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CLINICAL AND BIOLOGIC ACTIVITY OF AN ESTROGENIC HERBAL COMBINATION (PC-SPES) IN PROSTATE CANCER

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ABSTRACT

Background Herbal mixtures are popular alternatives to demonstrated therapies. PC-SPES, a commercially available combination of eight herbs, is used as a nonestrogenic treatment for cancer of the prostate. Since other herbal medicines have estrogenic effects in vitro, we tested the estrogenic activity of PC-SPES in yeast and mice and in men with prostate cancer.

Methods We measured the estrogenic activity of PC-SPES with transcriptional-activation assays in yeast and a biologic assay in mice. We assessed the clinical activity of PC-SPES in eight patients with hormone-sensitive prostate cancer by measuring serum prostate-specific antigen and testosterone concentrations during and after treatment.

Results In complementary yeast assays, a 1:200 dilution of an ethanol extract of PC-SPES had estrogenic activity similar to that of 1 nM estradiol, and in ovariectomized CD-1 mice, the herbal mixture increased uterine weights substantially. In six of six men with prostate cancer, PC-SPES decreased serum testosterone concentrations ($P<0.05$), and in eight of eight patients it decreased serum concentrations of prostate-specific antigen. All eight patients had breast tenderness and loss of libido, and one had venous thrombosis. High-performance liquid chromatography, gas chromatography, and mass spectrometry showed that PC-SPES contains estrogenic organic compounds that are distinct from diethylstilbestrol, estrone, and estradiol.

Conclusions PC-SPES has potent estrogenic activity. The use of this unregulated mixture of herbs may confound the results of standard or experimental therapies and may produce clinically significant adverse effects. (N Engl J Med 1998;339:785-91.)

HERBAL therapies are unconventional treatments in wide use for many diseases. They are sold as nutritional supplements for numerous illnesses, including the common cold (echinacea),[1] benign prostatic hypertrophy (saw palmetto),[2] and depression (Saint Johnswort).[3] Among patients with cancer, the use of unconventional medicines, including herbal therapies, has been reported to be as low as 5 percent and as high as 60 percent.[4,5] PC-SPES is an herbal combination used by patients with prostate cancer that consists of eight herbs: chrysanthemum, isatis, licorice, *Ganoderma lucidum, Panax pseudo-ginseng, Rabdosia rubescens,* saw palmetto, and scutellaria (skullcap).[6-8]

Herbal therapies can have important biologic activities. For example, saw palmetto inhibits 5α-reductase, an enzyme involved in testosterone metabolism,[9] and Saint Johnswort, like pharmacologic antidepressants, blocks monoamine oxidase activity.[10] Although PC-SPES is promoted as a nonestrogenic food supplement, some of its constituents have estrogenic activity.[6-8] Licorice competes with estradiol in an estrogen-receptor–binding assay,[11] and ginseng induces estrogen-regulated expression of pS2, a small protein found in breast cancer, in cultured MCF-7 breast-cancer cells.[12] The clinical implications of such activities are unknown.

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We recently observed that PC-SPES had clinical activity in a man with prostate cancer that had recurred after radical prostatectomy. His prostate-specific antigen concentration was 34 ng per milliliter when he started taking PC-SPES, without concurrent therapies. After one month, he reported breast tenderness and loss of libido, and his prostate-specific antigen had decreased to 0.4 ng per milliliter. These findings prompted a study to determine the mechanism of these effects. Our results indicate that PC-SPES has estrogenic activity, reduces concentrations of serum testosterone, exerts activity against prostate cancer, and causes untoward side effects.

