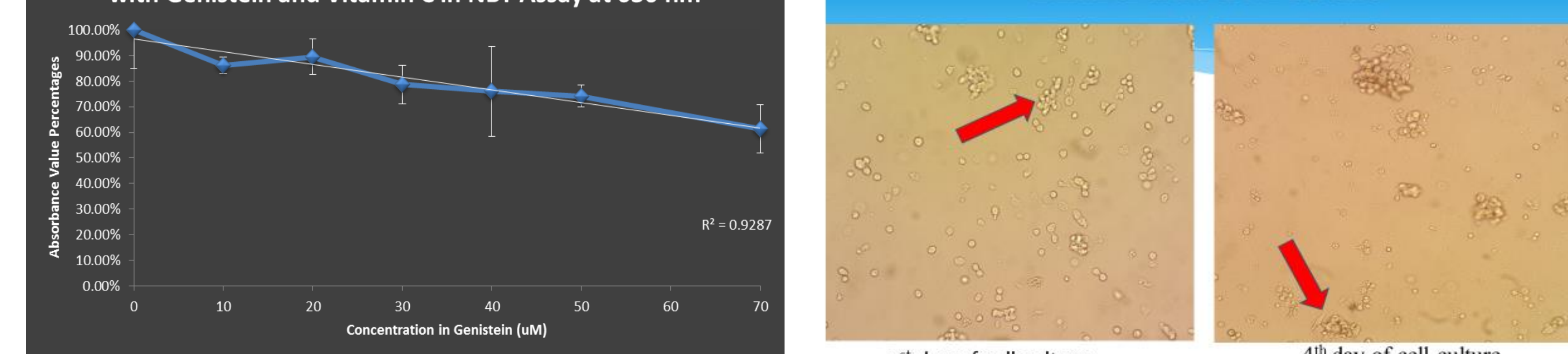
- 1 x 10⁶ LNCaP cells were cultured on RPMI 1640 growth media in an incubator (37^oC and 5% CO₂) until they reach their log early phase (24hrs). At this phase, they are counted using Trypan Blue Assay.
- Cells were then transferred to a 48 well ELISA plate. They were cultured once again to reach their log phase.
- The cells were treated with vitamin C, genistein and a combination of vitamin C and genistein in different treatment groups. The concentrations of each treatment ranges were 10 μ M, 20 μ M, 30 μ M, 40 μ M, 50 μ M and 70 μ M.
- For the combined treatment, a constant vitamin C concentration of 30 μ M was used along side concentrations of genistein ranging from 10 μ M to 70 μ M.
- After 24 hours from treatment, the cells were analyzed for viability using the MTT assay and ELISA plate reader.
- The absorbance readings from the ELISA plate were converted to percentages and plotted on the Y-axis against the concentration of each treatment drug on the x-axis.
- Statistical analysis was used to confirm whether a significant difference existed in each group.

