

## Phyto-oestrogens: do they have a role in breast cancer therapy?

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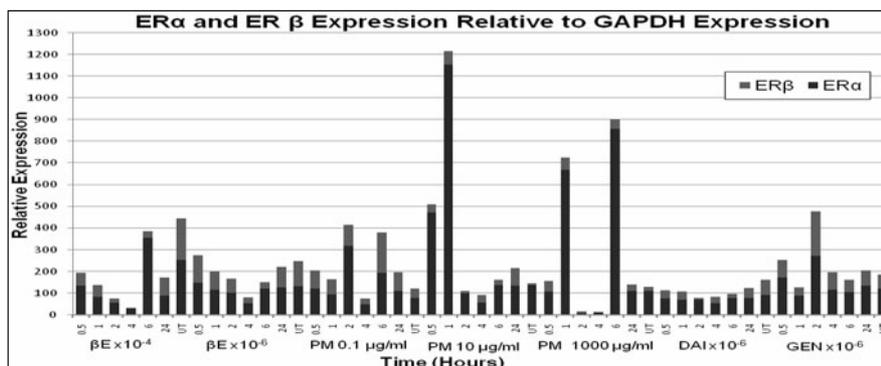
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Breast cancer is the most common malignancy in women<sup>(1)</sup>. Each year 44 335 women living in the UK are diagnosed with breast cancer<sup>(2)</sup>. Female sex hormones, such as oestrogen, are clearly implicated in breast cancer and conventional treatments include manipulation of the hormonal environment. There is both epidemiological<sup>(3,4)</sup> and experimental<sup>(5)</sup> evidence that plant oestrogens (phyto-oestrogens) in the diet may alter oestrogen metabolism, but the potential role of phyto-oestrogens in the management of breast cancer is unknown.

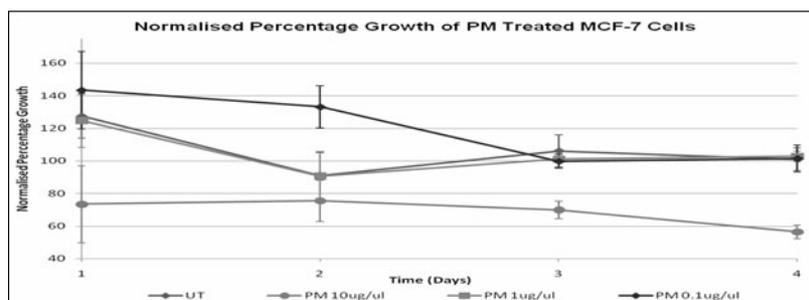
Ethnobotanical information has provided evidence of a potential anti-cancer effect of Kwai Krua root, *Pueraria Mirifica* (PM)<sup>(6,7)</sup>, a herb commonly used in Thailand for enhancing female health. The herb contains a mixture of phyto-oestrogens including genistein (GEN), daidzein (DAI) and miroestrol. The aim of the present study was to evaluate the possible role of phyto-oestrogens, in particular those present in PM, in the management of breast cancer. The investigation was carried out using an *in vitro* model that included: powdered extract of PM (concentrations ranging from 0.1 µg/ml to 1000 µg/ml); β-oestradiol (βE; concentrations 10<sup>-2</sup>M–10<sup>-6</sup>M); GEN (concentrations 10<sup>-8</sup>M–10<sup>-4</sup>M); DAI (concentrations 10<sup>-8</sup>M–10<sup>-4</sup>M); MCF-7 oestrogen receptor (ER)-positive breast cancer cell line (ATCC HTB-22). The effect of phyto-oestrogen exposure on cell growth over time was also monitored.

Tissue-culture techniques included: CyQuant™ assay (Invitrogen/Molecular Probes®, Paisley, UK); MTT and lactate dehydrogenase assays; immunostaining; flow cytometry; mRNA and protein extraction; RT-PCR; Western blotting; SDS gel electrophoresis. A powdered extract of Thai root PM was prepared at a stock concentration of 10 mg/ml in dimethyl sulfoxide and diluted in medium to various concentrations for use. βE, daidzein and GEN were prepared at stock concentrations in dimethyl sulfoxide and diluted as appropriate. MCF-7 ER-positive cells were maintained in culture for use.

Initial results indicated that ERα:ERβ mRNA expression appeared to change over time (0.5–24 h), suggesting a possible modulation of the receptors occurring at the level of the mRNA. This effect was particularly notable over the range of PM concentrations. GEN also produced similar effects on mRNA levels. Cell-growth studies over 4 d, as examined using the CyQuant™ assay, indicated low growth rates for the PM-treated cells (especially at 10 µg/ml) with an increasing effect over time that appeared to reach significance (*n* 4; ANOVA; *P* = 0.038) by day 3 or 4 when compared with untreated cells.



**Fig. 1.** For each treatment expression of ERα and ERβ mRNA is shown. Time points used were 0.5, 1, 2, 4, 6, and 24 h. Expression of ERα is higher for PM treatments (10 and 1000 µg/ml); PM at 0.1 µg/ml is in line with βE and GEN. Expression of ERβ is decreased for PM at 10 and 1000 µg/ml, and similar to BE and GEN for PM at 0.1 µg/ml. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.



**Fig. 2.** Effects of PM on MCF-7 growth over 4 d. The maximum concentration used was 10 µg/ml, as higher concentrations were previously found to be directly toxic to the cells. Reduction in growth was significant (*P* < 0.05) for PM at 0.1 µg/ul over 4 d.

These preliminary results are indicative of a potential anti-cancer action of PM that may be of use in the treatment of breast cancer. Further studies are required to confirm this possibility.

This study was funded by Solgar USA, Bio-Botanica and Smith Naturals. The MCF-7 ER-positive cells were a gift from CRUK.

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