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3 **Gynaecomastia linked to the intake of a herbal supplement fortified with**
4 **diethylstilbestrol**
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Abstract

This study reports the findings of a supplement marketed on the internet for prostate problems. The supplement was orally taken by a 60-year old man with divergent hormonal levels and who was surgically treated for gynaecomastia: development of abnormally large mammary glands in males. The supplement showed a strong effect in a yeast estrogen bioassay, expressing a yeast enhanced green fluorescent protein (yEGFP) upon exposure to estrogens. Using both NMR and a gradient liquid chromatographic time of flight mass spectrometric (LC/TOFMS) method, the response was shown to be caused by very high levels of diethylstilbestrol, known for causing gynaecomastia. The gynaecomastia was most probably caused by this orally taken 'natural' herbal supplement, as the patient hormonal levels also returned to normal again when stopping the use of it. This case demonstrates that physicians need to be aware of the use of supplements with illegal components that may be responsible for unwanted side-effects.

Introduction

Supplements for treatment of a variety of diseases can be easily obtained, without prescription by physicians, from various shops and through the internet. This is allowed since these products are based on natural compounds and do not contain pharmaceuticals. Pharmaceutical agents on the other hand can only be obtained through prescription by a physician. However, the use of supplements is not without risk, as shown by the following case, dealing with a vital 60-year old man who had undergone surgery for treatment of bilateral gynaecomastia.

Gynaecomastia is the development of abnormally large mammary glands in males and results in an enlargement of the male breast. Estrogens stimulate growth of breast tissue, androgens inhibit it. When the ratio between estrogens and androgens increases, breast tissue is stimulated to grow. Gynaecomastia is a common finding in the male population (Braunstein 2007). Three peaks in the age distribution can be identified: in neonates, in adolescent boys and in elderly. In neonates, the cases are due to the transfer of maternal estrogens and progesterone. Young men and adolescent boys are particularly sensitive to estrogens, while in the elderly gynaecomastia is associated with low testosterone levels. However, many cases of gynaecomastia have no clear cause, but about 10 to 20% are caused by drugs (Rohrich et al. 2003) for which over 300 pharmacological agents can be considered. Examples include the well known side effects of medications like cimetidine, ketoconazole, and spironolactone, but also medications specifically used to treat prostate cancer: cyproterone and flutamide, and even certain antipsychotics (Ismail and Barth 2001).

Here we report our findings with a patient who was treated for gynaecomastia. The case report describes a 60-year old man who was referred by a surgeon to evaluate

1
2
3 the cause of bilateral gynaecomastia. His medical history did not reveal any
4
5 abnormalities. The patient considered himself to be in a good physical condition,
6
7 doing exercises on a weekly basis and he reported normal libido and erectile
8
9 function. He had never used anabolic steroids, did not smoke and alcohol
10
11 consumption was moderate. On physical examination this man was remarkably vital.
12
13 His virilisation and body composition appeared normal (BMI of 26.6 kg/m²).
14
15 Examination of the chest and abdomen were normal. He had bilateral circumareolar
16
17 scars. Andrologic examination revealed normal testes and epididymes. However,
18
19 hormonal levels of sex steroids indicated hypogonadotropic hypogonadism (table 1).
20
21 While discussing these findings with the patient, it became apparent that his prostate
22
23 specific antigen (PSA) level had been evaluated on a regular basis. This had been
24
25 established and further examined by an urologist, but there had been no complaints
26
27 related to prostate hypertrophy or lower urinary tract symptoms. The family history
28
29 revealed a father and a grandfather with prostate carcinoma. The urologist performed
30
31 transrectal ultrasound guided prostate biopsies. These had been documented four
32
33 and three years earlier and revealed normal prostate tissue. Despite these findings,
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35 the patient remained worried about his mildly elevated levels of PSA, remained afraid
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37 of possibly developing prostate cancer, and perceived regular transrectal biopsies as
38
39 very stressful. Therefore he was not willing to agree with the proposed approach to
40
41 repeat them on a regular basis. To find a solution, the patient had consulted a
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43 physician in a private practice to look for alternative treatments. This physician
44
45 advised the use of a herbal supplement, labelled 'Prostasol', with the purpose to
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47 decrease the elevated PSA level and reduce the chance of developing prostate
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49 cancer. For approximately two years, the patient used the supplement with
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51 confidence, since his level of PSA became suppressed. However, in this period the
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3 patient had consulted a surgeon for his cosmetically bothersome gynaecomastia and
4 was surgically treated. Hereafter the surgeon referred the patient to an internist, in
5
6 order to find an explanation for the gynaecomastia.
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9
10 In this study the patient was asked to stop the use of the supplement and his
11 hormonal levels of sex steroids were examined again three and six months after
12 stopping the use, as it was speculated that the observed effects were caused by this
13 orally taken 'natural' herbal supplement. In particular, we studied the possibility that
14 this 'natural' herbal supplement might contain steroids, using a combination of
15 bioassays and confirmation techniques. The orally taken supplement was therefore
16 screened in a yeast estrogen bioassay and the presence of an estrogenic substance
17 was confirmed by NMR and LC/TOFMS analysis.
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32 **Materials and Methods**

