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Efficacy of cactus flowers miller treatment in benign prostatic hyperplasia due to inhibition of 5α reductase activity, aromatase activity and lipid peroxidation.

Jonas, Adi.; Rosenblat, G.; Krapf, D.;Bitterman, W.; Earon, G.; Neeman, I.

ABSTRACT

Two clinical trials were evaluated by Herbamed to test the beneficial properties of cactus flowers miller among patients who suffered from BPH symptoms. This treatment significantly improved nucturia, drops after voiding, filling of bladder fullness, and emergency for urination and urgency for urination.

The possible mechanism of action of cactus flower extracts to exert an effect on BPH was also examined. Cactus flower extracts inhibited aromatase and 5-alpha 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 extracts, while a marked antioxidative activity was associated with the aqueous extract. The finding suggests the efficacy of cactus flowers in BPH treatment and the possible mechanism of action.

1. Introduction

Benign prostate hyperplasia is the most common benign neoplasm in men, and has a high prevalence that increases with age. As the prostate enlarges, the layer of tissue surrounding it stops it from expanding, causing the gland to press against the urethra like a clamp on a garden hose. The bladder wall becomes thicker and irritable. The symptoms of BPH vary, but the most common ones involve changes or problems with urination, such as: a hesitant, interrupted, weak stream, urgency and leaking or dribbling, more frequent urination, especially at night. Moreover, Severe BPH can cause serious problems over time. Urine retention and strain on the bladder can lead to urinary tract infections, bladder or kidney damage, bladder

stones, and incontinence-the inability to control urination. If the bladder is permanently damaged, treatment for BPH may be ineffective. When BPH is found in its earlier stages, there is a lower risk of developing such complications. Much effort has been expanded 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 (1, 2, 3). The prostate gland is an androgen-sensitive organ and therefore androgen deprivation decrease the size of the prostate. Increasing evidence for involvement of the estrogen in the pathogenesis of BPH (4,5,6). Has lead to the initiation of including aromatase inhibition as a medical strategy in clinical trials of BPH. Aromatase inhibitors block the estrogen biosynthesis stemming from aromatization of androstenedione and testosterone. Phytoterapoetic preparations have had a long tradition of use in the medical treatment of BPH in Europe and all still commonly used for this porpose (7, 8). Various plant extract such as those from *Sernoa repens*, *Sabalis serrulate*, *Urtica dioica*, are marketed for the treatment for 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 membrane (9, 10, 11). No precise biochemical mode of action has been elucidated for various extracts from plants such as *Pygenum africanum* (12), *Hypoxis rooperi* (13), or the rye pollen extract known as "Cernitin" (14).

The purposes of this study are to check the influence of *Opuntia ficus-indica* (L.) Miller in caps on patients suffering from disturbances in urination due to BPH. In addition, the possible mechanism of action of *Opuntia ficus-indica* (L.) Miller extracts to exert an effect on BPH was also examined in regards to 5α reductase and aromatase inhibition and antioxidative capacity.

2. Materials and methods

2.1. In vivo studies

2.1.1. Patients and study design- clinical trial no.1

Thirty male patients 58 to 76 years were selected from those who visited the urology outpatient clinic of Soroka medical center suffering from disturbances in urination due prostate hypertrophy. Each of the patients was subjected to the following: 1. Medical history. 2. Physical test including rectal test. 3. Blood test for kidney function. 4. Ultra sound examination for the urinary tract. 5. Urodinamic test (flow-mater). 6. Microbiological test of the urine. Patients were treated three times per day with two vcap containing 250 mg of *Opuntia ficus-indica* (L.) miller and were tested for improvement in nucturia, drops after voiding, filling of bladder fullness, emergency for urination and urgency for urination. The trial lasted two months.

