



## SAW PALMETTO BERRY EXTRACT INHIBITS CELL GROWTH AND COX-2 EXPRESSION IN PROSTATIC CANCER CELLS

WOLFGANG H. GOLDMANN<sup>1</sup>, AMITA L. SHARMA<sup>1</sup>, STEVE J. CURRIER<sup>2</sup>, PAUL D. JOHNSTON<sup>2</sup>,  
AJAY RANA<sup>3</sup> and C. PAL SHARMA<sup>1\*</sup>

<sup>1</sup>Boston BioProducts Inc., Ashland, MA 01721; <sup>2</sup>PharmaPrint Inc., Irvine, CA 92612; and <sup>3</sup>Massachusetts General Hospital, Boston, MA 02114, U.S.A.

Received 14 August 2000; accepted 2 April 2001

The cytotoxicity of a commonly used material to alleviate the symptoms of benign prostatic hyperplasia (BPH), Saw Palmetto Berry Extract (SPBE), was examined as neat oil using a set of prostatic cell lines; 267B-1, BRFF-41T and LNCaP. Proliferation of these prostatic derived cell lines is inhibited to different degrees when dosed for 3 days with SPBE. The amount of SPBE required to inhibit 50% growth (IC<sub>50</sub>) of these cell lines was 20–30 nl equivalents of SPBE per ml of medium for cell lines 267B-1 and BRFF-41T and approximately 10-fold more for the LNCaP cell line. The effect of SPBE dosing on these cell lines is not irreversible, since a 30 min treatment with SPBE at an IC<sub>50</sub> concentration does not inhibit their growth. Normal prostate cells were inhibited by 20–25% when grown in the presence of 200 nl SPBE equivalent per ml media. Growth of other non-prostatic cancer cell lines, i.e. Jurkat and HT-29, was affected by approx. 50% and 40%, respectively. When LNCaP cells were grown in the presence of dihydrotestosterone and SPBE, the IC<sub>50</sub> concentration decreased significantly compared to LNCaP cells grown in the presence of serum and SPBE. Reduced cellular growth after SPBE treatment of these cell lines may relate to decreased expression of Cox-2 and may be due to changes observed in the expression of Bcl-2. Expression of Cox-1 under similar conditions is not affected because of its constitutive expression. Since increased Cox-2 expression is associated with an increased incidence of prostate cancer, and decrease in its expression by SPBE would provide a basis for further investigation of its use against BPH and in prostatic cancer chemoprevention.

© 2001 Academic Press

KEYWORDS: Saw Palmetto berry extract; cell growth inhibition; MTT and SRB assays; Cox-2; Cox-1; Bcl-2.

### INTRODUCTION

Phytotherapeutic formulations based on Saw Palmetto berry extract (*Serenoa repens*) (SPBE) have traditionally been used for treating prostate-related problems, and clinical research has supported the application of SPBE in the fight against benign prostatic hyperplasia BPH (Wilt *et al.*, 1998;

Plosker and Brogden, 1996; DiSilverio *et al.*, 1998; McKinney, 1999; Lowe and Ku, 1996). The combination of this extract with other herbs has been shown to lower not only testosterone concentrations but also the concentration of prostate-specific antigens (DiPaola *et al.*, 1998). Researchers have further demonstrated in animal studies that SPBE inhibits the binding of dihydrotestosterone DHT to its receptor (Carilla *et al.*, 1984; Sultan *et al.*, 1984) and blocks the conversion of testosterone to DHT by inhibiting the activity of 5- $\alpha$ -reductase. So far the mechanism of action is not known for this phytotherapy but it may include an anti-oestrogenic, anti-androgenic, anti-spasmodic,

\*To whom all correspondence should be addressed. C. Pal Sharma, Boston Bioproducts Inc., 200 Homer Ave, Ashland, MA 01721, U.S.A. Tel: +1 508 881 7755; Fax: +1 508 881 7368; E-mail: Cpalsharma@msn.com. Dr Goldmann's current address: Renal Unit, Massachusetts General Hospital, Room 8200, Building 149, 13th Street, Charlestown, MA 02129, U.S.A. This article is dedicated to Willy Goldmann who died in March 2001 at the age of 78.

or anti-inflammatory effect, or the inhibition of 5- $\alpha$ -reductase, since all of these activities have been documented for it in *in vitro* experiments (Stoner, 1996; Carraro *et al.*, 1996).

In the present study, we investigate the role of SPBE in prostate cancer by comparing the growth of prostatic cancer cell lines in the presence and absence of SPBE. The data presented here demonstrate that SPBE inhibits the growth of a normal prostatic derived cell line and two prostatic carcinoma cell lines. The results may suggest an 'operating mechanism' involving growth inhibition via alterations in the expression of Bcl-2 and prevention of prostate carcinoma development through the inhibition of expression of Cox-2.