33 **Chemicals**

34 Diethylstilbestrol (DES) was obtained from ICN, 17 β -estradiol (E2) from Sigma, 17 β -
35 testosterone (T) from Steraloids and dimethyl sulfoxide (DMSO) from Merck.
36
37 Chemicals to prepare the growth media and the preparation of the growth media for
38 yeast cells were as described elsewhere (Bovee et al. 2004). Two batches of the
39 supplement were obtained from the patient, who had ordered them from the internet,
40 in a package labelled 'Prostasol' and containing capsules with 450 mg. The first batch
41 carried the charge number 050926 and was valid until 25-09-2008 and the second
42 batch carried the charge number 070328 and was valid until 27-03-2010. The
43 package was identical to those shown on the internet. The patient was advised by a
44 physician to buy the supplement through the internet. According to the label,
45 Prostasol contains beta-sitosterol, cellulose, serenoa repens, quercetin, pygeum,
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3 scutellaria baicalensis, magnesium stearate, potassium hydrogen phosphate,
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5 camposterol, stimgasterol, brassicasterol, ganoderma lucidum, panax pseudo-
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7 ginseng, colloidal silicium dioxide, urtica dioica, and zingiber officinale. A third batch
8
9 was obtained from the internet and contained tablets of 910 mg. This third batch
10
11 carried the charge number 080118 and was valid until 17-01-2011. Compared to the
12
13 capsules, the tablets contained similar ingredients, but scutellaria baicalensis, urtica
14
15 dioica, and zingiber officinale were left out and 5 other ingredients were included:
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17 triglycerides, resveravine (8% resveratrol extract), sodium starch glycolate,
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19 hypromellose, and propylene glycol.
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24 *Blood analysis*

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26 Analysis of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin
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28 and thyroid stimulating hormone (TSH) were performed by an immunoenzymatic
29
30 assay, free thyroid hormone (FT4), testosterone (T) and estradiol (E2) were
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32 performed by a competitive immunoassay, all conform the procedure of the
33
34 manufacturer (Beckman Coulter). Sex hormone binding globulin (SHBG), prostate
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36 specific antigen (PSA), human chorionic gonadotropin and alpha-fetoprotein were
37
38 performed by a chemo-luminescence immunoassay conform the procedure of the
39
40 manufacturer (Siemens). Free Testosterone was estimated according to the equation
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42 as described by Vermeulen et al. in 1999.
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48 *Yeast estrogen and androgen bioassays*

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50 A capsule of the supplement was opened and 100 mg of the powder was extracted
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52 as described previously for feed (Bovee et al. 2006). The tablet was powdered and
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54 treated in the same way. In short, 100 mg Prostasol was mixed with 4 ml methanol
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56 and 4 ml sodium acetate buffer (0.25 N, pH 4.8). The sample was incubated for 10
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58 min in an ultrasonic bath and subsequently mixed head over head for 15 min,
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3 centrifuged at 3500 g, and 4 ml of the upper liquid phase was transferred to a glass
4 tube. Next, the pH was adjusted to 4.8 using 4 N acetic acid and the extract was
5 subjected to solid phase extraction (SPE) on a C18 column, previously conditioned
6 with 2.5 ml methanol and 2.5 ml sodium acetate pH 4.8. Subsequently, this column
7 was washed with 1.5 ml 10% (w/v) sodium carbonate solution, 3.0 ml water, 1.5 ml
8 sodium acetate pH 4.8, 3.0 ml water and finally with 2 ml methanol/water (50/50 v/v).
9 The column was air-dried and eluted with 4 ml acetonitrile. The eluate was applied to
10 an NH₂-column that was previously conditioned with 3.0 ml acetonitrile. The
11 acetonitrile eluate thus obtained was evaporated to 3 ml under a stream of nitrogen
12 gas. A 200 µl aliquot of this extract, equivalent to 3.3 mg of the supplement, and
13 several dilutions in acetonitrile were transferred to a 96 well plate in triplicate and 50
14 µl of a 4% DMSO solution in water was added to each well. To remove the
15 acetonitrile, the plate was dried overnight in a fume cupboard and was then ready to
16 be screened on estrogenic activity with the yeast estrogen bioassay. In the same way
17 a reagent blank was prepared.

18
19 Cultures of the yeast estrogen and androgen biosensor were grown overnight at
20 30°C with vigorous orbital shaking. At the late log phase, the cultures of both
21 cytosensors were diluted in the selective minimal medium supplemented with L-
22 leucine (MM/L) to an optical density (OD) value at 630 nm was reached between 0.04
23 and 0.06. For exposure, aliquots of 200 µl of this diluted yeast culture were pipetted
24 into each well of a 96-well plate and 2 µl of a stock solution in DMSO was added to
25 test the agonistic properties of the compounds. To test for anti-androgenic properties,
26 1 µl amounts of the stock solutions were co-exposed with 1 µl of 17β-testosterone
27 stock solutions known to cause either a half-maximal or a near maximal response.
28 DMSO and control containing only 17β-estradiol (E2) or 17β-testosterone (T) were
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3 included in each experiment and each sample concentration was assayed in
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5 triplicate. Exposure was performed for 24 h at 30°C and orbital shaking with 125 rpm.
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8 Fluorescence and OD were measured at 0 and 24 h directly in a Synergy™ HT
9
10 Multi-Detection Microplate Reader (BioTek Instruments Inc., USA) using excitation at
11
12 485 nm and emission at 530 nm. The fluorescent signal was corrected with the
13
14 signals obtained with the supplemented MM containing DMSO solvent only. In order
15
16 to check whether a sample was toxic for the yeast cells, densities of the yeast culture
17
18 were determined by measuring the OD at 630 nm.
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21 22 *NMR analysis*

