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Cactus flower extracts may prove beneficial in benign prostatic hyperplasia due to inhibition of 5α reductase activity, aromatase activity and lipid peroxidation

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Abstract The cactus flower is deemed to be helpful in benign prostatic hyperplasia (BPH) therapy, although there is no published information regarding its clinical effect in patients and on the mechanism of its biological activity. The present study evaluated the ability of cactus flower extracts to exert an effect on BPH through possible inhibition of such processes as lipid peroxidation, androgen aromatization and testosterone reduction. Cactus flower extracts indeed inhibited aromatase and 5α reductase activity in cultured foreskin fibroblasts, and also in human placental and prostatic homogenates. The inhibitory activity in both instances was associated with the dichloromethane or ethanol (methanol) extracts, while a marked antioxidative activity was associated with the aqueous extract. The finding that cactus flower extracts interfere concurrently in vitro with aromatase and reductase activity as well as with free radical processes suggests that these substances may prove beneficial in BPH treatment.

Key words Cactus flowers extracts · 5α reductase inhibitor · Aromatase inhibitor · Antioxidants

Introduction

Numerous compounds have been tested during the last decade for possible therapeutic and prophylactic effect on benign prostatic hyperplasia (BPH), which is the most common neoplastic disease in the human male [10]. Much effort has been expended on the formulation of inhibitors of prostatic steroid 5α reductase, aromatase inhibitors, and alpha-blockers. Regarding the 5α reductase inhibitors, it was reasoned that these could block the conversion of testosterone to the more potent tissue-specific androgen 5α dehydro-testosterone, which is believed to be involved in the etiology of prostatic hyperplasia and prostatic cancer [11, 28, 30]. The prostate gland is an androgen-sensitive organ and therefore androgen deprivation decreases the size of the prostate. Increasing evidence for involvement of the estrogen in pathogenesis of BPH [9, 14, 32] has led to the initiation of including aromatase inhibition as a medical strategy in clinical trials of BPH. Aromatase inhibitors block the estrogen biosynthesis stemming from the aromatization of androstenedione and testosterone. Phytotherapeutic preparations have had a long tradition of use in the medical treatment of BPH in Europe and are still commonly used for this purpose [1, 2]. Various plant extracts such as those from *Serenoa repens*, *Sabalis serrulatae*, *Urtica dioica*, etc. are marketed for the treatment of BPH and are reported to have 5α reductase inhibitory activity, aromatase inhibitory activity or the ability to modulate the binding of sex hormone-binding globulin to its receptor on membranes [18, 25, 34]. No precise biochemical mode of action has been elucidated for various extracts from plants such as *Pygeum africanum* [21], *Hypoxis rooperi* [6], or the rye pollen extract known as "Cernitin" [15]. The purpose of the present study was to evaluate the 5α reductase inhibition, aromatase inhibition and antioxidant potential of cactus flower extract known as opuntia. Opuntia is included in the British Herbal Pharmacopoeia as a medicine with astringent and antihemorrhagic effects, which is indicated for colitis, diarrhea and prostatic hypertrophy.

Materials and methods

Chemicals

[1,2,6,7-^{3}H(N)]-testosterone (1 mCi/ml) and [1β, 2β-^{3}H(N)]-androst-4-ene-3,17-dione (1 mCi/ml) were purchased from Du Pont. The nonradioactive steroids testosterone and dihydrotestosterone, and linoleic acid, were obtained from Fluka (AG Buchs, Switzerland). 4-androstene-3,7-dione, NADP, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, n-octyl β-D-glucopyranoside, dithio-1,4- erythritol, EDTA, 2-mercaptoethanol,

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TRIS, β-carotene, Tween 40 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were all purchased from Sigma (St Louis, Mo.). TLC plates, silica gel 60 and F-254 were obtained from Merck (Darmstadt, Germany). The cactus flowers were obtained from Herbamed (Rehovot, Israel).