METHODS

Materials

PC-SPES was purchased from Botaniclab (Brea, Calif.) in the form of 320-mg capsules for laboratory and clinical studies. To determine whether the composition of PC-SPES varied from lot to lot, we made four separate purchases of PC-SPES and analyzed each batch. Ginseng (Action Labs, Long Island, N.Y.) and saw palmetto (Fingerprint Botanicals, General Nutrition, Pittsburgh) were purchased from a health-food vendor. Stock solutions of PC-SPES and herbal extracts were prepared by exposing them to ethanol (dilution, 1:10 wt/vol) for 24 hours. Estrone, 17β-estradiol, and diethylstilbestrol were purchased from Sigma Chemical (St. Louis). Ovariectomized CD-1 mice (weight, 18 to 20 g) were purchased from Charles River Laboratories (Wilmington, Mass.) and housed under standard conditions in the Robert Wood Johnson Medical School vivarium.

Estrogen-Receptor Activity and Estrogen-Dependent Growth

The estrogenic activity of PC-SPES was assessed with a β-galactosidase assay with the yeast strain Y253 *(ura3–52,his3–*Δ200, *leu2–*Δ1,*lys2–801,ade2–101,yap1::leu2)*. In Y253, two plasmids were introduced. One is the plasmid YEp90HEGO, which expresses the human estrogen receptor α. The other plasmid ($PYER_1$) carries the *lacZ* gene, which encodes the enzyme β-galactosidase. In this plasmid, the upstream activating sequence of the gene was replaced by two estrogen-response elements. In this system, β-galactosidase activity is proportional to the degree of activation of estrogen-response elements.[13]

A complementary growth-based assay used the *Saccharomyces cerevisiae* strain PL3 *(ura3–*Δ1,*his3–*Δ200,*leu2–*Δ1,*trp1::3ERE–URA3*), which was a gift of Dr. R. Losson.[13] PL3 carries a *URA3* gene that is under the control of the estrogen-response element. Transcription of *URA3,* which is required for the growth of the cells in medium lacking uracil, is dependent on the activation of the human estrogen receptor by a ligand. Cells were seeded in 96-well plates in 190 μl of medium lacking uracil. Serial dilutions of 10 μl of 17β-estradiol or PC-SPES extract were added to the cultures, and growth was observed for four days.

Studies in Animals

Ovariectomized CD-1 mice received 1 ml of either a suspension of PC-SPES (five mice) or vehicle alone (five) orally on days 0, 1, 2, and 3. The suspension contained five capsules of PC-SPES ground into powder and suspended in 20 ml of an aqueous solution containing 5 percent gum arabic. Three additional animals received no treatment throughout the course of the experiment. On day 4, the animals were weighed and then killed by cervical dislocation. The uteri were removed and weighed. All studies in animals were approved by the institutional animal care and use committee of the Robert Wood Johnson Medical School.

High-Performance Liquid Chromatography, Gas Chromatography, and Mass Spectrometry

PC-SPES capsules were treated with 5 ml of 100 percent ethanol, the insoluble herbal residues were precipitated by centrifugation at 3500 rpm for 20 minutes, and the ethanol extract was dried under a stream of nitrogen. The dried residues were dissolved in 1 ml of ethanol and filtered through 0.45-μm cellulose