## MATERIALS AND METHODS

### *Cell lines and cell culture*

Human prostate cancer cell lines, 267B-1 and BRFF-41T, were obtained from BRFF (Ijamsville, MD, U.S.A.), and LNCaP, Jurkat (lymphoma) and HT-29 cells (human colon adenocarcinoma) were from ATCC (Rockville, MD, U.S.A.). Normal human prostate epithelial cells were purchased as growing cultures and maintained as described by the supplier (BioWhittaker, Walkersville, MD, U.S.A.). Cell lines were grown in media as recommended by the suppliers i.e., 267B-1 cells in P4/8F media, BRFF-41T cells in basal media BRFF-HPC1, LNCaP and Jurkat cells were maintained in RPMI and HT-29 in DMEM supplemented with 10% serum. All cell lines were maintained at 37°C in humidified atmosphere of 5% CO<sub>2</sub> in air. The cells were maintained as sub-confluent monolayers in T-25 flasks and sub-cultured once or twice per week. Cell passage levels were between 2–8 after receipt of cell lines from the supplier. 267B-1 and BRFF-41T cell lines were developed from prostate carcinoma (Type P). Antibodies against Cox-1 and Cox-2 were obtained from Cayman Chemical Company (Ann Arbor, MI, U.S.A.) and Bcl-2 from Transduction Laboratory (San Diego, CA, U.S.A.). All other reagents were purchased from Sigma Chemicals.

### *Preparation of SPBE*

The neat SPBE oil was originally prepared as an ethanolic extract of Saw Palmetto berries obtained from PharmaPrint (Irvine, CA, U.S.A.). A homogeneous stock solution of the SPBE for test was prepared by diluting the SPBE with ethanol (20% solution, w/v) at room temperature and shaking

with constant agitation for at least 10 h. This preparation was passed through 3-mm filters and finally centrifuged at 1000  $\times$  g for 10 min to remove herbal debris. This preparation was then concentrated by passing a stream of nitrogen over it. The concentrated material was stored in the dark at room temperature until use. Fresh dilutions were prepared for each experiment to minimize any non-specific degradation of SPBE constituents.

### *MTT assay*

MTT assays were performed as described by Mosmann (1983) with minor modifications. Briefly, exponential growing cells were harvested by trypsinization, counted, and then plated on flat-bottomed 96-well culture dishes. Optimal seeding densities for each cell line were determined and used to ensure exponential growth during 4-day assays. Cells ( $1 \times 10^4$ ) in 100  $\mu$ l media per well were seeded and allowed to attach overnight. Initially, a 10% stock solution of SPBE was prepared in ethanol and all further dilutions were made in cell growth media. To each well, 100  $\mu$ l of media containing different concentrations of SPBE were added. After 3 days, 20  $\mu$ l of MTT solution (5 mg/ml) were added to each well. Following 4 h of incubation at 37°C, the media was removed and formazan crystals, which result from the reduction of MTT by active cells, were dissolved in 50  $\mu$ l of 0.04 N HCl-isopropanol. The optical density at 590 nm was determined by a microplate reader. All experiments were performed in triplicate.

### *Sulfo rhodaminesulfate-B (SRB) assay*

SRB assays were performed as described by Skehan *et al.* (1990). Cell plating and incubation were carried out in the same way as described for the MTT assay. The cells were then fixed with 50  $\mu$ l 50% trichloroacetic acid at 37°C for 1 h. The cells were washed five times with deionized water, dried, and stained by the addition of 50  $\mu$ l SRB solution (0.4% SRB in 1% acetic acid). Following staining, cells were first washed five times with 1% acetic acid to remove unbound dye and then air-dried. Subsequently, bound dye was solubilized with 100  $\mu$ l of 10 mM Tris base (pH 10.5) and detected at 560 nm by a microplate reader.

### *Cell survival assay, counting, and data analysis*

Cell plating was performed in the same way as in MTT assays. After cell growth, fresh media was used (0.2 ml) containing various concentrations of SPBE and cells were then incubated at 37°C. After

30 min of incubation, the cells were washed again with fresh medium and grown for 3 d in the absence of SPBE. MTT assays were performed as described above.

Trypsinized cells were seeded in 96-well plates as described for the MTT assay. After the cells had been cultured for three days, the medium was removed and the cells were incubated with 50  $\mu$ l of trypsin-EDTA solution for 5 min at room temperature. Cells were suspended in trypsin-EDTA solution and counted with a hemocytometer. Cell counting was carried out in duplicate, and for each sample, the mean of all fields was calculated.

Microplate absorbance data were exported to Microsoft Excel, and the average as well as the standard deviation were calculated. The final data were analysed in Cricket Graph, which was used to prepare the figures.

#### Western blotting

Cells were seeded at a density of 500,000 cells/well in 6-well plates. Once the cells reached 80–90% confluency, the medium was replaced by serum-free medium for 24 h. The cells were induced at different concentrations of FCS for 24 h in the presence and absence of various concentrations of SPBE. The cells were then washed in PBS, removed, centrifuged and, lysed for 15 min on ice using 50 mM Tris-HCl, 110 mM NaCl, 1% Triton-X100, 5 mM EDTA; 10  $\mu$ g/ml each of leupeptin, chymostatin, antipain, pepstatin, and 2 mM phenylmethylsulfonyl-fluoride (PMSF). The lysates were centrifuged in an Eppendorf minifuge for 10 min at 4°C, and aliquots of 5  $\mu$ l from each sample were used to quantify the protein using Bradford dye. Cell lysates were denatured in Laemmli sample buffer, and 50–100  $\mu$ g of protein were analysed by 12% SDS-Page under reducing conditions. Following SDS-Page, proteins were transferred electrophoretically onto sheets of Immobilon-P membrane (Millipore, Bradford, MA, U.S.A.) in a buffer containing 50 mM Tris-glycine, pH 8.3, and 20% methanol for 5–6 h or overnight at a constant current of 60 mA. Transferred proteins were visualized with Coomassie blue and identified separately with polyclonal antibodies against Cox-1, Cox-2, and Bcl-2.