2.1.2. Patients and study design- clinical trial no.2

Fifty eight patients suffering from disturbances in urination due prostate hypertrophy were selected from those who visited Dr. Gideon Earon private clinic in Tel-Aviv, Israel. Each of the patients was subjected to the following: 1. Medical history. 2. Physical test including rectal test. 3. Blood test for kidney function. 4. Ultra sound examination for the urinary tract. 5. Urodinamic test (flow-mater). 6. Microbiological test of the urine. Patients were treated three times per day with two vcap containing 250 mg of *Opuntia ficus-indica* (L.) miller and were tested for urgency for urination, emergency for urination, filling of bladder fullness, and drops after voiding. The trial lasted six to eight months.

2.2. *In vitro* studies

2.2.1. Chemicals

1,2,6,7 ^{3}H (N) testosterone (1mCi/mL), 1β, 2β ^{3}H(N)-anderost-4-ene-3,17-dione 1mCi/mL) were purchased from DuPont. The non-radioactive steroids testosterone and dehydrotestosterone, were obtained from Fluka (AG Buchs, Switzerland). 4-androstene-3,17-dione, NADP, NADPH, Glucose-6-phosphate, Glucose-6-phosphate dehydrogenase, n-octyl- β-D-glucopyranoside, di-thiol-1,4,erytritol, EDTA, 2-mercaptoethanol, Tris, β-carotene, Tween-40, 3,4,5,-dimethyltiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) were all purchased from Sigma (St. Louis, MO). TLC plate, Silica gel 60 and F-254 obtained from Merck (Darmstadt, Germany). The *Opuntia ficus-indica* (L.) miller flowers were obtained from Herbamed (Rehovot, Israel).

2.2.2. Plant Extracts

Plant extracts were prepared as described by Jonas *et al*. (15). Briefly, aqueous extract was prepared by incubating 30 g of *Opuntia ficus-indica* (L.) miller flowers in 250 mL of distilled water and autoclaved for 20 min. The extraction of smaller amounts (5 mL) of plant by 55 mL of organic solvents (pertolium ether, dichlorometane, ethanol or a mixture of dichlorometane:methanol (9:1)) was carried out by sohxlet apparatus for 5 h. later in an Erlenmeyer flask over-night at room temperature.

2.2.3. Fibroblast culture

Fibroblast culture was prepared as described by Jonas *et al*. (15). Briefly, fibroblasts were established from explants of human foreskins. Fibroblasts were grown in 75 cm^2 plastic flasks in Dulblecco's modified Eagle medium (DMEN) containing 4500 mg/L glucose supplemented with 10% fetal calf serum (FCS), 2.0 mM of L-Glutamine, 50 mg/mL of gentamycin sulfate, 2.5 mg/mL of amphoteracin B. (Kibutz Beit-Haemek, Israel). The cultures were incubated in 37^{o}C in 5% CO_2-95% air atmosphere until confluent.

2.3.4. Citotoxicity assay

Citotoxicity of plant extract assay was prepared by MTT assay as described previously by Jonas et al, and Romijin and co-workers (15,16). Briefly, The tetrazolium salt (MTT) reduced to a colored formazan product (changing from blue to yellow) in the cells in presence or without of extracts.

2.3.5. Preparation of 5-α-reductase

Soluble-5-α-reductase mixture was prepared from human hypertrophic prostate tissue obtained at surgery as described elsewhere (17). Briefly, frozen prostatic tissue was homogenized with 100 mM sodium phosphate buffer pH=8.0 containing 10% (v/v) glycerol, 1.0 mM EDTA, 5.0 mM dithio-1,4-erthritol and 0.5% (w/v) n-octyl-β-D-pyranoside. The homogenate was centrifuged at 20 000 g for 20 minute and the supernatant used as 5-α-reductase mixture.

2.3.6. 5-α-reductase activity assay

5-α-reductase activity assay was prepared as described by Jonas *et al*. (15). Briefly, Activity was measured by the reduction at 37^{o}C for 40 min of 1,2,6,7 ^{3}H (N) testosterone (0.34 mM) in a 3.0 mL enzyme mixture pH=6.5 containing 0.5 mM NADPH, with or without test extracts. The radioactivity of dehydrotestosterone was determines by liquid scintillation spectrometry.