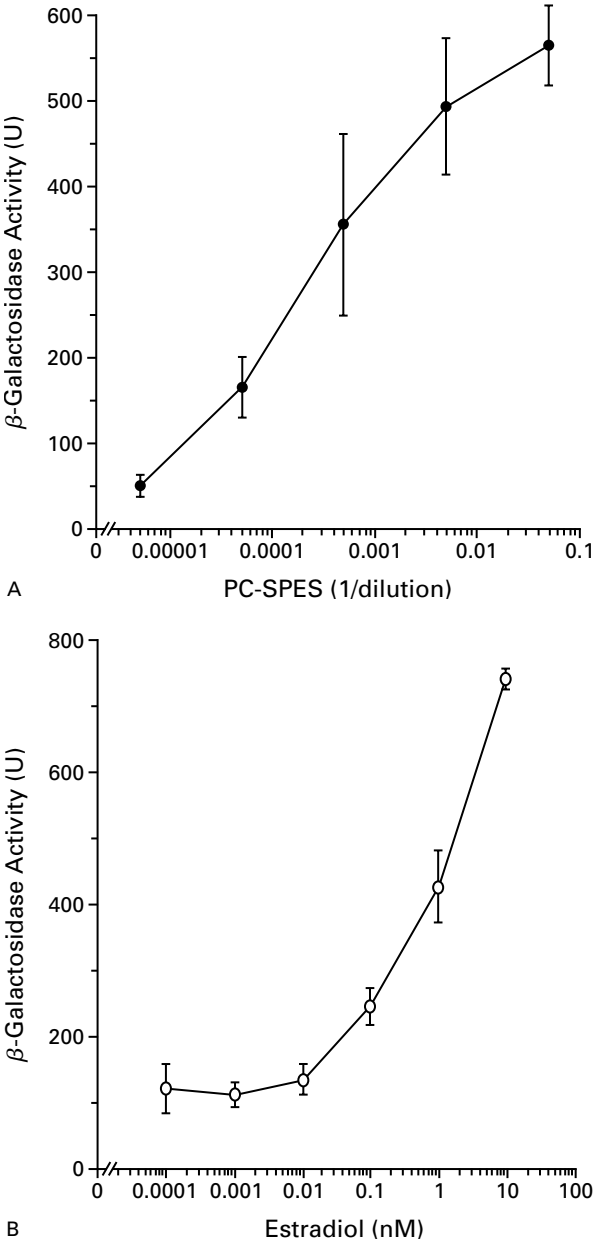

Figure 1. Effect of PC-SPES and Estradiol on β-Galactosidase Activity of Y253 Yeast with a *lacZ* Construct Downstream of a Human Estrogen-Response Element.

Both PC-SPES (Panel A) and estradiol (Panel B) activated the *lacZ* gene, as measured by β-galactosidase activity. PC-SPES had a dilution-dependent effect that was similar to the effect of estradiol. Each point represents the mean (±SE) of four separate experiments with estradiol and batches A, B, and C of PC-SPES.

acetate membranes, and 40-μl aliquots were analyzed by high-performance liquid chromatography as previously described.[14,15] Elutants were collected from the column, dried under a stream of nitrogen, and analyzed by gas chromatography and mass spectrometry. The dried elutants were redissolved in 30 μl of ethyl acetate and exposed to 50 μl of bis(trimethylsilyl)trifluoroacetamide at 68°C for 45 minutes. Gas chromatography and mass spectrometry were performed on a Varian Star gas chromatograph (model 3400, Varian, Walnut Creek, Calif.) coupled with a Varian Saturn ion-trap mass spectrometer (model 2000, Varian). A 30 m by 0.32 mm by 0.25 μm DB-5 capillary column (J and W Scientific, Folsom, Calif.) was used for the separation. The 3-μl sample was injected into the gas chromatograph at a temperature of 280°C. The column temperature was programmed to increase from 35°C to 300°C over a period of 55 minutes. The mass spectrometer was set to scan particles with a mass-to-charge ratio ranging from 17 to 600 every 0.6 second.

Clinical Studies

We studied eight patients: seven were enrolled in an observational study approved by the institutional review board at Robert Wood Johnson Medical School, and one met eligibility requirements before the study began (Patient 1). All eight patients had histologically proved prostate cancer, without progression during previous androgen-ablation therapy. The patients were required to have taken PC-SPES for at least one month and to have taken a minimum of four 320-mg capsules of PC-SPES daily for two weeks. During the study they could not receive any standard forms of androgen-ablation therapy. The effects of PC-SPES were assessed with a standardized questionnaire. Serum prostate-specific antigen and testosterone concentrations were measured while the patients were taking PC-SPES and two to six weeks after they had stopped taking the preparation. Pretreatment prostate-specific antigen concentrations were obtained from the patients' records.

Statistical Analysis

Data are presented as means ±SE. The uterine weights in the animals were compared with use of the Wilcoxon rank-sum test. The patients' prostate-specific antigen and testosterone values were compared with use of the Wilcoxon signed-rank test. All P values were two-sided.

