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HP8 herbal formula selectively inhibits prostate cancer cell line proliferation by inducing G2/M cell cycle arrest.

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Abstract:

HP8, a formula of Australian and European herbs and other natural ingredients, has been evaluated *in vitro* for its anti-proliferative effects against androgen independent prostate cancer cell line PC-3 and the androgen dependent prostate cancer cell line LNCap cultured by standard in vitro techniques. Its biological activity against these cell lines was compared to its activity against non-prostate cancer cell lines, HepG2 (liver cancer), and HL60 (human leukaemia).

Inhibition of the *in vitro* proliferation PC-3 and LNCap cell lines occurred in a dose dependent manner. Analysis by flow cytometry determined that cell division

was arrested at the G2/M phase of the mitosis cycle resulting in apoptosis within 24 hours. Significantly lower rates of G2/M arrest and apoptosis in the non-prostate cancer cell lines demonstrated that HP8's inhibitory effects were selective toward prostate cancer cell lines.

At the highest dose tested, 0.32 mg/mL, considered a physiologically relevant level, HP8 caused G2/M cell cycle arrest in 98.22% of the PC-3 cells in culture and in 77% of LNCap cells, but only 14.86% of HepG2 cells, and 52.68% HL60.

The results lead us to speculate that a potential mode of action of HP8 may be similar to that of Taxol which

exerts its effects by disrupting mitotic spindle function thus inducing cell cycle arrest. HP8 selectively inhibits the proliferation of prostate cancer cells and as such it has the potential for use as an anti-cancer agent in treatment of this disease.

In culture with the PC-3 prostate cancer cell line, HP8 inhibited proliferation in a dose dependent manner. Photomicrographs were conducted to physically assess cell damage using control cells (no HP8 – Figure A), lower concentration of HP8 0.08 mg/50uL (Figure B), and higher concentration at 3.2 mg/50uL (Figure C). The higher concentration indicates clearly using PC-3 androgen independent prostate cancer cell line both cell lysis and cell fragmentation are induced, however there is also evidence of significant lysis and cell fragmentation at the lower concentration.

Figure A. PC3 cell culture – control (no HP8)

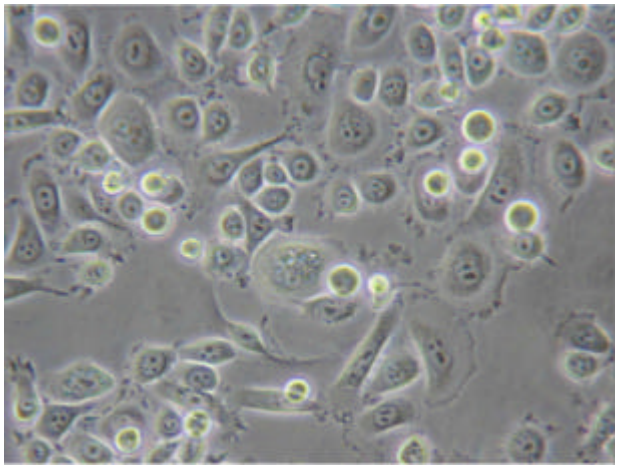


Figure B. PC3 culture with HP8 at .8mg/50uL
- moderate lysis and fragmentation.