2.3.7. Preparation of aromatase and aromatase assay

Aromatase mixture was prepared from human placentas and assay of aromatase was preformed according to Thompson and co-workers (18). Briefly, the test buffer contained 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)-anderost-4-ene-3,17-dione and unlabeled 4-androstene-3,17-dione together with the test extracts. Incubation period was 15 min at 37^{o}C. Subsequently, 200 mL of a 5% (w/v) charcoal suspension was added. After centrifugation, librated tritiated

water in the supernatant was counted and its quantity served as an index of enzymatic activity (Jonas).

2.3.8. Antioxidant activity assay

The antioxidant activity was assayed by destruction of β-carotene with linoleic acid emulsion in presence of the extracts by the method of Hammerschmidt and colleges with minor modifications (19). Briefly, β-carotene, linoleic acid and Tween 40 were dissolved in chloroform. A model emulsion was prepared by adding with stirring double distilled water (DDW) to the viscous lipid uniform after chloroform evaporation. The test mixture was placed at in a vial (final concentration 100 µg/mL), together with 5.0 mL of the model emulsion, and then incubated at 50^{O}C. Distraction of β-carotene was determined by reading the optical density at 470 nm.

3. Results

3.1. Clinical trials

3.1.1. Influence of *Opuntia ficus indica* (prickly) flowers miller treatment among patients suffered from BPH - clinical trial no. 1

All of the 30 participants showed normal kidney performance, negative urine microbiological results. The flow meter chart showed uro-mechanical disturbances due to prostatic hypertrophy at the beginning of the trial. Using two capsules three times daily for 2 months filled with *Opuntia ficus indica* flowers miller significantly improved nucturia (33%), drops after voiding (33%), filling of bladder fullness (48%), emergency for urination (52.2%) and urgency for urination (80%).

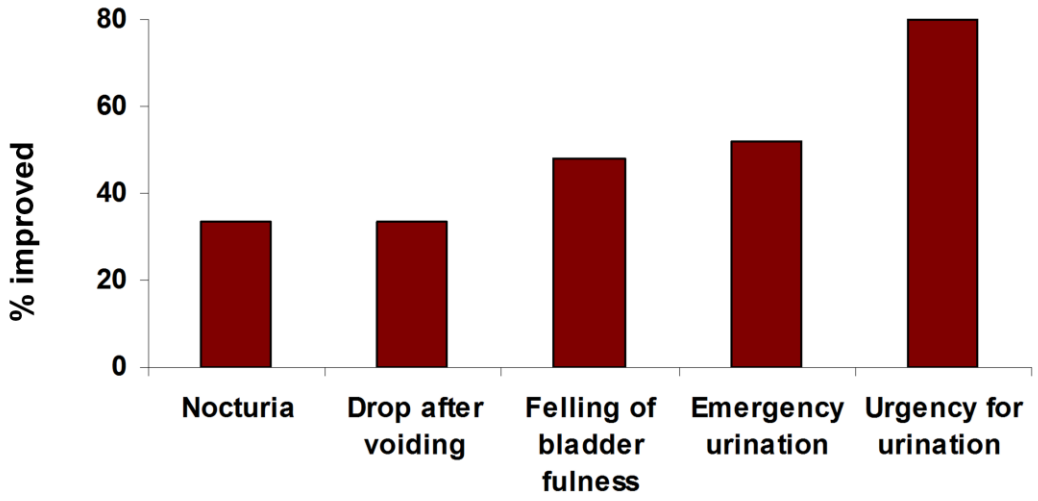

Figure 1. The influence of *Opuntia ficus indica* (prickly) flowers miller treatment during 2 months among patients suffered from BPH symptoms. *Opuntia ficus indica* flowers miller significantly improved nucturia (33%), drops after voiding (33%), filling of bladder fullness (48%), emergency for urination (52.2%) and urgency for urination (80%).