RESULTS

Estrogenic Activity in Yeast

We used two yeast strains to measure the estrogenic activity of PC-SPES. First, Y253 yeast harboring the *lacZ* gene under the control of the human estrogen receptor was treated with PC-SPES or estradiol. The PC-SPES extract caused a dose-dependent increase in β-galactosidase activity (Fig. 1A). A 1:200 dilution of the extract had estrogenic activity equivalent to that of 1 nM estradiol (Fig. 1).

We next analyzed the effects of estradiol and PC-SPES on the growth of the estrogen-dependent *S. cerevisiae* strain PL3. An ethanol extract from four separate batches of PC-SPES (1:200 dilution) or 1 nM estradiol supported the growth of PL3 in uracil-deficient medium (Fig. 2). Table 1 shows that all four batches of highly dilute PC-SPES extract (1:20,000) supported the growth of PL3. Estradiol supported growth of the yeast at a minimal concentration of 1×10^{-5} nM. Growth was not supported by herbal extracts of saw palmetto or ginseng.

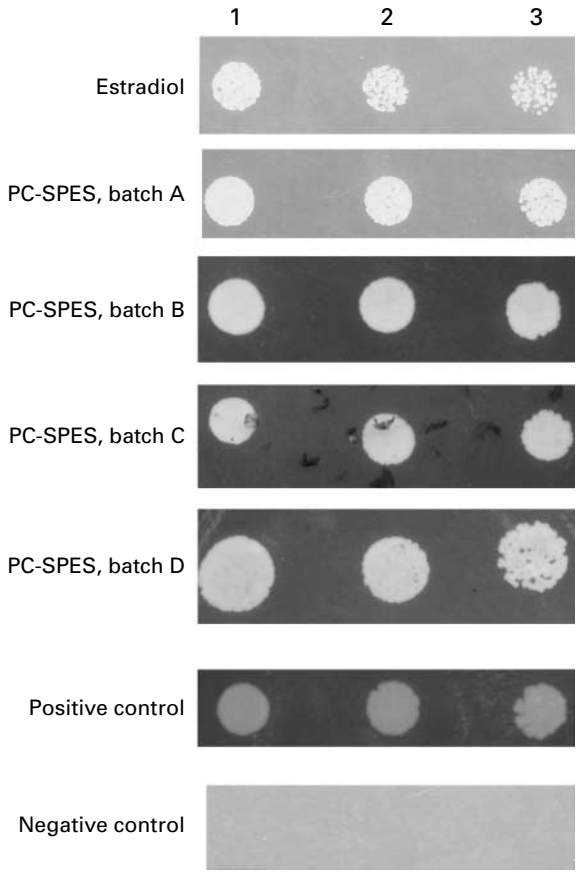

Figure 2. Effect of PC-SPES on the Growth of the PL3 Strain of *S. cerevisiae.*

In the PL3 strain the *URA3* gene is under the control of a human estrogen receptor, and this gene is required for the growth of cells in uracil-deficient medium. Growth is observed in the presence of ethanol-extracted PC-SPES (1:200) from four separate batches and 1 nM estradiol three days after 2000 cells (lane 1), 1000 cells (lane 2), and 500 cells (lane 3) had been plated. The negative control consists of ethanol in uracil-deficient agar. The positive control consists of ethanol in agar with uracil. The results are representative of four separate experiments.

Estrogenic Activity in Animals

To determine whether PC-SPES has estrogenic activity in animals, we treated ovariectomized CD-1 mice with 1 ml of a suspension of PC-SPES or vehicle daily for four days. As shown in Table 2, animals given PC-SPES had a mean uterine weight of 76.9±12.1 mg, as compared with a weight of 18.5±3.2 mg in mice given vehicle (P=0.008) and 19.5±2.8 mg in untreated animals.