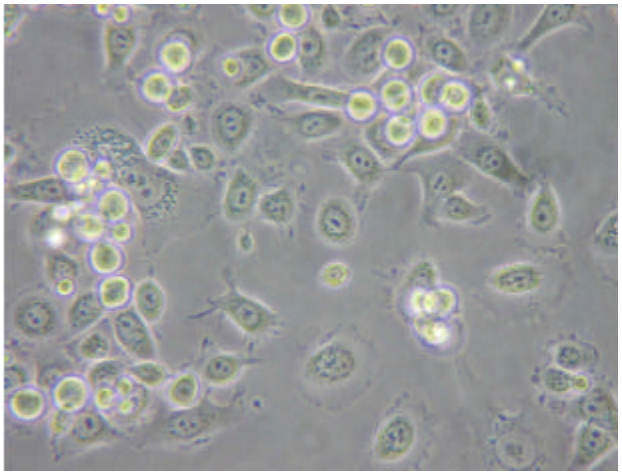
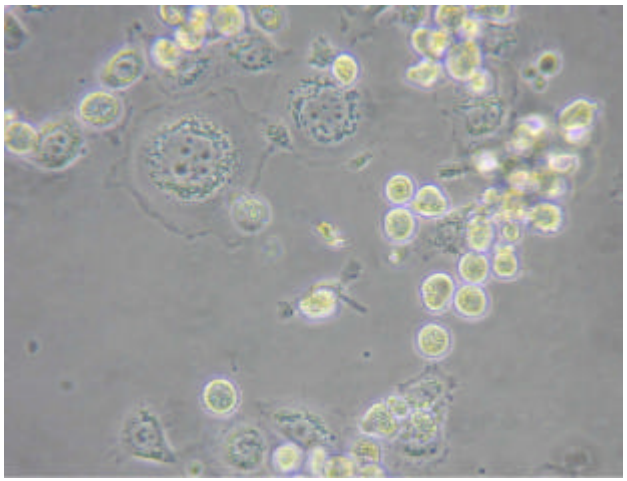
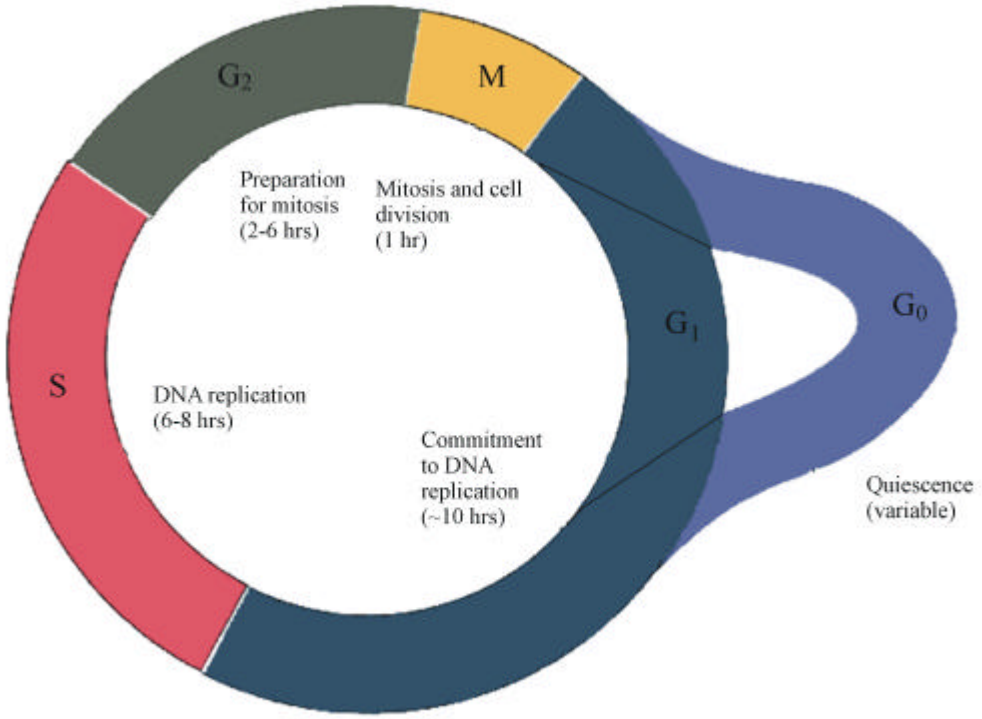


Figure C. PC3 culture with HP8 at 3.2mg/50uL,
- extensive lysis and fragmentation.



Studies suggested that HP8 exerts its effect by disrupting mitotic spindle function, inducing cell cycle arrest.



Cancerous cells are fast dividing cells and so will exhibit a well-defined cell cycle, separated into the several distinct phases. It starts at the G1 phase or the first gap phase. If the cells are permanently arrested in G1, as in non-dividing cells, we call this the G0 phase. At G1 the cell contains two copies of each chromosome. As the cell progresses from G1 phase it enters the synthesis or S phase, and during this phase DNA is replicated. When the replication is completed the cell enters the second

gap phase or G_2 phase. At the completion of G_2 phase the cell is ready to enter mitosis and at this point the cell divides to form two new cells. Many drugs used in “the fight against cancer” influence the cell cycle at these critical points.