Plant extracts

Aqueous extract was prepared by incubating 30 g of dry cactus flower powder in 250 ml of distilled water and autoclaved for 20 min. The extraction of a smaller amount (5 g) of plant by 55 ml organic solvents (petroleum ether, dichloromethane, ethanol or mixture of dichloromethane-methanol (9:1)) was carried out in Sohxlet apparatus for 5 h or, later, in an Erlenmeyer flask overnight at room temperature. This last dichloromethane-methanol extract (henceforth designated DM) was further separated by vacuum liquid chromatography column with silica gel (5 × 2 cm) by the consecutive elution with 100 ml of petroleum ether (60–80°C), petroleum ether-diethyl ether (1:1) and diethyl ether. The diethyl ether-eluted subfraction (henceforth DM3) was further separated by vacuum liquid chromatography column with silica gel (5 × 2 cm) by the consecutive elution with 100 ml of petroleum ether, petroleum ether- diethyl ether (1:1) and petroleum ether- diethyl ether (2:3). The last subfraction is henceforth designated DM33. All parallel extraction and separation were performed at least three times, giving very similar results. The schema of separation is shown in Fig. 1.

Fibroblast culture

Fibroblasts were established in our laboratory from explants of human foreskins. Fibroblasts were grown in 75-cm^2 plastic flasks (Corning Glass Works, Corning, N.Y.) in Dulbecco's modified Eagle medium (DMEM) containing 4500 mg/l glucose supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 50 mg/ml gentamycin sulfate, and 2.5 mg/ml amphoteracin B. The DMEM and supplements were all products of Biological Industries, (Kibbutz Beit Haemek, Israel). The cultures were incubated at 37°C in 5% CO_2–95% air atmosphere until confluent. Fibroblast cultures were subcultured by trypsinization and used between the third and eighteenth passage number.

Cytotoxicity assay

Cytotoxicity of plant extracts was studied by MTT assay in fibroblasts as described previously [27]. In this assay, the tetrazolium salt (MTT) reduction to a colored formazan product in the cells in presence (or without) of extracts was used as a cell viability index.

Preparation of 5α reductase

Soluble 5α reductase mixture was prepared from human hypertrophic prostate tissue obtained at surgery, as described elsewhere [24]. Briefly, frozen prostatic tissue was homogenized with 100 mM sodium phosphate buffer pH 8.0, containing 10% (v/v) glycerol, 1 mM EDTA, 5 mM dithio-1,4-erythritol and 0.5% (w/v) n-octyl-β-D-glucopyranoside. The homogenate was centrifuged at 20 000 *g* for 20 min and the supernatant used as the 5α reductase mixture.

5α reductase activity assay

5α reductase activity of soluble enzyme was measured by the reduction at 37°C for 40 min of [1,2,6,7,-^{3}H(N)] testosterone (0.34 mM) in a 3-ml enzyme mixture, pH 6.5, containing 0.5 mM NADPH, with and without the test substances. For the test substances preparation, 10–100 µl aliquots of ethanol or dichloromethane extracts were evaporated to a state of dryness, dissolved in 10 µl DMSO and in this form added to the incubation mixture. DMSO was used as a control solution to ensure that it had no inhibitory action on the enzyme. Plant water extract was added to the reaction mixture as it is. Steroids were extracted and separated by TLC as described previously [4].

The radioactivity of the dihydrotestosterone was determined by liquid scintillation spectrophotometry. Activities were corrected for enzyme free control. In cultured fibroblasts, 5α reductase activity was measured according to Evans et al. [7]. Briefly, cells were incubated overnight with serum-free medium at pH 7.0 containing [1,2,6,7,-^{3}H(N)] testosterone or [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione (20 nM). Following incubation aliquots of the incubation medium were extracted twice with ethyl acetate and then analyzed by TLC as described previously.

Preparation of aromatase and aromatase assay

Aromatase mixture was prepared from human placentas and assay of aromatase was performed according to [31]. Briefly, the test buffer contained an NADPH-regenerating system (glucose-6-phosphate, glucose-6-phosphate-dehydrogenase) human placental microsomal fraction, nicotinamide, $MgCl_2$, and dithiothreitol in phosphate buffer. Incubation was started by the addition of [1β, 2β-^{3}H(N)]-androst-4-ene-3,17-dione and unlabeled androst-4-ene-