Effect of PC-SPES on Serum Testosterone and Prostate-Specific Antigen in Patients with Prostate Cancer

We evaluated the activity of PC-SPES in eight patients with biopsy-proved prostate cancer before and during treatment with PC-SPES and in six patients

TABLE 1. EFFECT OF HERBAL EXTRACTS AND ESTRADIOL ON THE GROWTH OF ESTROGEN-DEPENDENT YEAST.*

ESTRADIOL CONCENTRATION (nM)	GROWTH	HERBAL-EXTRACT DILUTION	GROWTH					
			PC-SPES BATCH				GINSENG	SAW PALMETTO
			A	*B*	*C*	*D*		
1	+	$1:2\times10^{1}$	+	+	+	+	−	−
1×10^{-1}	+	$1:2\times10^{2}$	+	+	+	+	−	−
1×10^{-2}	+	$1:2\times10^{3}$	+	+	+	+	−	−
1×10^{-3}	+	$1:2\times10^{4}$	+	+	+	+	−	−
1×10^{-4}	+	$1:2\times10^{5}$	−	+	−	−	NT	NT
1×10^{-5}	+	$1:2\times10^{6}$	−	NT	NT	NT	NT	NT
1×10^{-6}	−	$1:2\times10^{7}$	−	NT	NT	NT	NT	NT

*NT denotes not tested.

TABLE 2. EFFECT OF PC-SPES IN MICE.

TREATMENT AND MOUSE NO.	FINAL BODY WEIGHT	UTERINE WEIGHT	UTERINE WEIGHT/ BODY WEIGHT
	g	mg	
PC-SPES			
1	18.9	68.9	3.65
2	19.8	37.4	1.89
3	22.0	79.8	3.63
4	21.8	86.6	3.97
5	23.7	111.8	4.72
Mean ±SE	21.2±0.9	76.9±12.1	3.6±0.5
Vehicle			
6	17.3	29.2	1.69
7	22.4	11.6	0.52
8	17.5	15.1	0.86
9	23.2	14.5	0.62
10	18.9	22.2	1.17
Mean ±SE	19.9±1.2	18.5±3.2	1.0±0.2
No treatment			
11	22.5	20.6	0.92
12	22.1	23.8	1.08
13	23.3	14.2	0.61
Mean ±SE	22.6±0.4	19.5±2.8	0.9±0.14

two to six weeks after treatment was stopped (Table 3). Serum testosterone concentrations decreased during the use of PC-SPES and increased within three weeks after PC-SPES was discontinued ($P<0.05$) (Table 3). The mean testosterone concentration was 237.0±81 ng per deciliter (822±281 nmol per liter; range, 25 to 494 ng per deciliter [87 to 1713 nmol per liter]) during treatment and 879±144 ng per deciliter (3047±499 nmol per liter; range, 301 to 1324 ng per deciliter [1044 to 4590 nmol per liter]) after treatment was discontinued. The serum testosterone concentration was below the normal reference range in four of the eight patients, and in two patients (Patients 1 and 3 in Table 3) it was below 30 ng per deciliter (104 nmol per liter), a concentration similar to that in patients receiving a standard regimen of androgen-ablation therapy.[16] Serum testosterone concentrations were within the normal reference range in four patients during treatment with PC-SPES (Patients 4, 5, 6, and 8 in Table 3) and increased within three weeks if the treatment was discontinued.

The serum prostate-specific antigen concentration was assessed before and during the use of PC-SPES. It decreased in all eight patients after the initiation of treatment with PC-SPES (Table 3), whether the initial concentration was high (as in Patients 1 and 3) or low (as in Patient 8). The concentration began to increase within three weeks after PC-SPES was discontinued (Table 3).

Other clinical effects observed during PC-SPES use were similar to those observed with pharmacologic doses of estrogen.[16-18] All eight patients had breast tenderness and loss of libido. One patient had a superficial venous thrombosis after taking nine capsules of PC-SPES per day for more than one month. No other serious adverse effects were noted.