## RESULTS

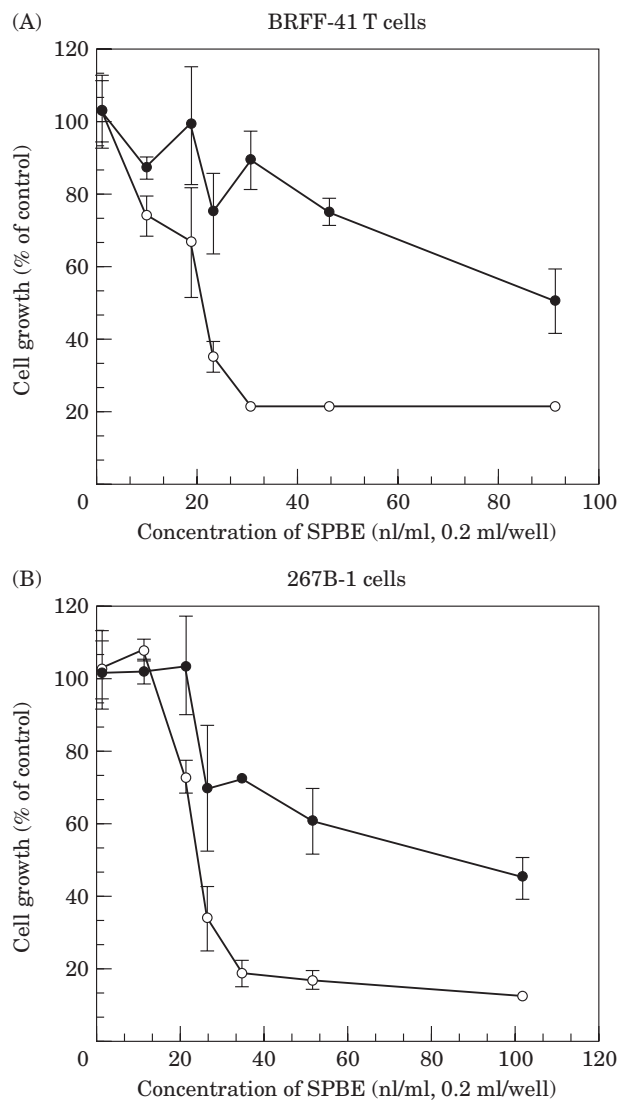
#### Effect of SPBE on cell growth

Prior to measuring the effect of SPBE on the proliferation or cytotoxicity of different prostate-

derived cell lines, we performed a series of MTT assays to optimize cell plating assay conditions. For BRFF-41T and LNCaP cells, we determined the range to allow for a linear relationship between cell number and the MTT assay readout, and decided to plate 20,000 cells per well in a 96-well plate. In all subsequent experiments using other cells, 10,000 cells were added per well.

Initial analyses determined the effects of SPBE on the growth of prostate-derived cell lines. SPBE was prepared as described in Materials and Methods. The cells were either grown in the presence of SPBE or incubated for 30 min and then grown in the absence of SPBE. BRFF-41T and 467B-1 cells in the presence of SPBE were completely inhibited at concentrations ranging between 40–50 nl equivalents per ml of media, resulting in an  $IC_{50}$  between 20–25 nl equivalents per ml of media (Figs 1A and 1B, cytotoxicity assay, open circles; Table 1). In contrast, the  $IC_{50}$  for LNCaP cells was in the range of 200 nl equivalents per ml of media (Fig. 2A, closed squares; Table 1). No inhibition of cell-growth was observed when cells were only grown in the presence of vehicles (Fig. 2A, closed circles). Normal prostate cells—when grown in the presence of 200 nl SPBE equivalent per ml media—were partially inhibited (20–25%), suggesting a specificity of SPBE for prostate cancer cells (Fig. 2B). To further demonstrate the specificity of SPBE, we also assessed the cytotoxicity of SPBE on HT-29 and Jurkat cells. When these cells were grown in the presence of 200 nl of SPBE, their growth decreased by about 40–50% (Fig. 2C), thus suggesting a more specific effect of SPBE on prostate cancer cell lines.

A 30-min pulse of the cell lines with SPBE at its  $IC_{50}$  concentration was not effective in inhibiting the growth of any of these cell lines (Figs 1A, B, cell survival assay, closed circles). Since prostate cell growth can be modulated by male hormones (Sonnenschein *et al.*, 1989; Lee *et al.*, 1995), we determined what impact the male hormone DHT has on the cytotoxicity/proliferation of the more SPBE-resistant LNCaP cell line. Usually, this hormone acts as a mitogen enhancing cellular growth. When these cells were grown in the presence of different concentrations of DHT, they responded favourably up to 1 nM. At higher concentrations, the growth of LNCaP cells decreased slightly in comparison to cells grown in 1 nM DHT (data not shown and Lee *et al.*, 1995). Under these conditions, it was found that there was a significant decrease in the  $IC_{50}$  concentration for SPBE when LNCaP cells were co-cultured in 1 nM DHT and



**Fig. 1.** Effect of SPBE on the growth of BRFF-41T and 267B-1 cells. Trypsinized cells suspended in HPC-1 media were seeded on fibronectin-coated, flat-bottomed 96-well culture dishes. After incubation for 24 h the medium was replaced with medium prepared in the presence/absence of different concentrations of SPBE. The MTT assay was performed after three days of cell culturing. The cell survival assay was carried out as follows: the cells were trypsinized and grown as mentioned above. The media containing different concentrations of SPBE were placed in wells for 30 min, and the cells were washed and further incubated in fresh media containing no SPBE. After three days of cell growth, the MTT assay was performed. —●—, Cell survival; —○—, cytotoxicity.

with different equivalents of SPBE (Fig. 2A, closed triangles, and Table 1).