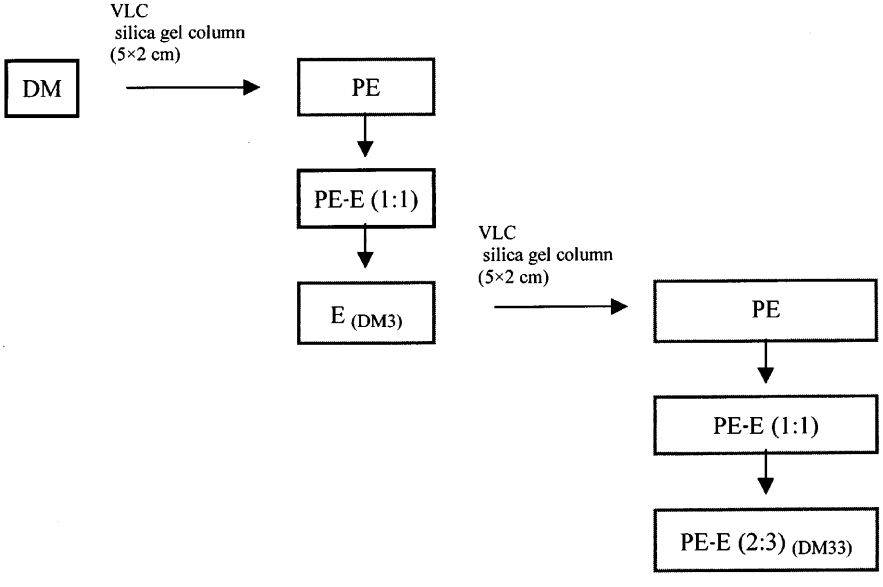

Fig. 1 Separation schema of dichloromethane-methanol (DM) extract of cactus flowers by vacuum liquid chromatography (VLC) column by consecutive elution with 100 ml of different solutions. *PE* petroleum-ether; *E* diethyl ether

3,17-dione together with test compound. Incubation period was 15 min at 37°C. Subsequently 200 ml of a 5% (w/v) charcoal suspension was added. After centrifugation, liberated tritiated water in the supernatant was counted and its quantity served as an index of enzymatic activity. Aromatase activity in cultured cells was measured according to Berkovitz et al. [3]. Before aromatase assay in monolayered fibroblasts, cells were seeded onto 25 cm^2 (for monolayer) and kept in culture until very confluent (2–3 weeks). The cells were transferred to serum-free medium 24 h prior to assay. Cells were incubated at pH 7 in media containing [1β,2β-^{3}H] androstenedione (2–50 nM) and progesterone (5 μM) for the 5α reductase inhibition. Following incubation (37°C, 4 h), the flasks were placed on ice, the medium was removed and extracted twice with ethyl acetate. The aqueous layer was then decanted upon charcoal pellets, mixed thoroughly and centrifuged (2000 *g*, 10 min). Finally, the supernatant was added to scintillation liquid and the tritiated water was counted.

Antioxidative activity assay

The antioxidant activity was assayed by destruction of β-carotene with linoleic acid emulsion in presence of the tested compounds and this by the method of [17] with minor modifications. Briefly, β-carotene, linoleic acid and Tween 40 were dissolved in chloroform. A model emulsion was prepared by adding, with stirring, double-distilled water to the viscous, uniform lipid after chloroform evaporation. The test mixture was placed in a vial (final concentration 100 μg/ml), together with 5 ml of the model emulsion, and then incubated at 50°C. Destruction of β-carotene was determined by reading the absorbance (optical density, O.D.) at 470 nm.

Results

Aqueous (40 mg/ml), ethanol (36 mg/ml) and dichloromethane (18 mg/ml) cactus flower extracts were tested for their 5α reductase inhibition activity. The effects of our plant extracts on human soluble prostate 5α reductase are summarized in Table 1. The greatest inhibitory effects on 5α reductase were obtained with the ethanol and dichloromethane plant extracts. Also tested were four fractions prepared by consecutive (step-by-step) extraction of the plant powder in solvents with increasing polarity (petroleum ether < dichloromethane < ethanol < water), and in these tests the 5α reductase inhibitory effect was found to be associated with the dichloromethane and ethanol fractions, albeit some inhibitory activity was found also with the aqueous fraction (data not shown). Additionally we resorted to cold extraction of cactus flower with dichloromethane-methanol (DM) mixture (9:1) in order to avoid any thermal degradation of the extractable compounds. Prepared in this, the DM plant extract did inhibit 5α reductase activity in prostate crude extract (Fig. 2A) and in foreskin fibroblast culture (Fig. 2B) although no cytotoxic effect of plant extracts was determined in cultured fibroblasts. Since DM extract contained fewer extractive compounds, its 5α reductase inhibition was less than that by the hot ethanol or dichlormethane comparative extracts. However, after further separation of the DM extract by silica gel, the resultant subfraction designated DM3 and DM33, exhibited a potent inhibitory effect on 5α reductase in prostate crude extract (Fig. 2A). Our plant extracts were also employed in in vitro aromatase assay on human placenta crude extract and human foreskin fibroblast culture. A potent aromatase inhibitory