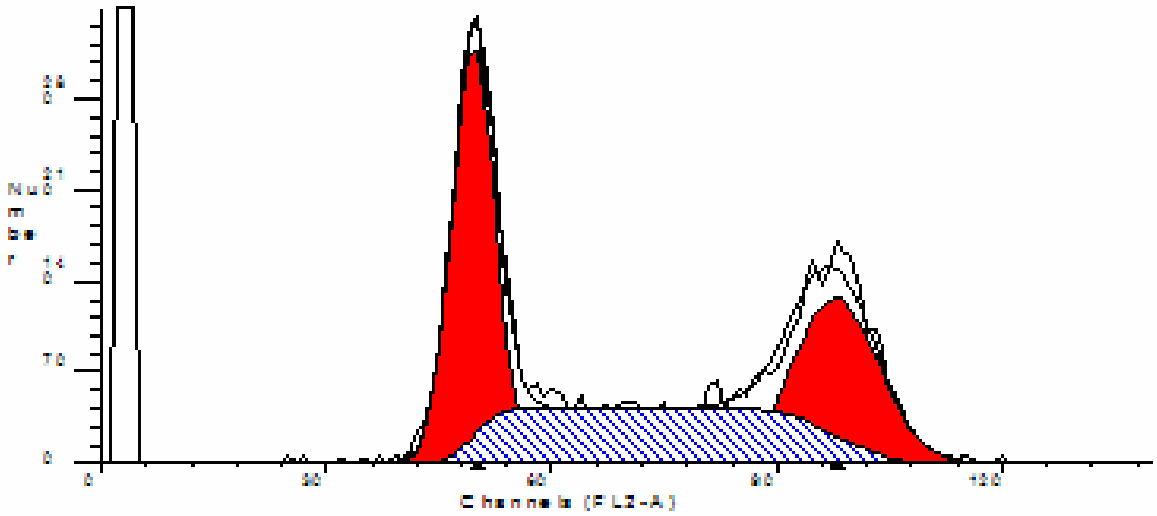
The changes induced during cell division by a compound or product, such as HP8, can be studied using the technique of flow-cytometry. This experimental method can sort a cell population into the various phases in which they exist after a treatment by a specific compound.

Method of cell cytometry to determine cell cycle effects: *A known weight of the test product was extracted with 50 mL of methanol. The extracts were sonicated for 15 minutes and then spun down at 4000 rpm. The supernatant was removed the extract was used in the cell cycle analysis. Cells were split into 16 x 25 cm^2 flasks at a low concentration and allowed to grow to 65-70% confluency (at the time of addition the cells were in log phase). Flasks were incubated for 24 hours and control flasks of Control media (no additions), a Control ethanol, a Control methanol, HP8 (Extract equivalent to 5.88mg of tablet/10ml of cell culture) and Taxol (10µL of 0.01 mg/mL=0.01ng/mL) were also run. Flow cytometry was performed on the fixed cells after washing in PBS and stained using propidium iodide (2% PI in 0.1% Triton X-100 containing 2 mg/mL Rnase A). A Becton Dickson FACSCaliber was used to assay the cells.*

Cell cycle analysis on the PC-3 hormone independent prostate cancer cell, indicate in a) control, the normal proportion of cells to be found in the G_1/G_0 and in the G_2/M phases of the cell cycle; b) results for HP8 clearly indicate that almost all cells were exhibiting cell cycle arrest at the G_2/M phase (>95%). c) Taxol, a widely used drug known to arrest cell division in the G_2/M phase, an effect confirmed in this experiment. This demonstrates that HP8 has a specific mode of action that is similar to Taxol.