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24 The methodology for sample preparation, analysis and measurement is described
25
26 elsewhere (Lommen et al. 2002). Basically, a few mg's of the contents of the capsule
27
28 were extracted with 1 ml methanol-d₄. In addition, ca. 1 mg of the reference standard
29
30 diethylstilbestrol was dissolved in 1 ml methanol-d₄ (Merck 99.8%). The ¹H NMR
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32 analysis was performed on a Bruker AMX 400 WB spectrometer. Presaturation was
33
34 performed on the H₂O resonance. A 90° pulse was used; the total relaxation delay
35
36 was 3.7 s; spectral width was 5000 Hz. The data were acquired in 16 K data points at
37
38 300 K. Before Fourier transformation and phasing, a 1/3 shifted quadrature sine bell
39
40 filter was applied and a zero-filling to 128 K. Calibration of spectra was achieved by
41
42 setting the HCD₂-resonance of deuterated methanol to 3.27 ppm.
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48 *LC/TOFMS analysis*

49
50 A capsule of the supplement was opened and 10 mg of the powder was transferred
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52 to a vessel and 10 ml methanol was added. The tablet was powdered and treated in
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54 the same way. After ultrasonic treatment for 30 min, 25 µl of the extraction solvent
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56 and 950 µL of an internal standard DES-d₆ in ethanol were transferred to a 2 ml
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58 autosampler vial. This extract, containing DES-d₆ (100 ng/ml), was analysed in
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3 duplicate on a high resolution UPLC/TOFMS. Separation was carried out with an
4
5 ultra performance liquid chromatographic (UPLC™) system, consisting of a vacuum
6
7 degasser, autosampler, and a binary pump (Acquity UPLC system, Waters, Milford,
8
9 MA, USA) equipped with a reversed phase Waters Acquity UPLC BEH C18 analytical
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11 column (50x2.1 mm, 1.7 µm particle size). The gradient (solvent A, water–acetonitrile
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13 (90:10, v/v); solvent B, water–acetonitrile (10:90, v/v)) was: 0-2 min 25% B; 2-15 min
14
15 linear increase to 90% B with a final hold for 1 min. The injection volume was 10 µl
16
17 and the flow rate was 0.2 ml/min. The UPLC system was connected to a Time-of-
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19 Flight Mass Spectrometer Waters-Micromass LCT Premier ToF equipped with an
20
21 electrospray interface operating in the negative ion mode, using the following
22
23 parameters: cone voltage 50V, capillary voltage 2700V, source temperature 120°C,
24
25 and desolvation temperature 350°C. Full scan spectra from 100 to 1000 Da were
26
27 acquired with a scan time of 0.25 s. Mass accuracy was maintained by using a lock
28
29 spray with lock mass of leucine-enkefaline 12C [M-H]⁻ ion m/z 556.2771. Resolution
30
31 was at least 10,000 FWHM at m/z of the lock mass. Dynamic range enhancement
32
33 (DRE) was switched on.

34
35 For quantification, a detector response versus concentration plot was constructed
36
37 with six different concentrations of DES in methanol (0-250 ng/ml and DES-d6 at 100
38
39 ng/ml) by plotting the ratio of the peak area of the extracted ion chromatogram (EIC)
40
41 for DES m/z 267.1385 (mass of the [M-H]⁻ ion) and the peak area of the EIC for DES-
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43 d6 m/z 273.1761 against the concentration. The concentrations in the samples were
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45 calculated using the linear regression method.
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Results and Discussion

Plasma hormone levels

When it became clear that the patient used a herbal supplement, which could be a possible factor in the cause of the hypogonadotropic hypogonadism and the development of gynaecomastia, he was asked to stop this use. His hormonal levels of sex steroids were examined again three and six months after stopping the use. As shown by the plasma hormone levels in table 1, the patient showed hypogonadotropic hypogonadism with a normal prolactin level while using the supplement. There were no signs or symptoms of bitemporal hemianopsia, no clinical signs of a Cushing syndrome, acromegaly nor hypothyroidism. There were neither signs of severe illness, stress or excessive exercise, which can also cause a reversible deficiency of gonadotropins. (McLeod and Iversen 2000). The patient showed decreased levels of LH and T and elevated levels of SHBG.

Hypothalamic gonadotrophin releasing hormone (GnRH) regulates the production of the pituitary gonadotrophins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH acts primarily on the Leydig cell to stimulate testosterone synthesis. The regulatory control of androgen synthesis is mediated by testosterone and estrogen feedback on both the hypothalamus and pituitary. The majority of androgens, more than 90%, are produced by the testes. Approximately 80% of estrogens are of extra-gonadal origin and originate from peripheral aromatisation of androgens. This is illustrated schematically in figure 1 (McLeod and Iversen 2000). Thus the patient's decreased level of LH was accompanied by a related suppressed level of testosterone.