Table 1 The effect of cactus flower extracts on human prostate 5α reductase activity

Samples	Final concentration (mg/ml)[a]	DHT[b] (cpm)	Activity (% of control)
Control	0	15563 ± 639	100
Water extract (100 μl)	1.3	10080 ± 1711	64.7
Ethanol extract (10 μl)	0.05	4938 ± 570	31.7
Dichloromethane extract (10 μl)	0.1	6723 ± 381	43.2

[a] Amount of extracted compounds in incubation mixture
[b] Mean result from two parallel determinations

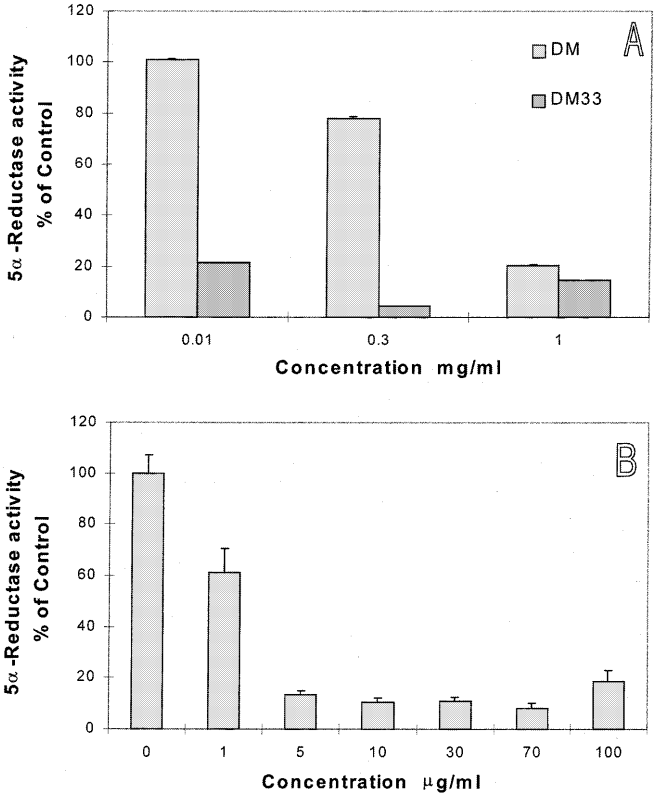

Fig. 2A, B Inhibition of 5α-reductase by cactus flower extracts (**A**) in an extract from human prostate; (**B**) in cultured foreskin fibroblasts. Amount of DHT synthesized from [1,2,6,7-^{3}H(N)]-testosterone was used as an index of 5α reductase activity

effect was obtained with both dichloromethane and ethanol hot extracts (data not shown), but a marked effect was also achieved with the DM extract. Both DM and the further subfraction DM3 and DM33 showed aromatase inhibitory effect. 80% of placental aromatase activity and 30% of foreskin fibroblast aromatase activity were inhibited by extracts (Fig. 3B). Effects of the 5α reductase inhibitor finasteride and of progesterone on the aromatization of [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione in foreskin fibroblasts culture are shown in Fig. 4. As can be seen both progesterone and finasteride markedly diminished the total concentration of androgens (androstandi-

