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Beneficial Effect of a Multifunctional Polyphytocompound in Experimental Prostatic Hyperplasia in Rats

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Abstract

The aim of the present study was to assess the efficacy of a poly-phytocompound in a model of experimental BPH. Adult 8 weeks male Wistar rats were subjected to complete orchiectomy under anesthesia (i.p. injection of 100 mg/kg body weight of sodium pentobarbital). After castration, experimental BPH was reproduced by subcutaneous injection of testosterone (20 mg/kg) for 4 weeks and, at the same time, rats randomly divided in 3 groups (15 rats each): (A) untreated BPH model; (B) BPH plus TR10/P3795 orally and (C) BPH plus finasteride (10 mg/kg body weight) administered orally as positive control group. A third group (D) of sham-operated rats served as control. Both TR10/P3795- and finasteride-treated groups showed a significant ($p<0.05$) and comparable reduction of all morphometric parameters (volume, weight and weight/body weight ration) which were grossly abnormal in untreated BPH model ($p<0.01$ vs. sham-op.). Moreover, both treatment schedule maintained a near-to-normal 3 h urinary output ($p<0.01$ vs. untreated BPH). Untreated BPH showed a significant increase of epithelial size and thickness and these features were equally decreased by TR10/P3795 and finasteride ($p<0.05$). Either TR10/P3795 or finasteride brought about a significant decrease of serum level of DHT and PAP ($p<0.05$ vs. sham). There was no difference among the two treatments. Prostatic tissue concentration of MDA, IL-6, TNFα and TGFβ1 significantly increased in untreated BPH model ($p<0.001$). All these parameters significantly decreased, although not normalised, in TR10/P3795-treated group ($p<0.05$ vs. sham and vs. finasteride). Finasteride determined only a not significant trend decrease of IL-6 and TNFα. Given the multifactorial aetiology of BPH, the data from this experimental model show the promising larger spectrum of mechanisms of action of the tested poly-phytocompound.

Keywords: Benign prostatic hyperplasia; Dihydrotestosterone; Prostate gland; Testosterone; Caviarlieri; Celergen

Introduction

Benign Prostatic Hyperplasia (BPH) is a slowly progressing process of micro and macronodular appearance characterized by hyperplastic epithelial modifications together with stromal growth. This process has a multifactorial etiology and represents the commonest cause of Lower Urinary Tract Symptoms (LUTS) in the aging male [1,2]. It has been reported that about 90% of men between 45 and 80 years old complain of some degree of LUTS (3 mcWary). More precisely, it seems that by the age of 50 [3,4] 50% of men may show the symptoms related with BPH and in those aged above 70 years this condition is the most significant cause of bladder outflow obstruction. From the histological viewpoint, the process which starts from the transitional or periurethral zone determines hyperplasia of glandular and stromal tissue with papillary buds and increased smooth muscle, lymphocytes and ducts [5,6]. The consequent prostate enlargement will bring about urethral constriction with following weak urinary stream, incomplete bladder emptying, nocturia, dysuria up to overt bladder outlet obstruction [7,8]. Thus, BPH-related LUTS can have a significant impact on quality of life and should not be underestimated [9,10]. Hormones affect the development and progression of BPH since the development and growth of the prostate gland very closely depends on androgen receptor stimulation [11-13]. Indeed, especially during aging process, prostate is mainly influence by Dihydrotestosterone (DHT), i.e., an active metabolite generated by the enzymatic conversion of testosterone by steroid 5α-reductase although other metabolites may play a role in health and disease [14]. Long before surgery may be required, well-known pharmacotherapeutic options are currently employed such as 5-alpha-reductase Inhibitors, alpha-adrenergic antagonists, anticholinergic agents and combination therapy [15,16]. Although these treatments have enabled consistent benefits [17-19], their use is associated to a different degree of side effects such as decreased libido, erectile dysfunction gynecomastia and poor ejaculatory function [20-22]. This limitation holds particularly relevant when very early cases of BPH are faced or when a tentative "preventive" strategy is planned. Till recently, there is a constant flow of experimental articles, reviews and clinical studies highlighting the role

of phytocompounds in this condition [23-26], given also its multifaced pathophysiological mechanisms [27-29]. The aim of the present study was to assess the efficacy of a poly-phytocompound in a model of experimental BPH.