Composition of PC-SPES

We used high-performance liquid chromatography to analyze the composition of PC-SPES. Representative chromatograms for estrone, estradiol, and diethylstilbestrol are shown in Figure 3A. High-performance liquid chromatography of PC-SPES demonstrated four peaks over a period of 53 to 64 minutes (Fig. 3B). Elutants corresponding to each of these four peaks had estrogenic activity in the yeast-growth assay (data not shown).

The peaks representing estrone and estradiol (Fig. 3A) differed from the major peaks found in PC-SPES (Fig. 3B). Since peak X of PC-SPES resembled that of diethylstilbestrol, we analyzed this peak using gas chromatography and mass spectrometry. Peak X elutants contained a mixture of organic chemicals (Fig. 3D). Among them, only one peak (labeled peak Y in Fig. 3D) resembled diethylstilbestrol (Fig. 3C). In-line mass spectrometric analysis of this peak (inset in Fig. 3D) showed that it differed from that of diethylstilbestrol (inset in Fig. 3C).

DISCUSSION

We found that PC-SPES, an unregulated herbal dietary supplement, has potent estrogenic activity in yeast, mice, and humans. In patients with prostate cancer, it causes clinically significant reductions in serum testosterone concentrations, decreases in prostate-specific antigen concentrations, and side effects similar to those of pharmacologic doses of estrogen.

In all eight patients we studied, PC-SPES reduced serum testosterone concentrations to levels shown in the randomized trial by the Leuprolide Study Group to have antitumor activity.[16] PC-SPES also reduced

TABLE 3. EFFECT OF PC-SPES ON TESTOSTERONE AND PROSTATE-SPECIFIC ANTIGEN CONCENTRATIONS.*

PATIENT NO.	PRIOR LOCAL THERAPY FOR PROSTATE CANCER	TESTOSTERONE		PROSTATE-SPECIFIC ANTIGEN			INTERVAL BETWEEN DISCONTINUATION OF PC-SPES AND MEASUREMENT
		DURING PC-SPES	AFTER PC-SPES	BEFORE PC-SPES	DURING PC-SPES	AFTER PC-SPES	
		ng/dl			ng/ml		wk
1	Prostatectomy	25	674	34.6	0.4	5.4 17.0	3 6
2	Radiation therapy	149	301	1.7	1.1	1.7	2
3	None	26	1072	122	1.2	36.0	2
4	Prostatectomy followed by radiation therapy	494	1324	1.2	0.5	0.8	2
5	Radiation therapy followed by cryosurgery	362	986	1.3	0.4	0.8	2
6	None	363	918	12.6	6.3	7.1	2
7	None	53	NA	6.7	5.6	NA	NA
8	Prostatectomy	316	NA	0.39	0.0	NA	NA

*The references ranges for serum testosterone at four standardized commercial laboratories were 241 to 827, 220 to 940, 220 to 940, and 300 to 1000 ng per deciliter. To convert values for testosterone to nanomoles per liter, multiply by 3.467. NA denotes not applicable, since these patients did not stop taking PC-SPES.

the serum prostate-specific antigen concentration in all eight patients; moreover, in the six patients who stopped taking PC-SPES, prostate-specific antigen increased within two to six weeks after treatment was discontinued (Table 3). The magnitude of this effect varied, perhaps because of the wide range of initial prostate-specific antigen values or differences in the types of local therapy the patients had received. The increase in prostate-specific antigen after the discontinuation of PC-SPES occurred at about the same time as the increase in testosterone. For example, three weeks after Patient 1 stopped taking PC-SPES, the testosterone concentration had increased from 25 to 674 ng per deciliter (87 to 2337 nmol per liter) and the prostate-specific antigen concentration had increased from 0.4 ng per milliliter to 5.4 ng per milliliter, with an increase to 17.0 ng per milliliter at six weeks.