3.1.2. Influence of *Opuntia ficus indica* flowers miller treatment among patients suffered from BPH - clinical trial no. 2

All of the 56 participants showed normal kidney performance, negative urine microbiological results. The flow meter chart showed uro-mechanical disturbances due to prostatic hypertrophy at the beginning of the trial. Using two capsules three times daily for 6 to 8 months filled with *Opuntia ficus indica* flowers miller significantly improved BPH symptoms. Urgency for urination (50%), emergency for urination (62%), filling of bladder fullness (46%), and drops after voiding (63%).

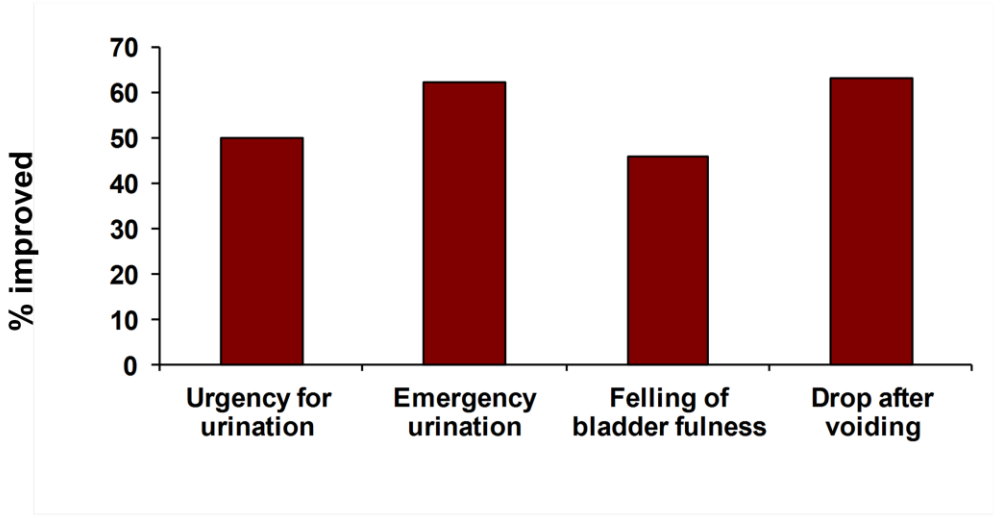

Figure 2. The influence of *Opuntia ficus indica* flowers miller treatment during 6 to eight months among patients suffered from BPH symptoms. *Opuntia ficus indica* flowers miller significantly improved urgency for urination (50%), emergency for urination (62%), filling of bladder fullness (46%), and drops after voiding (63%).

Table 1: The influence of *Opuntia ficus indica* (prickly) flowers miller treatment among patients suffered from BPH symptoms.

Urinary complaint	Two Clinical Trials		
	Private clinic (% improved)	Urology Outpatient (% complaints)	(% improved)
Urgency for urination	50	92.5	80.0
Emergency urination	62	85.2	52.2
Feeling of bladder fullness	46	92.5	48.0
Drops after voiding	63	66.7	33.3
Nocturia (> 2X/night)	N/A	100.0	33.3

3.2.1. 5-α-reductase inhibitory effect of *Opuntia ficus indica* (prickly) flowers miller extracts

Plant extracts were tested for their human soluble 5-α-reductase inhibition activity. The higher inhibitory effects on 5-α-reductase were obtained with the hot ethanol and dichloromethane plant extracts. Some inhibitory activity was found also with the aqueous fraction. By step-by-step extraction of the crude powder in solvents with increasing polarity (petroleum ether < dichloromethane < ethanol < water), the soluble 5-α-reductase inhibitory effect was found to be associated with dichloromethane and ethanol. Some inhibitory activity was found also with the aqueous fraction **(Table 2)**. In order to avoid any thermal degradation of the extractable compounds cold extraction of cactus (prickly) flowers with dichloromethane-ethanol mixture (9:1) was preformed (DM). The mixture inhibited 5-α-reductase activity in prostate crude extract and in foreskin fibroblasts culture. No citotoxicity was observed in tissue cell culture. After further separation of DM mixture, the resultant fractions designated as DM3 and DM33, exhibited potent inhibitory effect on 5-α-reductase in prostate crude extract **(Figure 3)**.