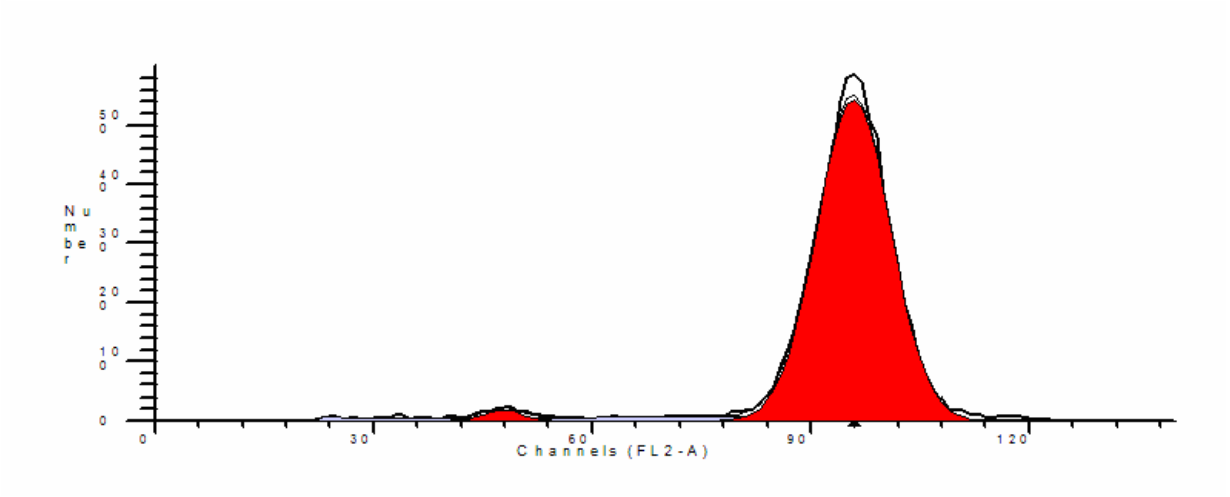
a) PC-3 / Control

G_1/G_O G_2/M



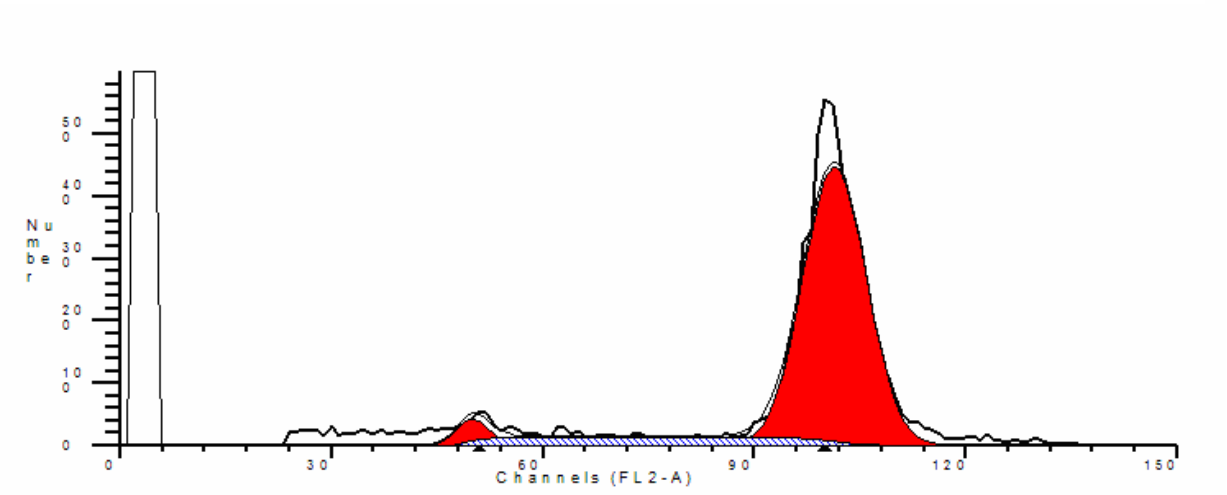
b) PC-3/ HP8

G_1/G_O G_2/M



c) PC-3 / Taxol

G_1/G_O G_2/M



HP8 induced G2/M phase cell cycle arrest, provoking apoptosis and inhibiting proliferation in a similar manner against the LNCap prostate cancer cell line.

Percentage Changes in G_0/G_1 and G_2/M for HP8 and PC-SPES on LNCap Cell Line

Treatment	Concentration (mg $10mL^{-1}$)	G_0-G_1	G_2-M
"HP8"	0.4	62.72	10.83
	0.8	72.23	9.91
	1.6	10.88	79.69
	3.2	10.73	77.23
Control		80.79	5.31

HP8 exhibited prostate cancer specific cytotoxicity as it was markedly less effective against the non-prostate cancer cell lines HepG2 (liver cancer), and HL60 (human leukemia).

Percentage Changes in G_0/G_1 and G_2/M for HP8 and PC-SPES on HL60 Cell Line			
Treatment	Concentration (mg $10mL^{-1}$)	G_0/G_1	G_2/M
"HP8"	0.4	31.65	13.74
	0.8	32.44	12.07
	1.6	28.65	21.89
	3.2	12.34	52.68
Control		34.79	12.77

Percentage Changes in G_0/G_1 and G_2/M for HP8 and PC-SPES on HepG2 Cell Line			
Treatment	Concentration (mg $10mL^{-1}$)	G_0/G_1	G_2/M
"HP8"	0.4	61.97	14.74
	0.8	60.14	16.5
	1.6	58.94	13.7
	3.2	54.16	14.86
Control		61.05	16.04