Since the MTT assay is dependent on the reduction of a tetrazolium salt to formazan by mitochondria of viable cells, its reaction can correlate with both cell number and incubation time. It is known that this reaction is also sensitive to the concen-

**Table 1.**  
Effect of SPBE on different cell lines' growth as measured by the MTT assay

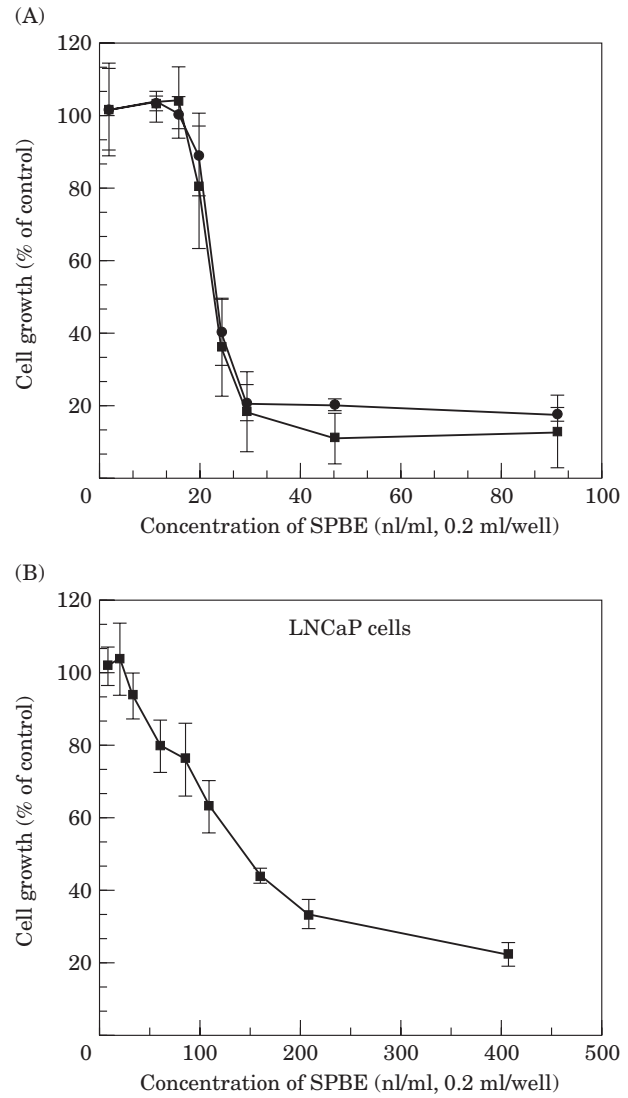
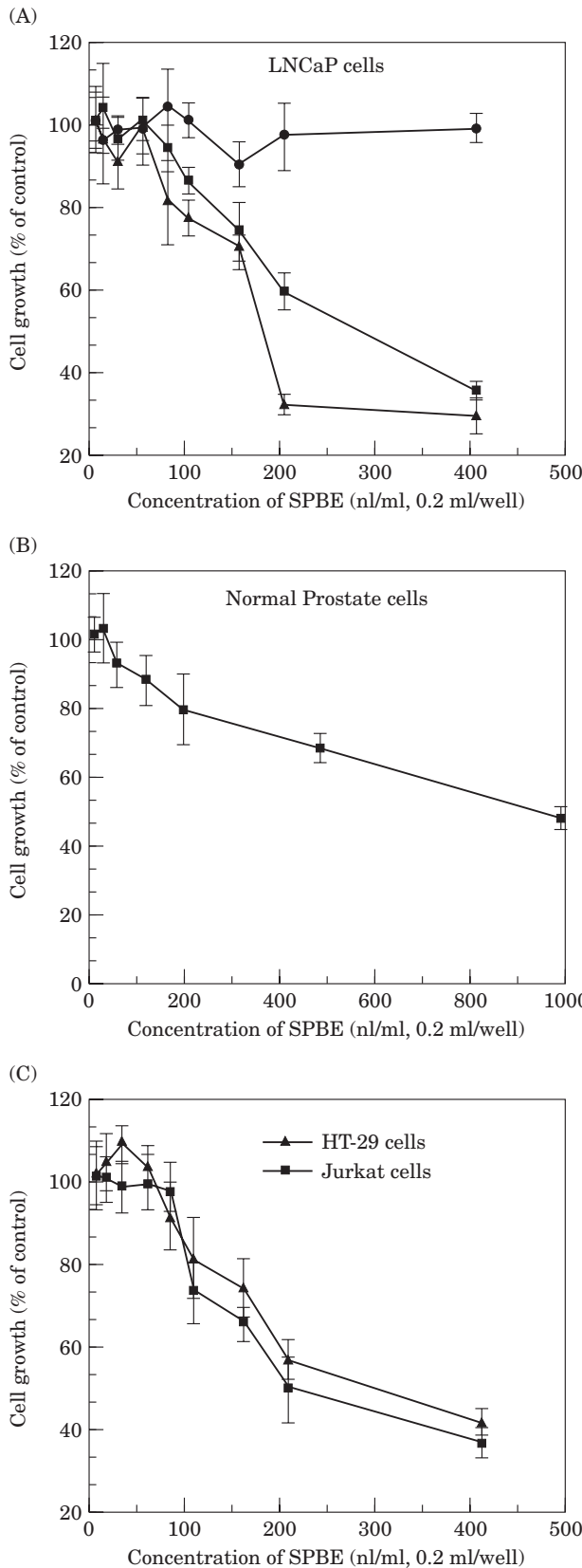
Cell lines	SPBE; $IC_{50}$ (nl/ml)
Normal prostate cells	>400
BRFF-41T	~20
267B-1	~20
LNCaP	~200
LNCaP+DHT	<200*
Jurkat	~200
HT-29	>200