Table 2. The effect of flower cactus extracts on human 5- α-reductase activity

Samples	Final concentration (mg/mL)	5- α-reductase Activity (% of control)
Control	0	100
Water extract (100 µL)	1.3	64.7
Ethanol extract (10 uL)	0.05	31.7
Dichloromethane extract (10 uL)	0.1	43.2

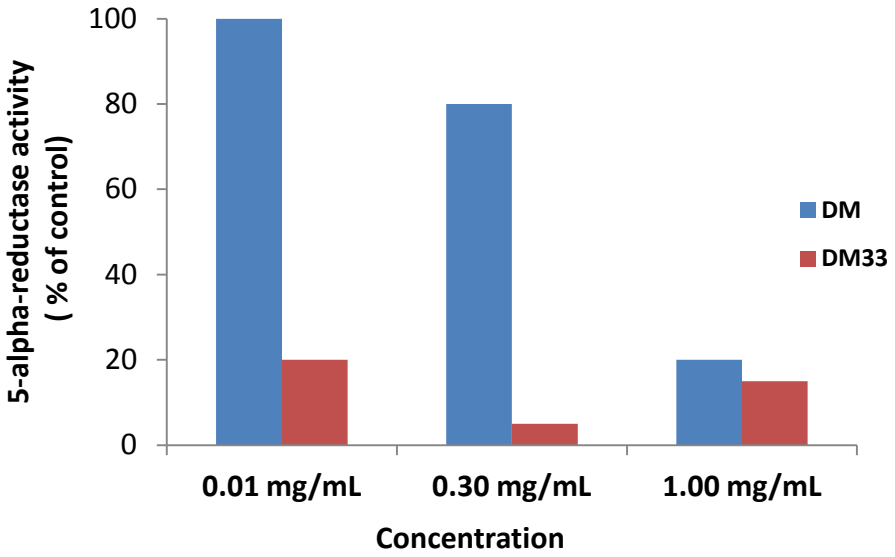

Figure 3. Inhibition of 5α-reductase activity by cactus flower extracts. In a extracts of human prostate. Amount of dehydeotestosterone synthesized from1,2,6,7 ^{3}H (N) testosterone was used as an index for 5- α-reductase activity.

3.2.2. Aromatase inhibitory effect of *Opuntia ficus indica* (prickly) flowers miller extracts

The plant extracts were also employed in *in vitro* aromatase assay on human placenta crude extract and in human foreskin fibroblast culture. A potent aromtase inhibitory effect was obtained with both dichloromethane and ethanol hot extracts (data not shown), but a marked effect was also achieved with the DM, DM3 and DM33 extracts. 80% of placental aromatase activity and 30% of froeskin fibroblasts aromatase activity were in habited by extracts **(Figure 4).**