However, the elevated level of SHBG was remarkable. Intravascular, the majority of the sex steroids are connected to SHBG, which has a higher affinity for androgens

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3 than for estrogens. Levels of SHBG increase with higher levels of estrogens and
4 decrease with higher levels of androgens. As the patient's E2 level was normal and
5 his T levels were decreased, the increased SHBG level was surprisingly. It was
6 therefore hypothesised that some ingredients of the herbal supplement might cause
7 an estrogen-like effect on the hypothalamic-pituitary-testis-axis or might cause an
8 inhibiting action on testosterone production. In that aspect, the patient's
9 gynaecomastia could also be explained, as gynaecomastia develops in case of
10 misbalance between hormones with estrogenic effects over hormones with
11 androgenic effects. Such an increased estrogen-androgen ratio can be the result of
12 increased levels of estrogens, decreased levels of androgens or increased levels of
13 estrogen precursors. Moreover, gynaecomastia can develop in association with
14 hypogonadotropic hypogonadism.

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32 The patient was then asked to stop the use of the supplement and his hormonal
33 levels, measured three and six months after stopping the intake of the supplement,
34 supported the hypothesis, as his levels of T, LH, and FSH became normal again
35 (table 1). Also, the SHBG level returned to normal and can be explained by the lower
36 estrogen-androgen ratio, as androgens inhibit production of SHBG. However, the
37 patient now clearly showed elevated E2 level, for which we have no explanation, and
38 PSA level. It was concluded that if the hypothesis was right and the supplement is
39 involved, it should contain a strong estrogenic compound. In addition, it might contain
40 a supplementary compound with an anti-androgenic mode of action.