\*LNCaP cells were grown in the presence of DHT instead of serum.

tration of glucose, hydrogen ions, NADH, and NADPH (Jabbar *et al.*, 1989; Vistica *et al.*, 1991). Since we do not know how SPBE affects those molecules that can influence the results of the MTT assay, they were verified using SRB assay. In this assay SRB stains proteins under weakly acidic conditions, a property that provides a quantitative measurement of the cell number. Thus, it gives no measure of cell viability. The previous MTT results were confirmed in this assay. As before, the SPBE treatment resulted in a concentration-dependent reduction in cell number for all three cell lines (Fig. 3A and 3B). Again, the inhibition of cell proliferation was dose-dependent for the SRB assay and no different to results from MTT assays. Comparing the two assays, the only difference was that the SRB assay was 2–4 times more sensitive in detecting cell numbers than the MTT assay. The reduction in cell numbers after 72 h of culture in the presence of SPBE was also verified by counting the actual number of cells after culturing in the presence of different concentrations of SPBE (data not shown).

#### Effect of SPBE on Cox-1 and Cox-2 expression in the LNCaP cell line

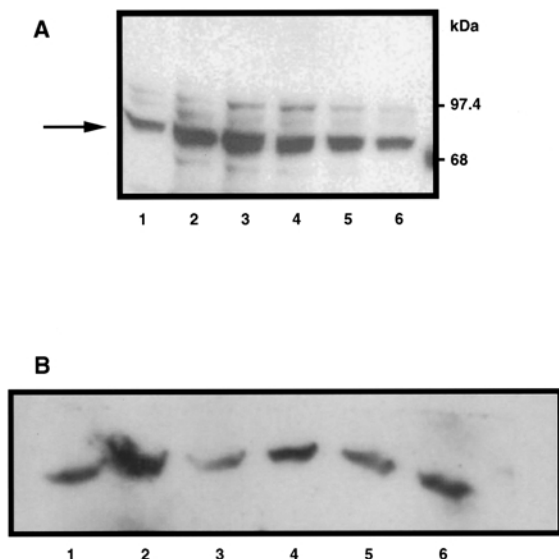
Having shown that SPBE inhibits the growth of prostate derived cell lines, we investigated its effect on the expression of Cox-1 and Cox-2. We chose the LNCaP cell line as a model for this investigation. LNCaP cells constitutively express high levels of the inducible Cox-2 mRNA and its protein (Tjandrawinata *et al.*, 1997; Liu *et al.*, 1998). It has been previously shown that specific Cox-2 inhibitors induce apoptosis in cell lines known to express high levels of Cox-2 (Kyprianou *et al.*, 1996). To determine the effect of SPBE on Cox-2 expression,



**Fig. 3.** Cytotoxicity of SPBE in different cell lines using SRB assay. (A) BRFF-41T (■) and 267B-1 (●) cells, (B) LNCaP (■) cells were each plated in wells and SRB assays were performed.

LNCaP cells were cultured in the presence or absence of both FCS and SPBE. Cell lysates were prepared after culturing the cells for 24 h and the proteins were determined using SDS-Page. The separated proteins were transferred onto an Immobilon-P membrane and subjected to Western blot, using polyclonal antibodies against Cox-1 or

**Fig. 2.** Effect of SPBE on cell growth of LNCaP (A), normal prostate (B) and HT-29 and Jurkat cells (C). Trypsinized cells suspended in RPMI medium were plated on flat-bottomed 96-well culture dish and cytotoxicity assays were performed as described in Materials and Methods. Cytotoxicity: ●, vehicle; ■, serum+SPBE; ▲, 1 nM DHT+SPBE.

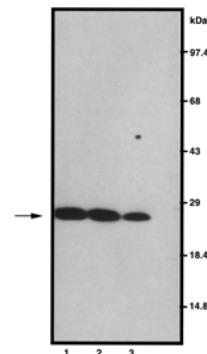


**Fig. 4.** Effect of SPBE on the regulation of Cox-2 (upper panel) and Cox-1 (lower panel) in LNCaP cells. Cells were grown in six-well plates as described in Materials and Methods in the absence (lane 1) or presence of 2% FCS (lane 2) or 10% FCS (lanes 3–6). Some cells were treated with increasing concentrations of SPBE (50, 100 and 200 nl per ml media, lane 4–6, respectively). Cells were harvested after 24 h and analysed by SDS-Page and Western blotting, using antibodies against Cox-1 and Cox-2.