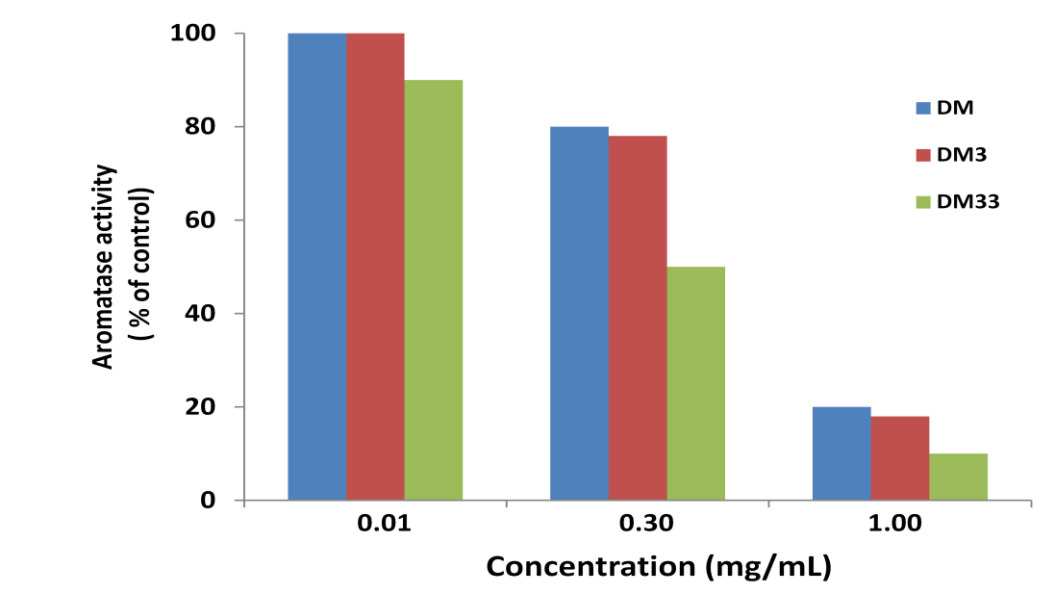

Figure 4. **The influence of cactus (prickly) flower extract on aromatase inhibition from human placenta. A marked effect was achieved with the DM, DM3 and DM33 extracts. X≥80% inhibition of placental aromatase activity was observed at concentration of 1.00 mg/mL. Amount of titrated water released during aromatization of 1β, 2β ^{3}H(N)-anderost-4-ene-3,17-dione, was used as an index of aromatase activity.**

3.2.3. Antioxidant activity

All the four fractions of plant step-by-step extraction, as well as the DM extract and its sub-fractions DM3, DM33, and also aqueous extract were tested for their antioxidant activity by assessing the protection against β-carotene oxidation in emulsions with linoleic acid. As shown in **Figure 5**, the ethanol and water extracts displayed significant antioxidant activity compared to butylated hydoxyanisole (BHA), which is a potent antioxidant.