Materials and Methods

Animals

Adult 8 weeks male Wistar rats (240-290 g) were used throughout the experiments and were housed individually in standard polypropylene cages (three rats/cage) under controlled standard conditions of light (12/24 h) and temperature (26 ± 1°C). Food pellets and tap water were provided ad libitum. For experimental purposes animals were fasted overnight but were allowed free access to water. Body weight was measured weekly in all rats. All animal procedures were performed according to approved protocols and in accordance with the Guiding Principles for the Care and Use of Animals, based on the Declaration of Helsinki.

Induction of BPH and treatments

Rats were hosted for 10 days to allow acclimatization and four days prior experiment they were subjected to complete orchiectomy with spermatic cord and blood vessels ligation under anesthesia (i.p. injection of 100 mg/kg body weight of sodium pentobarbital). After castration, experimental BPH was reproduced by subcutaneous injection of testosterone (20 mg/kg) for 4 weeks and, at the same time, rats, under a computerized randomization procedure ensuring a comparable body weight distribution were divided in 3 groups (15 rats each): (A) Untreated BPH model; (B) BPH plus 100 mg/kg of TR10/P3795, a poli-phytocompound of potential prostate protective effect (100 mg containing: *Serenoa repens* extract 56.5%, red clover 26%, pumpkin seed extract 13% and pomegranate extract 4.5%, Andronam, Named, Lesmo, Italy) orally and (C) BPH plus finasteride (0.5 mg/kg body weight) administered orally as positive control group. A third group (D) of sham-operated rats served as control. All compounds were administered to the animals in the morning. Care was taken as to put all the TR10/P3795 or finasteride supplementation in the morning food supply while checking that all was eaten up. Finasteride was stored in an air-tight, dark container at room temperature. The finasteride dosing was prepared in powder at the required concentrations and stored at 4°C.

Urinary output, blood and prostatic tissue samples

On the day before sacrifice (27^{th} day), all groups were transferred into metabolic cages to measure 3 h urinary outputs. On the next day (4 weeks study), all animals were fasted overnight. Blood samples were collected in EDTA and centrifuged at 3000 × g for 10 min; serum was instantly separated and stored at −20 °C. After animals were sacrificed, prostate were weighed and stored in 10% buffered formaldehyde solution. Tissue was then embedded in paraffin technique, 5 µm thick sections were cut and stained by haematoxylin and eosin for light microscopic examination. Separate aliquots of ventral prostatic tissue were snap frozen at −70°C until further analysis.

Histological examinations and photomicrographic image analysis

Each section was viewed under a light microscope at a magnification of ×40–400. A Windows-connected Photometric Quantix digital camera was set as to capture and optimize photomicrographs images and morphological changes were blindly evaluated by a pathologist unaware of the treatment group to calculate the HE stained gland area in the rat ventral prostate. The stained area in the gland indicated the degree of proliferation of epithelial components. This was assessed by randomly counting 10 glands in each section and then quantifying the glandular epithelial area as stained area per glandular area under 100-fold magnification. The mean percent area density was also evaluated for each group.

Assessment of serum dihydrotestosterone (DHT) and prostatic acid phosphatase (PAP)

Blood samples were allowed to clot into a serum separator tube for two hours at room temperature before centrifugation for 20 min at 1000 × g. Serum levels of DHT and PAP were assayed by Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available kits (Santa Cruz Biotechnology, Santa Cruz, California, USA). Briefly, triplicate aliquots of standards and samples were put into separate wells pre-incubated with DHT- and PAP-specific biotin-conjugated polyclonal antibody solution. Afterwards, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Same procedure was applied with the standard. Then, 3,3'5,5'-tetramethyl-benzidine substrate solution was added to each well and the reaction was stopped by adding a 0.16 M sulphuric acid solution. The reading of the colour change of each well was assessed by spectrophotometric at the absorbance of 450 nm wavelength using analytical grade laboratory reagents. The final concentration of DHT and PAP was calculated by comparing the O.D. of the samples to the standard curve.