Cox-2. Results from this experiment are shown in Figure 4. In lane 1 of Figure 4A, the basal Cox-2 expression is shown for LNCaP cells grown in culture medium not supplemented with FCS. However, when LNCaP cells were stimulated for 24 h with culture medium containing either 2% or 10% FCS, respectively, there was 3.7 and 4.2 fold increase in Cox-2 protein expression as measured by densitometry (Fig. 4A; lanes 2 and 3, respectively). In experiments where LNCaP cells were stimulated with culture medium supplemented with 10% FCS and 50, 100, or 200 nl equivalents of SPBE, we detected a significant decrease in Cox-2 expression, i.e., 51, 64 and 86%, respectively (Fig. 4A; lanes 4–6). Not unexpectedly, under the same experimental conditions, no change in Cox-1 protein expression was detected after stimulation with FCS or in co-culture with SPBE, except an inter-sample variation (Fig. 4B). It was assumed from the Coomassie stain of total proteins, that equal amounts of protein were loaded on SDS-Page gels (data not shown).

#### *Effect of SPBE on Bcl-2 expression*

To clarify why the cell number does not increase for the prostate cell lines when cultured with SPBE,



**Fig. 5.** Effect of SPBE on Bcl-2 expression. Jurkat cells were grown in the presence of media (lane 1), in the presence of media and 10% FCS (lane 2), and in the presence of media plus 10% FCS and 200  $\mu$ l equivalents of SPBE (lane 3). Western blotting was performed against Bcl-2 using specific antibodies.

we determined the level of Bcl-2 expression in the LNCaP prostatic carcinoma cell line (Fig. 5). There was insignificant expression of Bcl-2 in LNCaP cells. Therefore, we used a Jurkat cell line to demonstrate the effect of SPBE on the expression of Bcl-2, since this cell line is known to express higher levels of the Bcl-2 protein in comparison to LNCaP. Unlike the expression of the Cox-2 protein, FCS does not stimulate Bcl-2 protein expression in Jurkat cells. However, basal expression of the Bcl-2 protein is decreased by 39% when Jurkat cells are co-cultured in the presence of 200 nl equivalents of SPBE (Fig. 5, lane 3). A decrease in expression of Bcl-2 caused by SPBE suggests that these cells are susceptible to apoptosis, resulting in an inability to increase in cell number when cultured over a 72-h time period.

## DISCUSSION

We have demonstrated a concentration-dependent growth inhibition of three prostatic cancer cell lines, BRFF-41T, 267B-1, and LNCaP, when co-cultured with SPBE. The response of two of these cell lines was similar whereas the LNCaP cell line was inherently more resistant to the inhibitory activity of SPBE (Figs 1–3 and Table 1). Growth inhibition of all three cell lines' is not irreversible since pulsing the cell lines for 30 min with SPBE at its  $IC_{50}$  concentration—as determined by co-culturing the cells with the extract for a period of 72 h—is not inhibitory (Fig. 1 and data not shown). It is interesting to note that in LNCaP cells co-cultured with both SPBE and 1 nM of the usually mitogenic androgen DHT, the  $IC_{50}$  value

decreases significantly in comparison to cells that were grown in the presence of serum alone. Several possible mechanisms of action might explain this observation. It has been demonstrated in animal studies that SPBE inhibits the binding of DHT to its receptor (Carilla *et al.*, 1984; Sultan *et al.*, 1984) and blocks the conversion of testosterone to DHT by inhibiting the activity of 5- $\alpha$ -reductase (Bayne *et al.*, 1999; Iehle *et al.*, 1995; Delos *et al.*, 1994). The lack of interaction of DHT with its receptor or inhibition of 5- $\alpha$ -reductase may be responsible for the further decrease in the IC<sub>50</sub> value in comparison to cells grown in the presence of serum. Another possible reason may be the presence of large numbers of other growth factors or hormones in serum that are affected less severely by SPBE compared to DHT.

On similar lines, Hsieh *et al.* (1997) have shown that a complex herbal mixture containing Saw Palmetto, PC-SPES, inhibits the proliferation of LNCaP cells and that this decrease is accompanied by a 60–70% down-regulation in proliferating cell nuclear antigens (PCNA) and levels of secreted PSA. PC-SPES is believed to decrease androgen-receptor expression, as shown by Western blot analysis, and this may explain the lower expression of PSA. Recent work by Tiwari *et al.* (1999) also supports the *in vivo* anti-tumor effects of PC-SPES.

The induction of apoptosis is associated with decreased expression of Bcl-2 proteins. A potential role for the anti-apoptotic gene Bcl-2 as a survival mechanism for androgen-resistant prostate cancers is supported by the observation that Bcl-2 is not expressed in normal secretory prostatic epithelial cells. Yet, it is expressed in a variety of prostate hyperplasia and cancer specimens. It is known that over-expression of Bcl-2 protects cancer cells from apoptosis *in vitro* and confers resistance to androgen-deprivation therapy *in vivo* (Raffo *et al.*, 1995). A role for Bcl-2 in survival of the androgen-resistant cells is experimentally supported when analysing androgen-dependent and androgen-independent LNCaP cell-lines. Androgen-independent LNCaP-AI cells express much higher levels of Bcl-2 than the androgen-dependent cells. Expression of Bcl-2 was also not modulated by the addition of the androgen agonist R1881 (Lu *et al.*, 1999), but was shown in Figure 5 to be down-regulated in Jurkat cells when co-cultured with SPBE. Reduced expression of Bcl-2 may render the androgen-resistant prostate cancer cells more susceptible to apoptosis, resulting in a reduction in tumour burden in the cancer patients. Our inability to detect Bcl-2 in LNCaP cells may be due to

the variation in their growth conditions and/or differentiation.