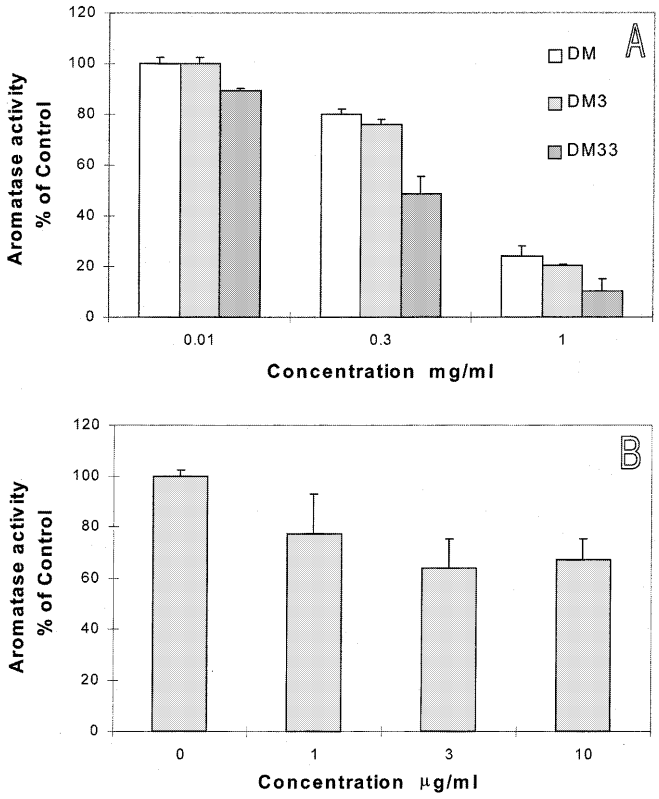

Fig. 3A, B The inhibition of aromatase activity by cactus flower extracts (**A**) in an extract from human placenta; (**B**) in cultured foreskin fibroblasts. Amount of tritiated water released during aromatization of [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione, was used as an index of aromatase activity.

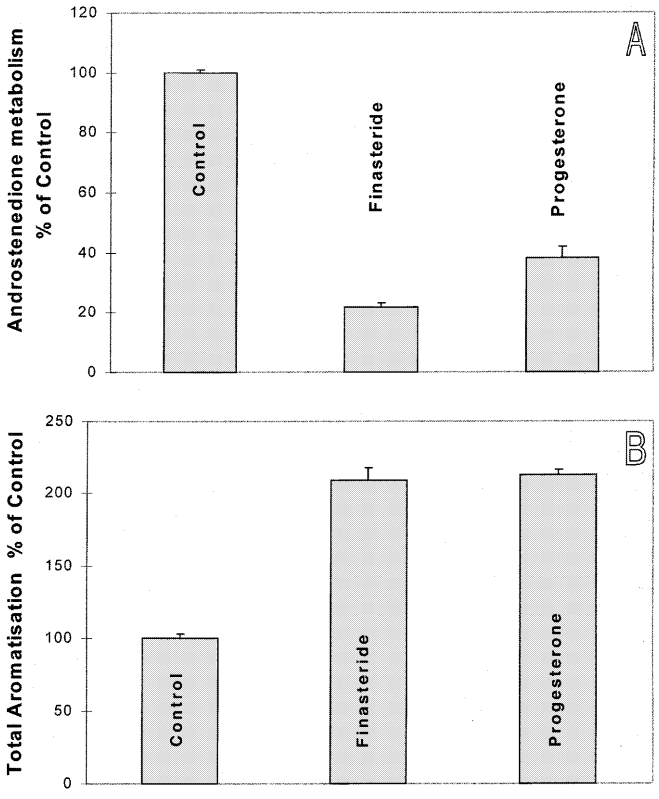

Fig. 4A, B Effect of the 5α-reductase inhibitors finasteride and progesterone. (**A**) On the transformation of [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione to its metabolites: androstandione, androstandiol, testosterone and DHT; (**B**) on aromatization of [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione, in cultured foreskin fibroblasts

one, androstandiol, testosterone and DHT) in fibroblasts. Yet, in the presence of 5α reductase inhibitors, the amount of the androgens involved in aromatization was double that in the nontreated control cells.

All the four fractions of plant step-by-step extraction, as well as the DM extract, its subfraction DM3 and DM33, and also aqueous extract proper were tested for antioxidant activity by assessing the protection against β-carotene oxidation in emulsions with linoleic acid. As shown in Fig. 5, the ethanol and water fractions displayed significant antioxidant activity, comparable to that of butylated hydoxyanisole (BHA), which is a potent antioxidant. Antioxidative properties of other extracts in test systems with β-carotene were not detected (data is not shown).