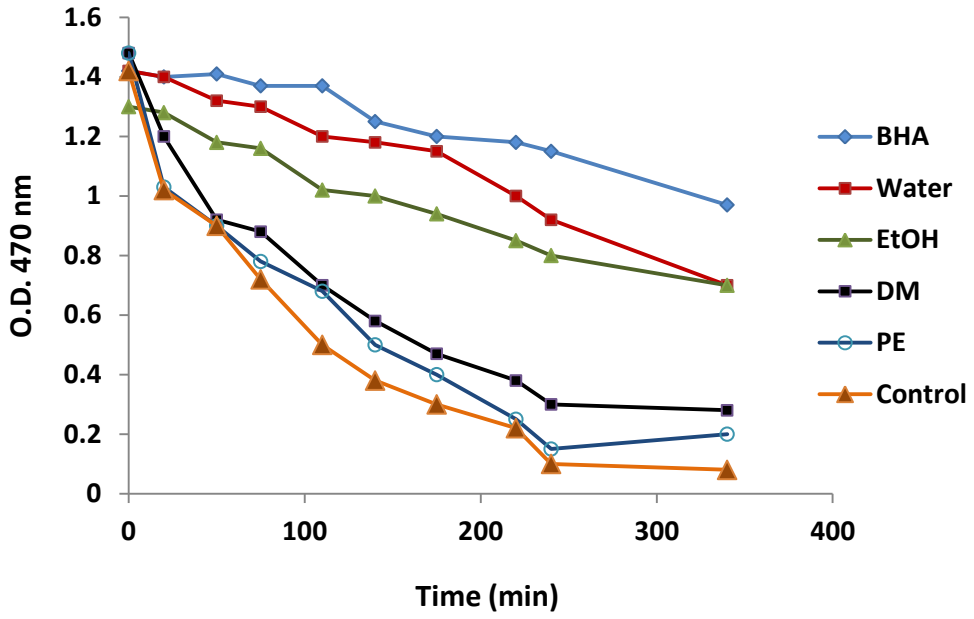

Figure 5. **Oxidation of β-carotene emulsion with linoleic acid in presence of cactus (prickly) flower extracts. Fractions were prepared by consecutive extraction of cactus flower powder in solvents with increasing polarity. The protection against oxidation of β-carotene was compared to BHA antioxidant capacity at concentration of 100 µg/mL. (Butylated hydoxyanisole (BHA), Ethanol (EtOH), Dichelоromethane (DM), Peterolium ether (PE)).**

4. Discussion

The cause of BPH is not well understood. Some researchers believe that factors related to aging and the testes may spur the development of BPH. Others connect BPH phenomena with in balanced sex hormone levels. As men age, the amount of active testosterone in the blood decreases, leaving a higher proportion of estrogen. Studies done on animals have suggested that BPH may occur because the higher amount of estrogen within the gland increases the activity of substances that promote cell growth. Some researchers suggest that BPH may develop as a result of "instructions" given to cells early in life. According to this theory, BPH occurs because cells in one section of the gland follow these instructions and "reawaken" later in life. These "reawakened" cells then deliver signals to other cells in the gland, instructing them to grow or making them more sensitive to hormones that influence growth.

A commonly raised question is whether men with benign prostatic hyperplasia (BPH) are at greater risk for prostate cancer than men who have no sign of BPH. A new, national cohort study of more than 3 million Danish males followed for up to 27 offers us some insights. Men with clinical symptoms of BPH are at increased risk of also having a diagnosis of prostate cancer and potentially dying from prostate cancer over time compared to the BPH-free male population. However, there is still ***no evidence at all*** to suggest that having BPH is in itself a cause of prostate cancer.

BPH affects the quality rather than the quantity of life. Therefore the management of BPH has undergone a rapid evolution over the past decade from a surgical emphasis to the treatment of the patient's symptoms through conventional or alternative medicine include herbal medicine and nutritional therapy.

The cactus (prickly) flowers were noted to be helpful in preventing the symptoms of benign prostate hyperplasia therapy by the British Herbal Pharmacopoeia at 1983, although there is no published any information regarding its clinical effects on patients or the mechanism of its biological activity. Two clinical trials were conducted by Herbamed in regards to the influence of *opuntia ficus indica* flowers miller supplementation among subjects who suffered from BPH symptoms. At the first clinical trial using two capsules three times daily for 2 months filled with *Opuntia ficus indica* flowers miller significantly improved nucturia (33%), drops after voiding (33%), filling of bladder fullness (48%), emergency for urination (52.2%) and urgency for urination (80%) **(Figure 1, Table 1)**. At the second trial using two capsules three times daily for 6 to 8 months filled with *Opuntia ficus indica* flowers miller significantly improved BPH symptoms i.e. urgency for urination (50%), emergency for urination (62%), filling of bladder fullness (46%), and drops after voiding (63%) **(Figure 2, Table 1)**.

In screening new therapeutic compounds for the treatment of BPH we have examined the ability of cactus flower to ameliorate BPH and this is through the inhibition of such processes as lipid peroxidation, androgen aromatization and testosterone reduction. All our experiments were geared to determine the inhibition of aromatase activity in the crude placenta extract and 5α-reductase

activity in crude prostate extract. Ancillary trials were also carried out in cultured human foreskin fibroblasts because the letters were shown to contain a variety of steroid-metabolizing enzymes, including two 5α-reductase isozymes as in human prostate (20, 21). Our results demonstrate that cactus flower extract contains antioxidants as well as 5α-reductase and aromatase inhibitors (figure . 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 forskin 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)-anderost-4-ene-3,17-dione which transforms to estrogen in the presence of 5α-reductase inhibitor. Aanderost-4-ene-3,17-dione is the chemical precursor of both testosterone (DHT) and estrogen **(Figure 6)** and inhibition of testosterone transformation to DHT may cause increased anderost-4-ene-3,17-dione aromatization. It has been already shown that progesterone, a competitive inhibitor of 5α-reductase, stimulates aromatization of androstendione without affecting the aromatase complex (22).