Assessment of oxidative and inflammatory status in BPH

Pre-weighted prostatic tissues were ice crushed in liquid nitrogen and then homogenized for 15 min in PBS (pH 7.4). The concentration of malondialdehyde (MDA), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) and transforming growth factor beta 1 (TGF-β1) were assayed by using commercially available kits (BioSource, San Diego, CA USA for MDA and Seikagaku Corp., Tokyo, Japan for ELISA Immunoassay of IL6, TNF-α and TGF-β1). All values were normalized and expressed per milligram of protein content.

Statistical analyses

The data obtained were expressed as mean values ± SD. Statistical analysis was performed using SPSS 17. Significant difference among the groups was performed using a one-way Analysis of Variances (ANOVA), followed by a non-parametric post Tukey test. Analysis using two-tailed and a p-value ≤0.05 was considered as statistically significant. Pearson's correlation analysis was used for correlation of parameters measured.

Results

weight and prostate parameters

Body weight physiologically increased in sham-operated group and this was comparable to both untreated BPH model and both treatments groups without any significant difference although the Finasteride-group showed to have a trend towards lower weight (data not shown, $p>0.05$). As compared to sham-operated control, prostate weight, weight ratio and volume significantly increased in untreated BPH model ($p<0.05$). Both TR10/P3795-and finasteride-treated groups showed a significant and comparable reduction (Figure 1, $p<0.05$ vs. sham group).

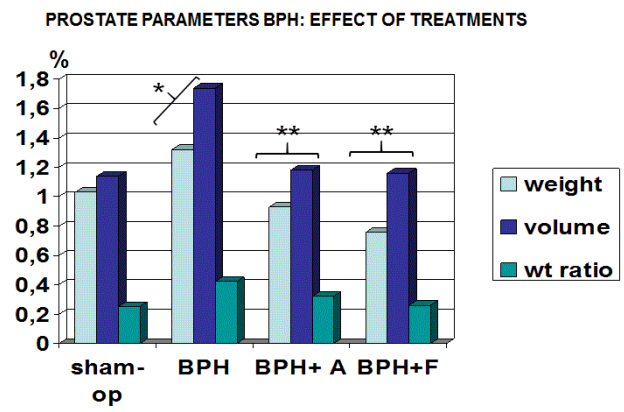

Figure 1: Morphological changes of prostate in BPH model and effect of treatment. *p<0.01 vs. sham-op group; **p<0.001 vs. sham-op rats.

Urinary output

As compared to sham-op control group, rats with untreated BPH showed a significant contraction of urinary output of over 50% ($p<0.01$). Both TR10/P3795 and finasteride brought about a near to normal urinary volume (Figure 2, $p<0.01$ vs. untreated BPH). There was no significant difference between the two treatment groups.

Routine blood chemistry

Liver and renal functional blood parameters were within normal range throughout the study, irrespective of the group considered and no statistically significant difference appeared (data not shown).

Histological morphology of the prostate

As expected, as compared to sham operated group, untreated BPH rats showed typical features such as epithelial hyperplasia with intraepithelial vacuoles. Moreover, an uneven acinar structure with evident increase of the glandular ducts branching and of in-folding of ductal columnar epithelial was recorded (Figure 3). When calculating these parameters, it appeared that there was a significant increase of epithelial size and thickness (Figures 4 and 5, $p<0.01$). Either TR10/P3795 or Finasteride brought about a statistically significant protection of these features ($p<0.05$ vs. BPH model) whit an overall milder feature of BPH.

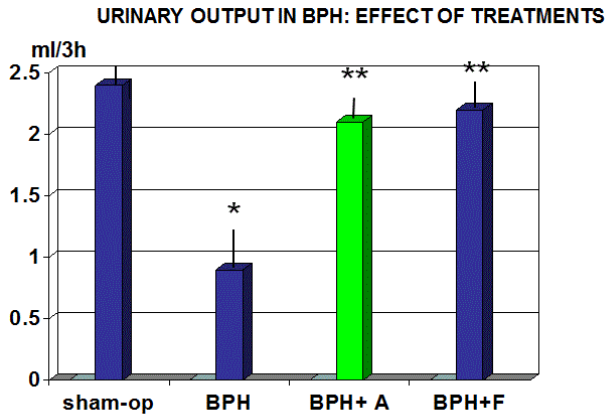

Figure 2: Urinary output in bph: effect of treatments. *p<0.01 vs. sham-op group; **p<0.05 vs. untreated BPH model.