Further, we investigated the effects of FCS and SPBE on the expression of either Cox-1 or Cox-2 in LNCaP cells. Exposing these cells to increasing concentrations of FCS induced the expression of Cox-2, but not Cox-1 (Fig. 4). However, this induction of Cox-2 was reversed in a dose-response manner when the LNCaP cells were co-cultured with SPBE (Fig. 4). The lack of induction of Cox-1 expression by FCS is not surprising since its expression is usually not amenable to induction by a variety of growth factors and cytokines. The importance of reducing the expression of Cox-2 is emphasized by the fact that NS398, a specific inhibitor of Cox-2, induces apoptosis in LNCaP cells but not in human fetal prostate fibroblasts (Guinan *et al.*, 1997). Studies have also revealed that treatment with highly selective Cox-2 inhibitors results in both decreased size and number of premalignant colonic lesions in several animal model systems and that decreased expression of Cox-2 potentially protects against colon cancer (Tsulii and DuBois, 1995). Comparing observations from this study with previously published results, we hypothesize that Cox-2 inhibition induced by SPBE may provide an important basis for potential chemopreventative action. Further experimental work will address this important issue.

[*Note:* The ‘mechanism of action’ of SPBE is reported to be its ability to inhibit the conversion of testosterone to DHT (Bayne *et al.*, 1999; Iehle *et al.*, 1995; Delos *et al.*, 1994). SPBE also inhibits the binding of DHT to its receptors (Sultan *et al.*, 1984; Carilla *et al.*, 1984). Further, it has been suggested that too much DHT could cause prostate cells to divide at an abnormal rate leading to enlargement of the prostate gland. Other effects of SPBE are anti-inflammatory (Di Silverio *et al.*, 1992) and anti-estrogenic (Di Silverio *et al.*, 1992; Briley *et al.*, 1983). In conclusion, SPBE is a well-tolerated drug that has an efficacy similar to Finasteride in improving symptoms in men with BPH (Plosker and Brogden, 1996; Gerber *et al.*, 1998). An additional effect of SPBE is the inhibition of growth factor-induced proliferation of human prostate cell-cultures (Paubert-Braquet, 1998). This drug also induces a significant reduction in DHT and epidermal growth factor concentrations in human BPH following three months of treatment (Di Silverio, 1998). Amongst other effects suggested for how SPBE functions, is the inhibition of signal transduction through the prolectin receptor (Vacher *et al.*, 1995)].