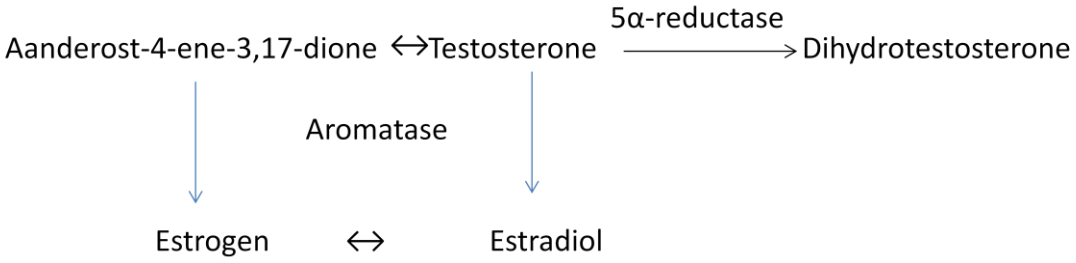

Figure 6. Scheme of Aanderost-4-ene-3,17-dione metabolic pathway.

Our results demonstrate that in the presence of another 5α-reductase inhibitor namely finasteride, the transformation of androstendione to estrogen in cultured forskin fibroblasts is markedly increased. This finding is in agreement with Lin et al. (23), who deduced from their experiments with rate smooth muscle cells that the aromatization pathway of testosterone to estradiol would prevail with DHT formation is inhibited. Accordingly, the relatively weak aromatase inhibitory effect of cactus flower extract on cultured forskin fibroblasts, is attribute to the increased

aromatization of 1β, 2β ^{3}H(N)-anderost-4-ene-3,17-dione stemming from the concurrent 5α-reductase inhibition. The findings that cactus flower extract 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 these enzymes 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 (24). It is generally assumed that SHBG synthesis is regulated by, and is, in fact, a reflection of the estrogen/androgen ratio (25). 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.

Another important finding of the present study is the high antioxidant activity of the aqueous plant extract **(Figure 5)**. Numerous previous investigations have shown that lipid peroxides and active oxygene species (ROS) (e.g. superoxide radicals, singlet oxygen, hydrogen peroxide, hydroxyl radicals) are involved in the regulation of cellular proliferation and in etiology of a variety of diseases, including accelerated aging and prostate cancer. Intracellular ROS are generated spontaneously as a result of oxygen interaction with reducing compounds, or as intermediates of some metabolic reactions (26). Under normal conditions, the ROS level in the tissue is controlled by antioxidants and antioxidants enzymes such as vitamin C, vitamin E, glutathione (GSH) , superoxide dismutase (SOD), catalase (CAT), glutathione reductase. The augmentation of ROS concentration (oxidative stress) is commonly associated with increasing age and with several diseases accompanied by tissue inflammation. Ripple and co-workers demonstrated that the oxidative stress is also increased by androgen treatment in androgen responsive human prostate carcinoma cells. It was proposed that redox alteration may play a key roll in a signal transduction pathway important for regulation cell growth and antioxidative defense (27, 28). Hence the ameliorative effect of cactus flower on BPH is likely through

inhibition the prostatic 5α reductase, aromatase activity and redox alteration **(Figures 3, 4, 5, 6, Table 2).**

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Herbamed Ltd. 7 Opphenhimer st. Rheovot Science park, 76701. Israel.

Websites: www. Herbamed.com. nutravida-health.com