Previous studies of patients with prostate cancer have shown that the effects of pharmacologic doses of estrogen are similar to those of PC-SPES.[16-19] Our study assessed patients whose tumors were not known to be refractory to androgen-ablation therapy; the effects of PC-SPES on cancer that is refractory to hormone therapy and whether tumors would become resistant to the effects of this compound are unknown.[20]

The adverse effects of PC-SPES were similar to those of estrogen. All patients in our study experienced impotence and breast tenderness, and one patient had a superficial venous thrombosis. Prior studies of estrogen therapy in men with prostate cancer have assessed the frequency of these effects.[16-19] For example, the Leuprolide Study Group reported that 49 percent of patients who were treated with diethylstilbestrol for metastatic prostate cancer experienced breast tenderness, and 7 percent had venous thrombosis.[16] Aprikian et al. also found that the most common adverse effect of diethylstilbestrol treatment was gynecomastia and that 2 of 55 patients had thrombosis (1 had a pulmonary embolus).[18]

Our laboratory data support the hypothesis that the clinical effects of PC-SPES are due at least in part to estrogenic activity. PC-SPES activates the human estrogen-response element in two yeast systems. The estrogenic activity of a 1:200 dilution of a stock extract of one 320-mg PC-SPES capsule was similar to that of 1 nM estradiol in both the yeast *lacZ* and growth assays. PC-SPES also had estrogenic activity in mice. Ovariectomized CD-1 mice given PC-SPES had significantly heavier uteri than control mice. Other groups have used this mouse model to study the estrogenicity of various compounds.[15,21] The CD-1 mouse has also been used to study the teratogenic effects of estrogens.[22,23] Prenatal exposure of CD-1 mice to diethylstilbestrol results in reproductive tract anomalies similar to those that occur in women who were exposed to diethylstilbestrol prenatally.[23] Given the potential toxicity of PC-SPES in pregnant women, studies of this herbal combination in animal models are warranted.

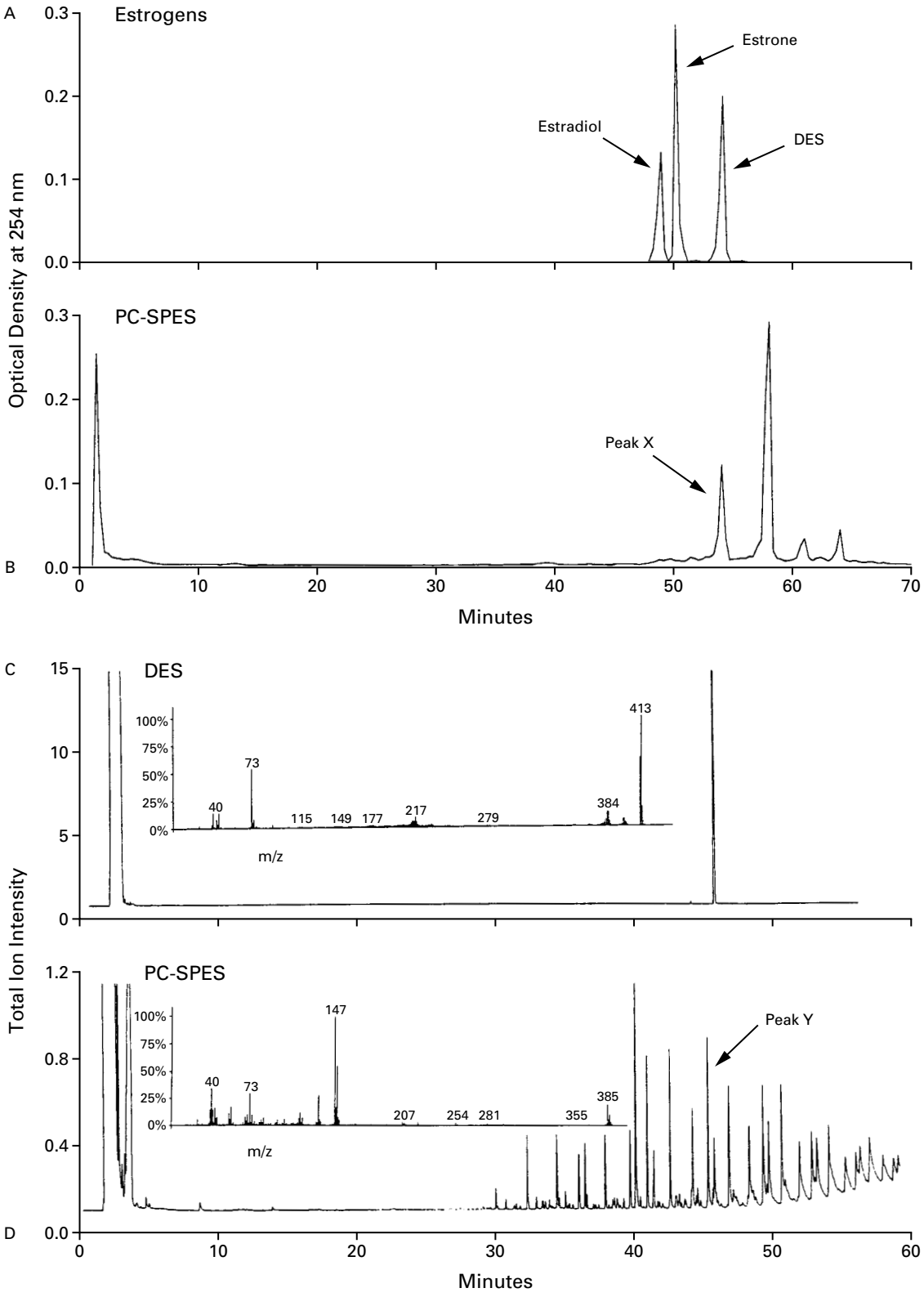
A
Estrogens
Estrone
Estradiol
DES
PC-SPES
Peak X
B
Optical Density at 254 nm
Minutes
C
DES
m/z
D
PC-SPES
Peak Y
Total Ion Intensity
Minutes