51 52 53 *Examination of the supplement*

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55 According to the manufacturers' website, Prostatol contains the so-called herbal
56 components saw palmetto, ginseng, skullcap, and reishi. These components are
57 apparent in comparable products called 'SPES' and 'PC SPES'. Both SPES and PC
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3 SPES are described by their manufacturer as herbal mixtures for a non-estrogenic
4 treatment for prostate cancer. However, the contents of SPES and PC SPES are
5 sometimes questioned. The California State Department of Health Services issued a
6 public health warning about SPES and PC SPES after they tested positive for the
7 drugs alprazolam and warfarin respectively
8 (sonic.net/~brianf/caoma/spes_recall_m.htm). Also the U.S. Food and Drug
9 Administration (FDA) came up with a safety alert
10 (www.fda.gov/medwatch/SAFETY/2002/safety02.htm). Regarding Prostatol, the
11 website mentions that Prostatol contains quercetin, sitosterol, zinziber officinalis,
12 urtica dioica, and pygeum (www.med-pro.org). This herbal food supplement is
13 marketed as 'a non-estrogenic mixture' in the treatment for mild prostate cancer and
14 is described by its manufacturer as a pharmaceutical that is tested on toxicity in a trial
15 with prostate cancer patients. According to the manufacturer, these studies revealed
16 that it has a positive effect on the quality of life as it helps against the pain due to
17 disseminations in the bones with both patients with hormone sensitive and insensitive
18 prostate cancer. Moreover, the website mentions that the PSA levels in more than
19 70% of the patients decreased. In a placebo controlled survey of 200 men with
20 benign prostate hypertrophy examining the substance beta-sitosterol, a component of
21 Prostatol, it was reported that there were no significant side effects of beta-sitosterol
22 and it was suggested that beta-sitosterol is a safe agent (Berges et al. 1995).
23 However, that study reports only self-reported urological variables and there are no
24 endocrine data on levels of sex steroids and gonadotropins. Quercetin, another
25 component of Prostatol blocks growth of androgen sensitive humane prostate cancer
26 cells (Yuan et al. 2005). Quercetin inhibits expression of the human androgen
27 receptor, playing a role in proliferation, differentiation and maintenance of function of
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3 the prostate. Finally, pygeum has been shown to diminish enzyme activity of 5 alpha-
4 reductase and to have inhibiting effects on inflammation (Breza et al. 1998). There is
5 one published study that showed a suppression of testosterone upon intake of
6 Prostatol and that effect was allocated to the presence of phytoestrogens (Clement
7 and Bublely 2008). However, Prostatols' contents were not thoroughly analysed in
8 that study. Because it is questionable whether the information on the label about the
9 contents of the supplement is reliable, it was decided to further investigate the
10 androgenic and estrogenic characteristics of this herbal mixture. Bioassays are an
11 ideal tool for tackling this kind of problems since they are also able to detect
12 previously unknown hormonal compounds. The different batches of the supplement
13 were therefore screened for estrogenic activity in a yeast estrogen bioassay. Figure 2
14 shows the responses obtained with several dilutions of an extract prepared from the
15 first batch. It clearly shows that the supplement contains at least one strong
16 estrogenic compound. The extract had to be diluted at least a 10.000 times in order
17 to obtain a signal lower than the maximum response in this bioassay. Based on the
18 E2 standard curve, it was estimated that this first batch contained about 0.3 mg "E2
19 equivalents" per gram.
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43 For identification of the estrogenic compound(s), we decided to first analyse a sample
44 by NMR as the amount seemed to be in the mg per gram range. This revealed the
45 possible presence of diethylstilbestrol (DES). With the aid of a DES standard, the
46 identity was confirmed, as all 4 multiplet signals of DES could be found in the extract
47 at the correct positions (data not shown). Identification and confirmation of DES was
48 then performed by LC/TOFMS analysis. Figure 3 shows the ion current
49 chromatograms of mass 267.1357 ± 0.0001 Da of a standard solution of DES (250
50 ng per ml) (3A) and the methanol extract that was prepared from the same Prostatol
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3 batch (3B). Figure 3C shows the ESI-TOF mass-spectrum taken at the retention time
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5 of DES. It confirms the presence of DES in the extract. The calculated amount from a
6
7 duplo analysis for this capsule was 0.9 ± 0.2 mg DES per gram.
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10 A capsule of a second batch, also obtained from the patient, was analysed and
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12 revealed the presence of an estrogenic activity corresponding to 3.6 mg “E2
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14 equivalents” per gram in the bioassay and 4.1 ± 0.1 mg DES per gram by LC/TOFMS
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16 analysis. DES has been shown to be about as potent as E2 in the yeast estrogen
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18 assay (Bovee et al. 2004). This means that the semi-quantitative calculated amounts
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20 from the bioassay should more or less be comparable with the amounts determined
21
22 by LC/TOFMS analysis. The results from both batches show that this is indeed the
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24 case. After the patient was informed a new batch of Prostatol was ordered from the
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26 internet and contained tablets instead of capsules. When analysed in the same way,
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28 the tablets contained no estrogenic activity and no DES.
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34 As NMR analysis did not reveal the presence of any other steroids in the first two
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36 batches, we tested whether the anti-androgenic effects, as observed in the patient,
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38 could also be due to DES. We therefore tested DES in the yeast androgen bioassay
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40 by testing DES with and without the addition of 17β -testosterone (T). In this way we
41
42 were previously able to demonstrate the specific anti-androgenic action of
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44 compounds such as flutamide, but also the known anti-androgenic properties of E2
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46 and 17α -ethinylestradiol (Bovee et al. 2008 and Bovee et al. 2009). These results are
47
48 shown in figure 4 and clearly demonstrated that DES can also act as an androgen
49
50 receptor antagonist.
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55 The patient was advised to take 4 capsules of Prostatol per day, corresponding to
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57 about 4.5 mg DES per day. As DES is about as estrogenic as E2 and 17α -
58
59 ethinylestradiol and as an average birth control pill contains about 0.03 mg 17α -
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3 ethinylestradiol per tablet, it means that this patient was orally taking an estrogenic
4 amount that is equal to 150 birth control pills per day. Moreover, compared to 17 α -
5 ethinylestradiol, DES is regarded as a less safe agent. In 1971, in utero exposure
6 was found to be associated with a greatly increased risk of clear cell carcinoma of the
7 vagina and cervix, known world wide as the DES daughters (Herbst et al. 1971).
8 Subsequently, DES use was found to be associated with an increased risk of breast
9 cancer in women who took the drug (Colton et al. 1993). The latest results suggest
10 that the DES daughters also have an increased risk of breast cancer after age 40
11 years (Palmer et al. 2006). Even the fertility of men exposed to DES has been
12 investigated, but revealed that there was no increased risk of infertility among men
13 exposed to DES before birth (Wilcox et al. 1995). However, several studies have
14 shown a misplaced opening of the penis (hypospadias), epididymal cysts (non-
15 cancerous growths on the testicle), and undescended testicles (cryptorchidism) (Wilcox
16 et al. 1995 and Brouwers et al. 2006). In addition, DES has been studied in the
17 treatment of prostate carcinoma, but high doses of DES, 5 mg per day, were
18 associated with increased mortality from cardiovascular disease. With a 1 mg per day
19 dose there was no increase in cardiovascular mortality, but it appeared to be as
20 effective as the high dose, although the testosterone level was not completely
21 suppressed (Brawer 2001 and Montgomery et al. 2007).
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48 The adulteration of natural supplements with synthetic compounds is not new. Katz et
49 al. (2002) mentioned about the related supplement PC-SPEC that 'at present, a more
50 extensive controversy surrounds this herbal agent because (unpublished, but highly
51 publicized) analyses suggest that some batches were contaminated with synthetic
52 hormone (DES) and/or blood coagulation effector (warfarin). If true, it is certainly
53 another indicator of a need for more rigorous regulation of the nutraceutical industry'.
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3 PC-SPES, a herbal additive comparable to Prostatol, is no longer allowed in the US
4
5 since 2002, as it was also shown to contain traces of warfarin. Oh et al. (2004) had to
6
7 terminate a clinical trial comparing PC-SPES with DES when it turned out that the
8
9 supplement contained variable amounts of DES. Sang et al. (2006) described the
10
11 presence of ethinylestradiol in batches of PC-SPES. With regard to Prostatol, the
12
13 Danish Medicines Agency in November 2006 issued a warning that it may contain
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15 diethylstilbestrol and encouraged people to stop using it and hand over the remaining
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17 part of the product to a pharmacy
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22 (www.dkma.dk/1024/visUKLSArtikel.asp?artikelID=10577).
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27 **Conclusions**

