#1503 Anti-cancer effects of a nutrient mixture in human melanoma cells A2058: inhibition of cell proliferation, MMP expression, invasion and apoptosis

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1. Introduction:

Melanoma, an extremely aggressive cancer, causes the majority of skin cancer-related deaths, secondary to metastais to other organs of the body, such as lymph nodes, lungs, liver, brain or bone. Currently there are no viable treatments for melanoma.

2. Objective:

We investigated the effect of a special mixture (PB) of quercetin, cruciferex, curcumin, green tea extract and resveratrol on human melanoma cell line 2058 for viablity, MMP expression, invasion, apoptisis and cell morphologoy.

3. Materials and Methods:

Human melanoma cells A2058 (ATCC) were maintained in DMEM culture, supplemented with fetal bovine serum (FBS) and antibiotics in 24-well tissue culture plates. AT near confluence, cells were treated with PB at 0, 10, 25, 50, 75 and 100 µg/mL concentration, in triplicate at each does. Cells were also treated with 100 ng/mL phorbol 12-myristate 13-acetate (PMA). Cell proliferation was assessed by MTT assay, MMPs by gelatinase zymography, invasion through Matrigel, cell migration by scratch test, apoptosis using live green caspase detection kit (Molecular Probe), and morphology by H&E staining.

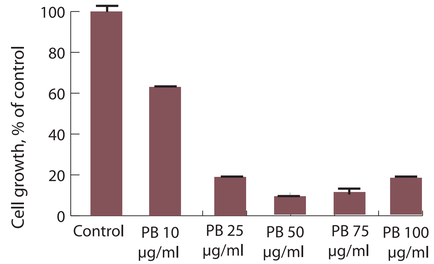
Composition of the phytobiological mixture (PB)

Nutrient	Amount (% of total weight)
Quercetin	400 mg (27.6%)
Cuciferex	400 mg (27.6%)
Curcumin	300 mg (20.7%
Standardized Green Tea Extract (80% polyphenol)	300 mg (20.7%)
Resvertrol	50 mg (3.4%) mg

4. Results:

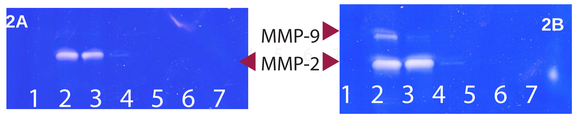
1. PB inhibited proliferation of melanoma cells A2058 by 45% at 10 µg/ml and ~80% at 25-100 µg/ml concentration, as shown in Figure 1.

Figure 1 - Effect of PB on growth of melanoma A2058 cells



2. Zymography demonstrated MMP-2 in untreated melanoma A2058 cells and induction of MMP-9 by PMA. MMP-2 and MMP-9 were inihbited by PB in a dose-dependent fashion with virtual blockage of both MMPs at 50 µg/ml, as shown in Figure 2.

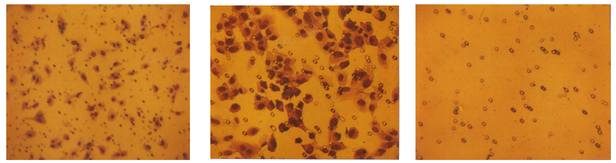
Figure 2- Effect of NM on secretion of MMP-2 and -9 by normal (2A) and PMA (100 ng/mL)-treated (2B) melanoma A2058 cells



Legend: 1 -Markers, 2- Control, 3-7 PB 10, 25, 50, 75, 100 µg/ml

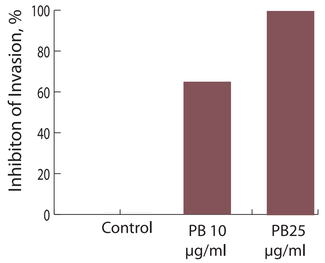
3. Invasion through Matrigel was inhibited by 65% at 10 and 100% at 25 µg/ml PB. See Figures 3 and 4.

Figure 3- Photomicrographs of effect of PB on melanoma A2058 invasion through Matrigel



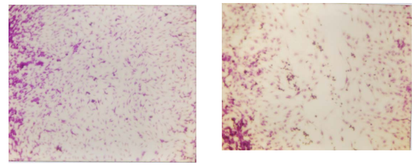
Control PB 10 µg/ml PB 25 µg/ml

Figure 4- Effect of PB on melanoma A2058 invasion through Matrigel

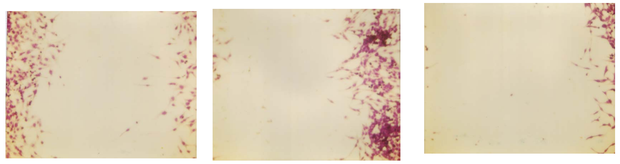


4. The cell migration assay (scratch test) revealed dose-dependent inhibition of melanoma A2058 cell migration by PB with complete block at 75 µg/mL, as shown in Figure 5.

Figure 5- Effect of PB on migration of melanoma A2058 cells: scratch test



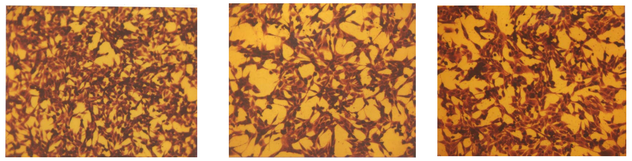
Control PB 10 µg/ml



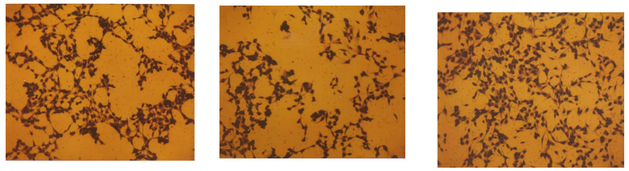
PB 25 µg/ml PB 50 µg/ml PB 75 µg/ml

5. H& E staining showed no morphological changes in melanoma A2058 exposed to PB at lower concentrations and slight changes at higher concentrations, as shown in Figure 6.

Figure 6- Effect of PB on morphology of melanoma A2058 cells



Control PB 10 µg/ml PB 25µg/ml



PB 50 µg/ml PB 75 µg/ml PB 100 µg/ml

6. PB induced apoptosis in a dose-dependent fashion. (No figures shown.)

5. Conclusion:

In conclusion, PB significantly inhibited melanoma cell growth, invasion through Matrigel, MMP-2 and -9 expression, and cell migration, as well as induced apoptosis, improtant parameters for cancer prevention, suggesting PB as a potential candidate for therapeutic use in the treatment of melanoma.