## REFERENCES

- BAYNE CW, CONNELLY F, ROSS M, HABIB FK, 1999. Serenoa repens (Permixon): a 5 $\alpha$ -reductase types I and II inhibitor—new evidence in a coculture model of BPH. *Prostate* **40**: 232–241.
- BRILEY ME, SULLIVAN S, STEVENSON S, HULL CL, 1983. Validation and application of nutrition education objectives. *J Am Diet Assoc* **82**: 385–388.
- CARRARO JC, RAYNAUD JP, KOCH G, CHISHOLM GD, DiSILVERIO F, TELLAC P, SILVA FC, CAUQUIL J, CHOPIN DK, HAMDY FC, HANUS M, HAURI D, KALINTERIS A, MARENCAK J, PERIER A, PERRIN P, 1996. Comparison of phytotherapy (Permixon) with finasteride in the treatment of benign prostate hyperplasia: a randomized international study 1.098 patients. *Prostate* **29**: 231–240.
- CARILLA E, BRILEY M, FAURAN F, SULTAN C, DU VILLIERS C, 1984. Binding of Permixon, a new treatment for prostatic benign hyperplasia, to the cytosolic androgen receptor in the rat prostate. *J Steroid Biochem* **20**: 521–523.
- DELOS S, IEHLE C, MARTIN PM, RAYNAUD JP, 1994. Inhibition of the activity of 'basic' 5  $\alpha$ -reductase (type 1) detected in DU 145 cells and expressed in insect cells. *Steroid Biochem Molec Biol* **48**: 347–352.
- DiPAOLA RS, ZHANG H, LAMBERT GH, MEEKER R, LICITRA E, RAFI MM, ZHU BT, SAULDING H, GOODIN S, TOLEDANO MB, HAIT WN, GALLO MA, 1998. Clinical and biologic activity of an estrogenic herbal combination (PC-SPES) in prostate cancer. *N Eng J Med* **339**: 785–791.
- Di SILVERIO F, D'ERAMO G, LUBRANO C, FLAMMIA GP, SCIARRA A, PALMA E, CAPONERA M, SCIARRA F, 1992. Evidence that Serenoa repens extract displays an antiestrogenic activity in prostatic tissue of benign hypertrophy patients. *Eur Urol* **21**: 309–314.
- Di SILVERIO F, SCIARRA A, D'ERAMO G, CASALE P, LORETO A, SECCARECCIA F, 1998. Relationship among age, prostate-specific antigen, and prostate volume in men with and without benign and malignant prostate diseases. *Prostate* **36**: 1–7.
- GERBER GS, ZAGAJA GP, BALES GT, CHODAK GW, CONTRERAS BA, 1998. Saw palmetto (*Serenoa repens*) in men with lower urinary tract symptoms: effects on urodynamic parameters and voiding symptoms. *Urology* **51**: 1003–1007.
- GUINAN P, DIDOMENICO D, BROWN J, SHAW M, SHARIFI R, RAY V, SHOTT S, RUBENSTEIN M, 1997. The effect of androgen deprivation on malignant and benign prostate tissue. *Med Oncol* **14**: 145–152.
- HSIEH T, CHEN SS, WANG X, WU JM, 1997. Regulation of androgen receptor (AR) and prostate specific antigen (PSA) expression in the androgen-responsive human prostate LNCaP cells by ethanolic extracts of the Chinese herbal preparation, PC-SPES. *Biochem Mol Biol Int* **42**: 535–544.
- IEHLE C, DELOS S, GUIRO O, TATE R, RAYNAUD JP, MARTIN PM, 1995. Human prostatic steroid 5  $\alpha$ -reductase isoforms—a comparative study of selective inhibitors. *J Steroid Biochem Mol Biol* **54**: 273–279.
- JABBAR SA, TWENTYMAN PR, WATSON JV, 1989. The MTT assay underestimates the growth inhibitory effects of interferons. *Br J Cancer* **60**: 523–528.
- KYPRIANOU N, TU H, JACOBS SC, 1996. Apoptosis versus proliferative activities in human benign prostate hyperplasia. *Hum Pathol* **27**: 668–675.
- LEE C, SUTKOWSKI DM, SENSIBAR JA, ZELNER D, KIM I, AMSEL I, SHAW W, PRINS GS, KOZLOWSKI JM, 1995. Regulation of proliferation and production of prostate-specific antigen in androgen-sensitive prostatic cancer cells, LNCaP, by dihydrotestosterone. *Endocrinology* **136**: 796–803.
- LIU XH, YAO S, KIRSCHENBAUM A, LEVINE AC, 1998. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates Bcl-2 expression in LNCaP cells. *Cancer Res* **58**: 4245–4249.
- LOWE FC, KU JC, 1996. Phytotherapy in treatment of benign prostatic hyperplasia: a critical review. *Urology* **48**: 12–20.
- LU SL, TSAI S, TSAI MJ, 1999. Molecular mechanisms of androgen-independent growth of human prostate cancer LNCaP-A1 cells. *Endocrinology* **140**: 5054–5059.
- McKINNEY DE, 1999. Saw Palmetto for benign prostatic hyperplasia. *JAMA* **281**: 1699.
- MOSMANN T, 1983. Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**: 55–63.
- PAUBERT-BRAQUET M, COUSSE H, RAYNAUD JP, MENCIA-HUERTA JM, BRAQUET P, 1998. Effect of the lipidosterolic extract of *Serenoa repens* (Permixon) and its major components on basic fibroblast growth factor-induced proliferation of cultures of human prostate biopsies. *Eur Urol* **33**: 340–347.
- PLOSKER GL, BROGDEN RN, 1996. *Serenoa repens* (permixon). A review of its pharmacology and therapeutic efficacy in benign prostatic hyperplasia. *Drugs Aging* **9**: 379–395.
- RAFFO AJ, PERLMAN H, CHEN MW, et al., 1995. Overexpression of Bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. *Cancer Res* **55**: 4438–4445.
- SKEHAN P, STORENG P, SCUDIERO D, et al., 1990. New colorimetric cytotoxicity assay for anticancer drug screening. *J Natl Cancer Inst* **82**: 1107–1112.
- SONNENSCHN C, OLEA W, PASANEN ME, SOTO AM, 1989. Biological effects of sex hormone-binding globulin on androgen metabolism in LNCaP prostate cells. *Cancer Res* **49**: 3474–3481.
- STONER E, 1996. 5 $\alpha$ -reductase inhibitors/finasteride. *Prostate (Suppl.)* **6**: 82–87.
- SULTAN C, TERRAZA A, DEVILLIER E, BRILEY M, LOIRE C, DESCOMBS B, 1984. Inhibition of androgen metabolism and binding by a liposterolic extract of '*Serenoa repens* B' in human foreskin fibroblasts. *J Steroid Biochem* **20**: 515–519.
- TIWARI RK, GELIEBTER J, GARIKAPATY VP, YEDAVELLI SP, CHEN S, MITTELMANN A, 1999. Anti-tumor effects of PC-SPES, an herbal formulation. *Int J Oncol* **14**: 713–719.
- TJANDRAWINATA RR, DAHIYA R, HUGHES-FULFORD M, 1997. Induction of cyclooxygenase-2 mRNA by prostaglandin E2 in human prostatic carcinoma cells. *Br J Cancer* **75**: 1111–1118.
- TSUJII M, DuBois RN, 1995. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* **83**: 493–501.
- VACHER P, 1995. Gn-RH agonists in the treatment of prostatic carcinoma. *Biomed Pharmacother* **49**: 325–331.
- VISTICA DT, SKEHAN P, SCUDIERO D, et al., 1991. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. *Cancer Res* **51**: 2515–2520.
- WILT TJ, ISHANI A, STARK G, MACDONALD R, LAU J, MURLOW C, 1998. Saw Palmetto extracts for treatment of benign prostatic hyperplasia: a systematic review. *JAMA* **280**: 1604–1609.