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29 An elevated estrogen-androgen ratio in a 60-year old male patient probably caused
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31 the development of gynaecomastia, for which he was surgically treated. As
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33 demonstrated by the decreased levels of T and LH, there was hypogonadotropic
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35 hypogonadism. This patient was using a herbal supplement. It was hypothesised that
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37 some ingredients of this supplement might have caused an estrogen-like effect on
38
39 the hypothalamic-pituitary-testis-axis or an inhibiting action on testosterone
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41 production. In particular the increased SHGB level led to this idea. When the patient
42
43 stopped the use of the supplement, his T, SHGB, FSH, and LH reversed to normal
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45 levels.
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50 Since Prostatol contains some natural ingredients that might lead to estrogenic and
51
52 anti-androgenic effects *in vivo*, we investigated whether these natural compounds
53
54 could have had the strength to cause the observed gynaecomastia. Using a yeast
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56 estrogen bioassay, it was shown that the supplement used by the patient, caused a
57
58 very high estrogenic activity, corresponding to mg amounts of E2 equivalents per
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3 gram of supplement. NMR and LC/TOFMS analysis subsequently showed that this
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5 'natural' product contained huge amounts of the synthetic estrogen DES. When
6
7 tested in the yeast androgen bioassay, it was shown that DES can also inhibit the
8
9 response of testosterone. These results show that DES can act both as a strong
10
11 estrogen receptor agonist and a strong androgen receptor antagonist. The presence
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13 of this huge amount of the synthetic drug DES in the supplement is incontrovertible
14
15 related to the observed abnormal hormonal levels in this patient as all these
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17 abnormalities can be explained by the activities of DES. Moreover, DES is orally
18
19 active and has previously been shown to cause gynaecomastia (Wortsman et al.
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21 1989 and Malkowicz 2002).
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27 At this stage we are not able to explain the increased level of estradiol as observed
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29 after stopping the use of the supplement. After stopping, the patient also showed an
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31 increased PSA level again, but lowering the PSA level to reassure a patient is a
32
33 questionable advice.
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37 In summary, as lower urinary tract symptoms in an increasing number of men
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39 threaten their quality of life, they are stimulated to use health improving products, but
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41 it is a misconception to think that so called natural medications, herbal drugs or food
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43 additives cannot cause side effects. Moreover, this case shows that these products
44
45 might contain ingredients that are not mentioned on the label. Although highly
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47 publicized, it is hardly published and the main of consumers are probably not familiar
48
49 with all the issues involved. This in combination with insufficient communication
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51 between physicians and patients can result in potentially dangerous interactions
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53 (Capodice and Katz 2006). In our case it led to an unnecessary removal of breast
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55 tissue.
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Table 1: Hormonal values of a 60-year old patient who suffered from gynaecomastia.

| | Normal values | During use of 'Prostasol' | 3 months after stopping the use of 'Prostasol' | 6 months after stopping the use of 'Prostasol' |
|--------------------|----------------|------------------------------|--|--|
| PSA | <3.5 µg/l | 0.96 | 5.7 | 11.6 |
| Estradiol | 40-120 pmol/l | 108 | 150 | 253 |
| Testosterone | 9-38 nmol/l | 0.4 | 11.7 | 11.9 |
| Free testosterone | 175-750 pmol/l | 3 | 260 | 288 |
| LH | 2-15 U/l | 1.6 | 3.8 | 12.6 |
| FSH | 2-10 U/l | 2.2 | 11.8 | 3.8 |
| SHBG | 10-70 nmol/l | 127 | 26 | 22 |
| Prolactin | 0-250 mU/l | 157 | 126 | 115 |
| TSH | 0,3-5,2 mU/l | 1.7 | 1.2 | 1.7 |
| F-T4 | 12-28 pmol/l | 14.5 | 16 | 16.5 |
| Alpha foetoprotein | 0-15 µg/l | normal | | |
| Beta HCG | <5 U/l | normal | | |

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8 Figure 1.

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10 Glandular and extraglandular origins and interrelations of the androgens:
11 testosterone, DHT, androstenedione, and the estrogens: estradiol and estrone, and
12 their effect on breast tissue. The effect of prostate cancer treatments on these
13 pathways is also shown. Thick arrows denote the major sources of the hormone
14 (obtained from McLeod and Iversen 2000).
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25 Figure 2.