Discussion

The cactus flower is deemed helpful in benign prostate hyperplasia therapy (British Herbal Pharmacopoeia, 1983), although there is no published any information regarding its clinical effect on patients or the mechanism of its biological activity. In screening new therapeutic agents for the treatment of BPH we have examined the ability of the cactus flower to ameliorate BPH and this through the inhibition of such processes as lipid peroxidation, androgen aromatization and testosterone reduction. All our experiments with cactus flower extracts were geared to determine the inhibition of aromatase activity in the crude placenta extract and of 5α reductase activity in crude prostate extract. Ancillary trials were also carried out in cultured human foreskin fibroblasts because the latter were shown to contain a variety of steroid-metabolizing enzymes, including two 5α reductase isozymes as in human prostate [16, 35]. Moreover, the fibroblasts proved useful for screening aromatase inhibitors [3, 29]. Our results demonstrate that cactus flower extract contains antioxidants as well as 5α reductase and aromatase inhibitors. Thus, about 80% of the enzymatic activities of both aromatase and 5α reductase in crude placenta or prostate extracts were inhibited by our cactus flower extracts, but the properties of subfractions DM3 and DM33 were even greater. Contrariwise, in cultured foreskin fibroblasts only 30% of aromatase activity was inhibited compared with the control. One possible reason for this is the elevated level of [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione which transforms to estrogen in the presence of 5α reductase inhibitor. Androst-4-ene-3,17-dione is the chemical precursor of both testosterone (DHT) and estrogen (Fig. 6) and inhibition of testosterone transformation to DHT may cause increased androst-4-ene-3,17-dione aromatization. It has already been shown that proges-

terone, a competitive inhibitor of 5α reductase, stimulates aromatization of androstenedione without affecting the aromatase complex [3]. Our study demonstrates that in the presence of another 5α reductase inhibitor namely finasteride, the transformation of androstenedione to estrogen in cultured foreskin fibroblasts is markedly increased. This finding is in agreement with Lin et al. [20], who deduced from their experiments with rat smooth muscle cells that the aromatization pathway of testosterone to estradiol would prevail when DHT formation is inhibited. Accordingly, the relatively weak aromatase inhibitory effect of cactus flower extract on cultured foreskin fibroblasts, is attributable to the increased aromatization of [1β,2β-^{3}H(N)]-androst-4-ene-3,17-dione stemming from the concurrent 5α reductase inhibition. The finding that cactus flower extracts simultaneously interfere in vitro with both aromatase and reductase activities suggests their possible beneficial use in BPH treatment. Indeed, the contributory role of steroid 5α reductase (and its product DHT) to prostate enlargement has already been confirmed and there is increasing evidence that the inhibition of this enzyme would induce regression of the hyperplastic gland. The contribution of estrogen to the pathogenesis of BPH has also been proposed. One such hypothesis conjectures that under the mediation of sex hormone-binding globulin (SHBG), estrogen participates in setting the pace for prostatic growth and function [23]. It is generally assumed that SHBG synthesis is regulated by, and is, in fact, a reflection of the estrogen/androgen ratio [8]. Be that as it may, there is a highly significant fall in plasma testosterone and DHT levels in men above 50 years of age, so that the estrogen/androgen ratio is increased.

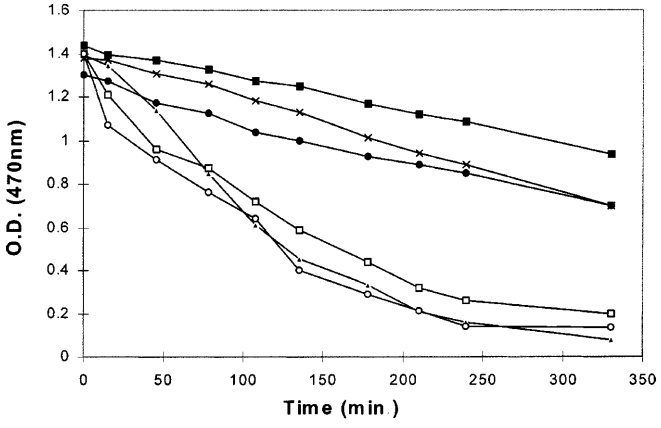

Fig. 5 Oxidation of β-carotene emulsion with linoleic acid in presence of cactus flower extracts. Fractions were prepared by consecutive extraction of cactus flower powder in solvents with increasing polarity. The protection against oxidation afforded by extract fractions to β-carotene in emulsion was compared with that of BHA at concentration 100 μg/ml. *Filled squares* BHA, *crosses* aqueous fraction, *filled circles* ethanol fraction, *Open squares* dichloromethane fraction, *filled triangles* petroleum ether fraction, *O* control