RESULTS

NBT Assay Results



Third set of result

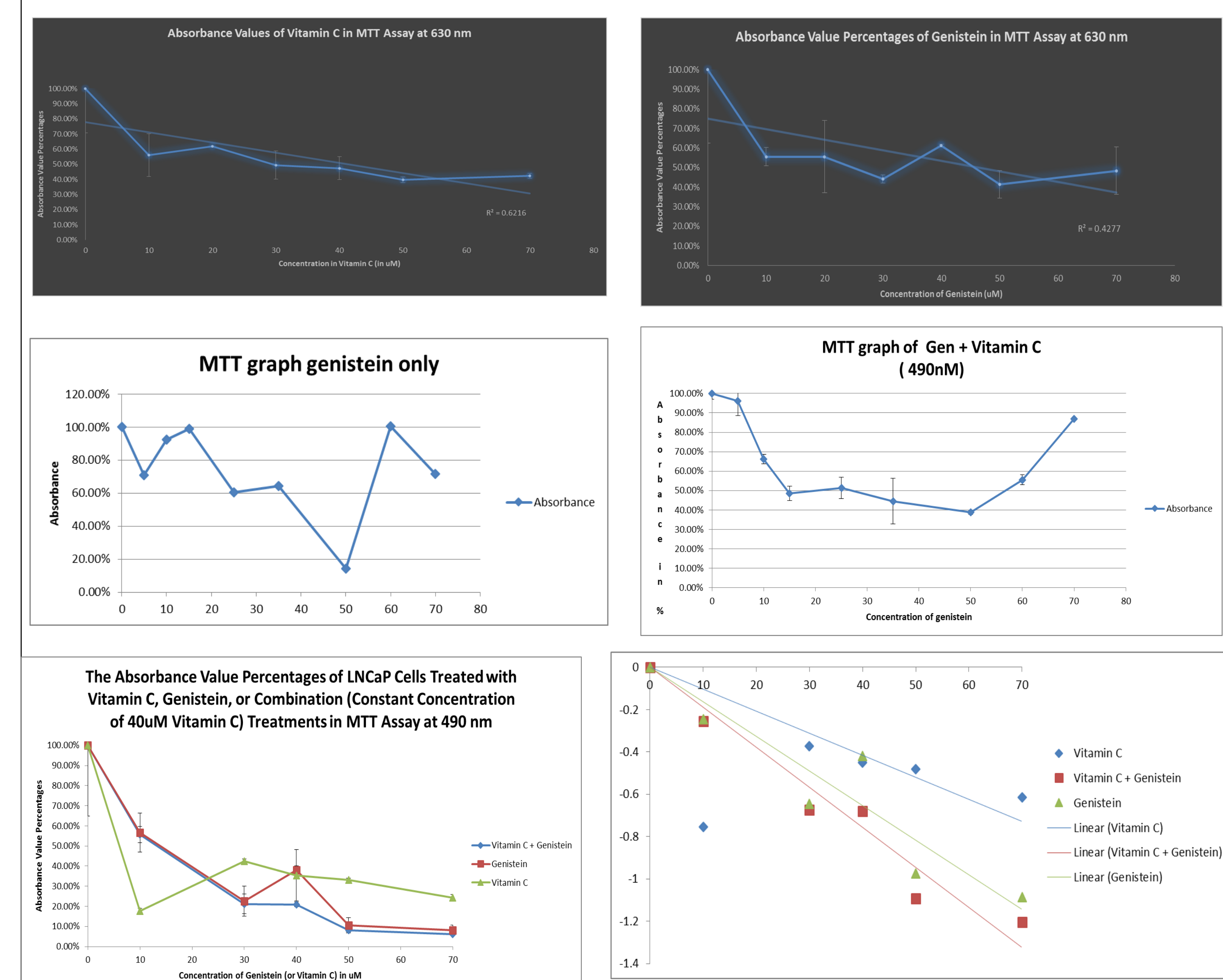


CONCLUSION

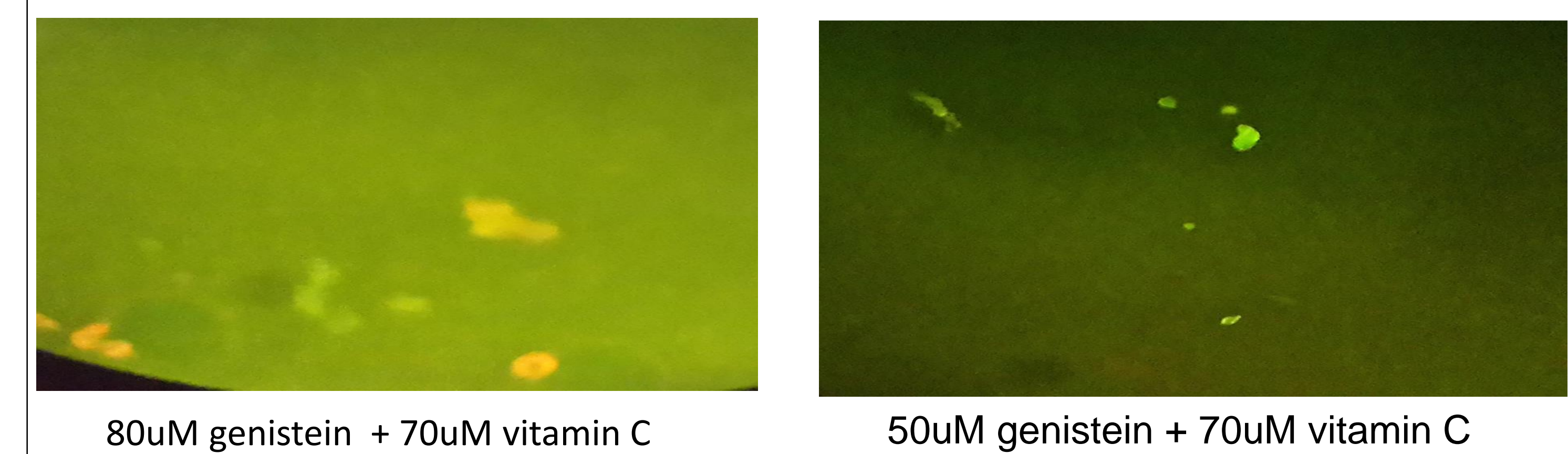
Combination treatment is more effective against prostate cancer. Fluorescence assay shows different rate of apoptosis at different combination concentration. Decrease in absorbance reflects a decreased level of intracellular ROS at various concentrations. Combination treatment reduce the concentration of genistein needed to achieve IC-50 as compared to genistein only.

DISCUSSION

MTT Assay Results



Fluorescence assay of combination treatment



The preliminary data support the hypothesis. This is because the bi-phasic nature of genistein was impacted on the treatment group. A decrease in the population of LNCaP cells was then proceeded by this spike. Induced apoptosis is one of the pathway that vitamin C and/ or genistein exert their cytotoxic effects. The significance of this is that vitamin C can be used as adjuvant in chemotherapy. Through induced apoptosis, vitamin C and genistein can kill cancer cells in a manner that is much less deleterious to the surrounding cells. Thus, these limit the damage done to the surrounding cells of the body through necrosis. The fluorescence assay result shows that that cells treated with the combination treatment are actually dying as a result of apoptosis.

REFERENCES

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