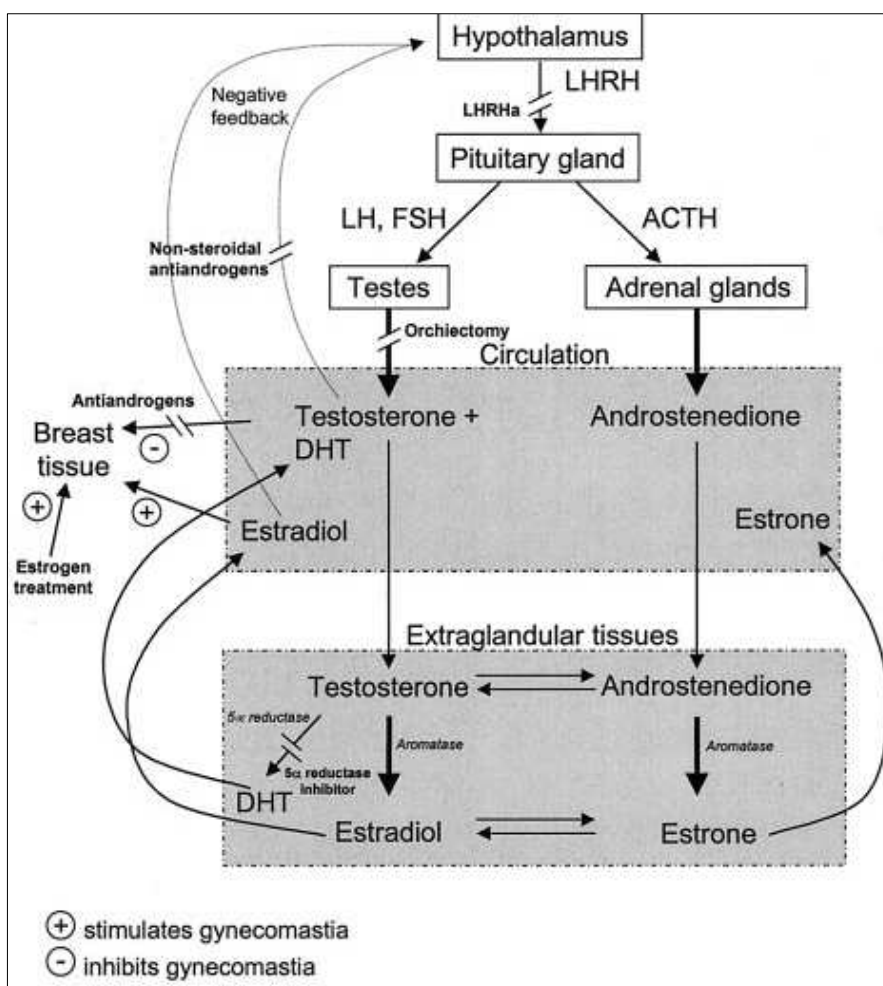
26 Responses of E2 (◆ closed diamonds [nM]) and the first batch of Prostatol (▲
27 closed triangle [μl]) in the yeast estrogen bioassay expressing human estrogen
28 receptor α and yeast enhanced green fluorescence protein upon exposure to
29 estrogens.
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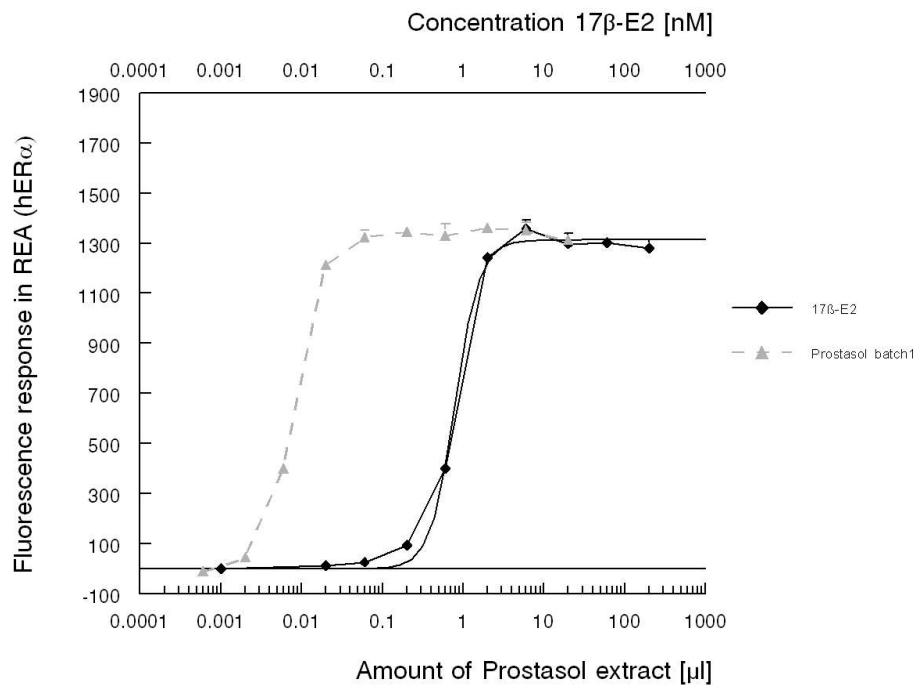
39 Figure 3.

40 Accurate mass LC-TOFMS analysis of the first batch of Prostatol. A Standard
41 solution DES (250 ng per ml). B Methanol extract of Prostatol. C The ESI-TOF mass-
42 spectrum of the Prostatol extract taken at the retention time of DES.
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51 Figure 4.

52 Responses of testosterone (T) (+ plus), DES (Δ open triangle) and DES in
53 combination with a high (▲ closed triangle) and low (▼ closed triangle) dose of T, in
54 the yeast androgen bioassay expressing human androgen receptor and yeast
55 enhanced green fluorescence protein upon exposure to androgens.
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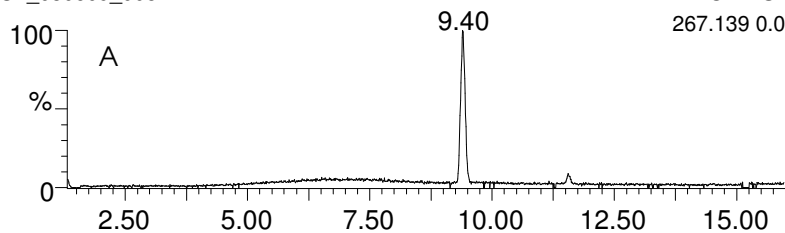