It is not clear as yet whether the in vitro finding on the effect of 5α reductase inhibitors on androgen aromatization is consistent with the in vivo situation. The reported results regarding the estrogen/androgen ratio in 5α reductase inhibitor-treated patients have not really resolved this problem, while Volpi et al. [33] have reported a bilateral gynecomastia in a 62-year-old man following finasteride treatment, which was probably caused by an increase of the estrogen to androgen ratio. From all the foregoing, however, we feel justified in concluding that the capacity of cactus flower extracts to inhibit aromatase as well as 5α reductase activity, augurs its usefulness in the treatment of the BPH. We further believe that the extremely high effective dose requirements of our cactus extracts and fractions as compared with those of potent synthetic 5α reductase or aromatase inhibitors, do not reflect the true picture but simply mirror inadequate extraction procedures. We are confident that the further and better fractionation of our cactus extracts will yield a much more highly concentrated product.

Another important finding of the present study is the high antioxidant activity of our aqueous plant extract. Numerous previous investigations have shown that lipid peroxides and reactive oxygen species (ROS) (e.g. superoxide radicals, singlet oxygen, hydrogen peroxide, hydroxy radicals) are involved in the regulation of cellular proliferation and in the etiology of a variety of diseases, including accelerated aging and prostate can-

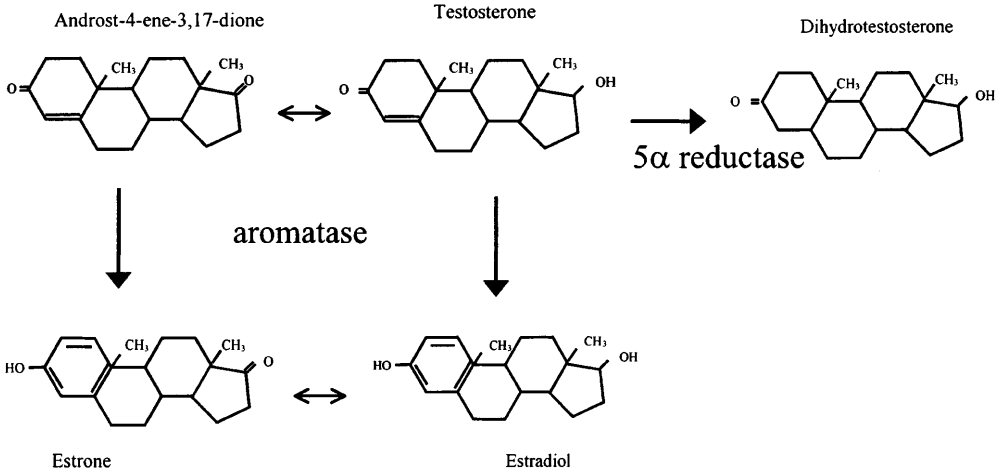

Fig. 6 Schema of androst-4-ene-3,17-dione metabolic pathway

cer. Intracellular ROS are generated spontaneously as a result of oxygen interaction with reducing compounds, or as intermediates of some metabolic reactions. Under normal conditions, the ROS level in the tissue is controlled by antioxidants and antioxidant enzymes such as glutathion, vitamin C, vitamin E, superoxide dismutase, catalase, glutathion reductase, etc. The augmentation of ROS concentration (oxidative stress) is commonly associated with increasing age and with several diseases accompanied by tissue inflammation.

Recently Ripple et al. [26] demonstrated that the oxidative stress is also increased by androgen treatment in androgen responsive human prostate carcinoma cells LNCaP. It is proposed that redox alteration may play a key role in a signal transduction pathway important for regulation cell growth [5]. Antioxidants are believed to reduce the risk of prostate cancer [12]. Publications generate sizable evidence that plant antioxidants play an important role in biological systems as agents of antioxidative defense [19]. Hence the ameliorative effect of cactus flower extract on prostate hyperplasia is likely to be attributable to numerous, as yet unidentified compounds, which inhibit the prostatic 5α reductase and aromatase activity and may possibly also regulate free radical processes. The full significance of cactus flower extracts in the treatment of BPH is now being evaluated by us in clinical trials.

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