Figure 3. Results of High-Performance Liquid Chromatography, Gas Chromatography, and Mass Spectrometry of PC-SPES and the Estrogens Estrone, Estradiol, and Diethylstilbestrol (DES).

The results of high-performance liquid chromatography, shown in Panels A and B, are representative of three experiments with two separate batches of PC-SPES. The results of gas chromatography and mass spectrometry show that peak X of PC-SPES in Panel B differs from the peak representing diethylstilbestrol in Panel A. Gas chromatograms of diethylstilbestrol (Panel C) and peak X of PC-SPES (Panel D) are shown along with in-line mass spectrometric analysis of diethylstilbestrol (inset in Panel C) and peak Y of peak X (inset in Panel D). The mass spectrometer was set to scan particles with a mass-to-charge ratio (m/z) ranging from 17 to 600 every 0.6 second.

Halicka et al. found that PC-SPES inhibited the growth of PC-3 cells (prostate-tumor cells) and MCF-7 cells (breast-tumor cells) in cell culture.[7] Hsieh et al. demonstrated that PC-SPES decreased both the production of prostate-specific antigen and the expression of androgen receptors in cultures of LNCaP prostate-tumor cells.[8] We do not know whether these effects of PC-SPES on cultured tumor cells are independent of estrogenic activity, since estrogen alone induces apoptosis in tumor-cell lines.[24]

PC-SPES has potent estrogenic activity and contains multiple organic compounds, but not the known estrogens estrone, estradiol, and diethylstilbestrol or chemicals with similar structures and metabolites. Since our laboratory found estrogenic activity in elutants of PC-SPES corresponding to separate peaks obtained from high-performance liquid chromatography, multiple phytoestrogens may be responsible for the observed activity. Our results suggest that PC-SPES may prove useful in the treatment of hormonally sensitive prostate cancer; but when used concurrently with standard or experimental therapies PC-SPES may confound the results. In addition, estrogens can have substantial toxic effects, and the safety of nutritional supplements with substantial estrogenic activity needs to be evaluated. These data demonstrate that unregulated, commercially available dietary supplements may have biologic activity that can affect diseases, standard medical therapy, and general health.

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