



# A Novel Nutrient Mixture Induces Apoptosis in Human Prostate Cancer Cell Line Du-145

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## **Abstract**

### **Introduction:**

Prostate cancer, the major cancer affecting males and second most deadly cancer in the United States, primarily affects males aged 55 and older and African Americans more than Caucasians. Once prostate cancer has metastasized, current treatment methods are generally ineffective. We have identified a novel antineoplastic agent, a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract that demonstrates a broad spectrum of antitumor activity against a number of human cancer cell lines.

### **Objective:**

We investigated whether the cytotoxic effect of NM on DU145 was consistent with its apoptotic effect on these cells.

### **Method and Materials:**

Prostate cancer DU-145 (ATCC) cells were cultured in MEM and treated with NM in different concentrations, 0, 100, 500 and 1000 µg/ml concentration. Growth inhibition was detected by MTT assay, morphology by H&E staining, and apoptosis by Live Green Caspases Detection Kit.

### **Results:**

NM was slightly toxic to DU145 cells at 100-µg/ml and profound at 500 and 1000 µg/ml. DU-145 cells treated with NM at 500 and 1000 µg/ml concentration and stained by H&E demonstrated obvious apoptotic cells. Apoptotic cells showed shrinkage with darkly stained and condensed nuclei and strongly acidophilic cytoplasm. Using caspases kit, slight apoptosis was observed at 100 µg, moderate at 500 and significant at 1000 µg/ml concentrations.

### **Conclusions:**

The results suggest that NM induces apoptosis in prostate cancer cell line DU-145 and raises the possibility of its use as a new chemotherapeutic agent.

## Comment:

A novel antineoplastic agent, a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract demonstrates a broad spectrum of antitumor activity against a number of human cancer cell lines. We investigated whether the cytotoxic effect of NM on DU145 was consistent with its apoptotic effect on these cells. DU-145 cells treated with NM at 500 and 1000 µg/ml demonstrated profound toxicity and obvious apoptotic cells morphologically. Using caspases kit, slight apoptosis was observed at 100 µg, moderate at 500 and significant at 1000 µg/ml concentrations. The results are significant as they suggest NM as a therapeutic agent for prostate cancer, the major cancer affecting males and second most deadly cancer in the United States.

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### Method and Materials:

1. Prostate cancer DU-145 (ATCC) cells were cultured in MEM and treated with NM in different concentrations, 0, 100, 500 and 1000 µg/ml.  
2. Growth inhibition was detected by MTT assay, morphology by H&E staining, and apoptosis by Live Green Caspases Detection Kit.

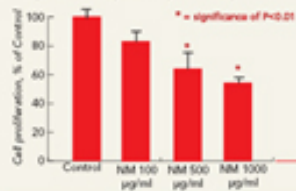
### Composition of the Nutrient Mixture (NM)

Nutrient	Proportion
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	710 mg
L-Lysine	1000 mg
L-Proline	750 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (80% polyphenol)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

### Results:

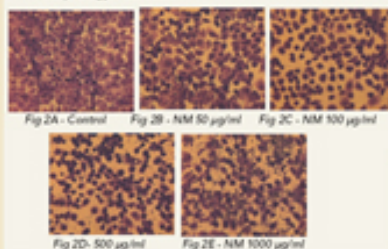
1. The nutrient mixture (NM) was slightly toxic to prostate DU-145 cells at 100 µg/ml and profound at 500 µg/ml (36%, p=0.01) and 1000 µg/ml (46%, p=0.001), as shown in Figure 1.

Figure 1 - Effect of NM on prostate DU-145 cell proliferation/cytotoxicity: MTT assay



2. Prostate DU-145 cells treated with the nutrient mixture (NM) at 500 and 1000 µg/ml and stained with H&E demonstrated obvious apoptotic cells. Apoptotic cells showed shrinkage with darkly stained and condensed nuclei and strongly acidophilic cytoplasm, as shown in Figures 2A - E.

Figure 2 - Effect of NM on prostate DU-145 cell morphology: H&E stains



3. Using the live green caspases kit, slight apoptosis was observed at 100 µg/ml, moderate at 500 µg/ml and significant at 1000 µg/ml, as shown in Figures 3A-D and Figure 4.

Figure 3 - Effect of NM on prostate DU-145 cell apoptosis: live green caspase detection kit

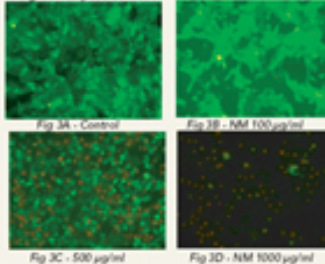
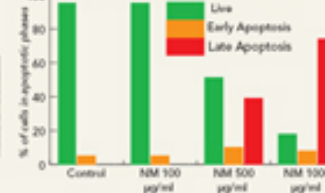


Figure 4 - Effect of NM on prostate DU-145 cell apoptosis: percent of cells in apoptotic phases



### Conclusion:

The results suggest that the nutrient mixture induces apoptosis in prostate cancer cell line DU-145 and raises the possibility of its use as a new chemotherapeutic agent.