Responses of E2 (• closed diamonds [nM]) and the first batch of Prostatol (▲ closed triangle [μ l]) in the yeast estrogen bioassay expressing human estrogen receptor alpha and yeast enhanced green fluorescence protein upon exposure to estrogens.
270x208mm (120 x 120 DPI)

RIK0215677 MeOH extract (1.04 mg/ml)

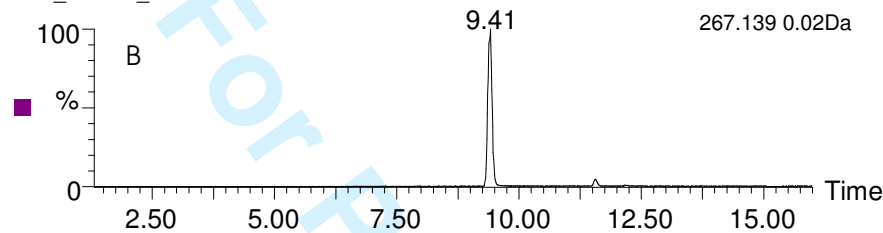
LCT_080609_005

1: TOF MS ES-
267.139 0.02Da



LCT_080609_007

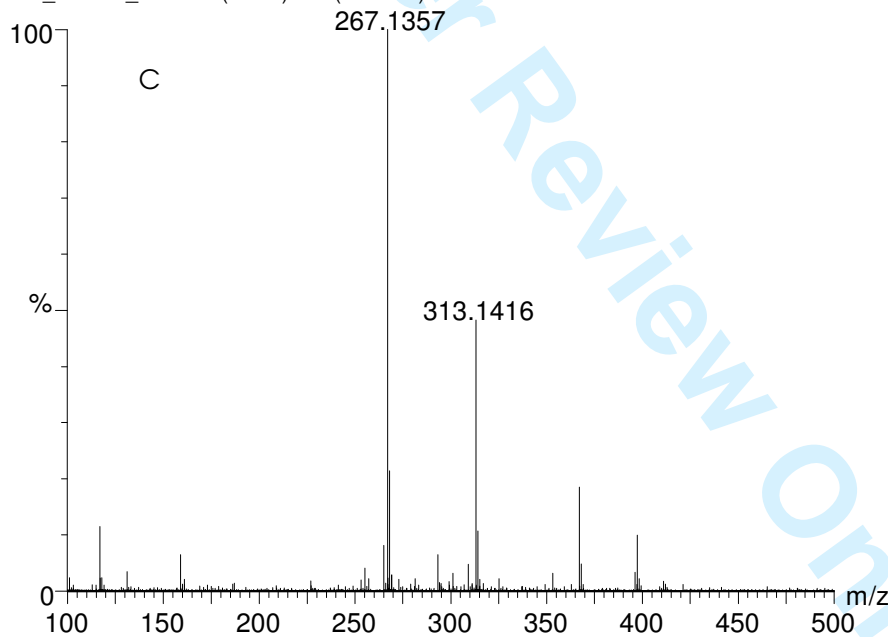
1: TOF MS ES-
267.139 0.02Da

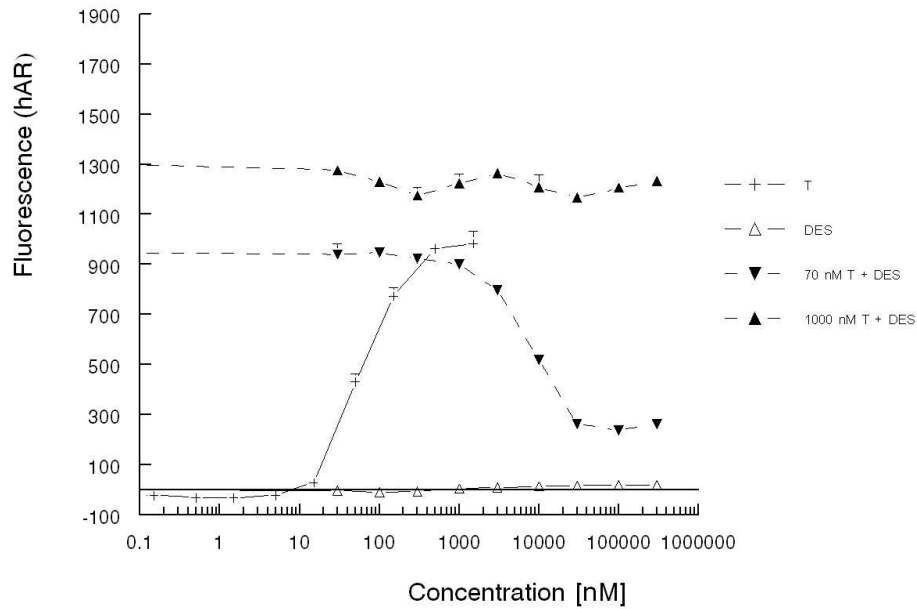


RIK0215677 MeOH extract (1.04 mg/ml)

LCT_080609_007 785 (9.415) Cm (777:792)

1: TOF MS ES-





Responses of testosterone (T) (+ plus), DES (Δ open triangle) and DES in combination with a high (▲ closed triangle) and low (▼ closed triangle) dose of T, in the yeast androgen bioassay expressing human androgen receptor and yeast enhanced green fluorescence protein upon exposure to androgens.
270x208mm (120 x 120 DPI)