Assessment of serum dihydrotestosterone (DHT) and prostatic acid phosphatase (PAP)

Rats with untreated BPH showed a significant increase of both DHT and PAP serum level (Figures 6 and 7, $p<0.001$ vs. sham-op group). Both TR10/P3795 and finasteride brought about a significant decrease of both parameters ($p<0.05$ vs. untreated BPH), finasteride showing a not significant better performance as for PAP serum level.

Assessment of oxidative and inflammatory status in BPH model

Oxidative stress, as measured by MDA concentration, was significantly elevated at a tissue level in BPH model when compared to same area in sham group (Figure 8, $p<0.01$). On the other hand, TR10/P3795 significantly decreased MDA concentrations compared to the untreated BPH group ($p<0.01$).

Accordingly, prostatic sample taken from untreated BPH rats showed a significantly abnormality of all tested inflammatory parameters [IL-6, TNF-α and TGF-β1 (Figures 9-11, $p<0.001$ vs. sham-op rats)].

Discussion

Natural compounds maintain a growing popularity in the treatment of Benign Prostatic Hyperplasia (BPH) and related Lower Urinary Tract Symptoms (LUTS) mainly due their overall general acceptance and reported lack of substantial side-effects. While the hormonal factor does represent a relevant pathophysiological variable of BPH occurrence and related drugs have been synthesized accordingly, several mechanisms have been advocated for its development. These include, among others, tissue and intracellular redox unbalance [30,31]. Indeed it is well known that human prostate tissue has a peculiar vulnerability to oxidative DNA damage due to more rapid cell turnover and also to the low activity of superoxide dismutase and catalase and increased endogenous levels of DNA base products, these two variables having being reported as to be inversely correlated in in BPH samples has received further recent confirmations [32].

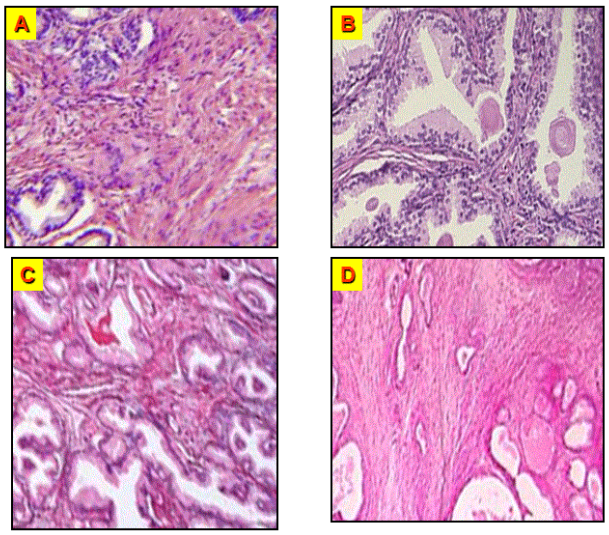

Figure 3: Photomicroscopy of ventral prostate tissue in all groups. A) Sham-operated group (400x); B) Untreated BPH group (600x); C) Finasteride-treated BPH group (400x); D) Phytocompound-treated BPH group (400x). Gross epithelial hyperplasia and abnormal acinar structure with marked glandular ducts branching and in-folding of ductal columnar epithelial appeared in BPH (A). These features were mitigated in either finasteride and, at higher extent, in phytocompound treated rats.

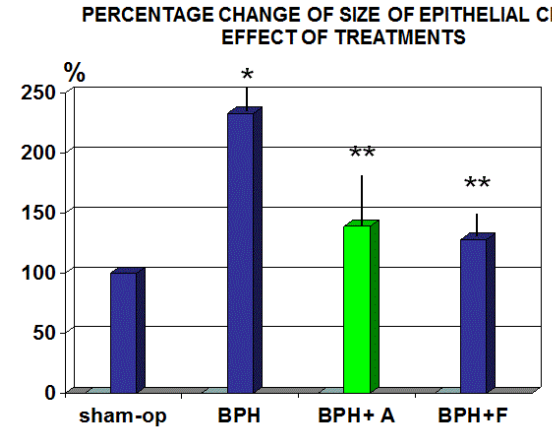

Figure 4: Percentage change of size of epithelial cells: effect of treatments. *p<0.01 vs. sham-op group; **p<0.05 vs. untreated BPH model.

In BPH, the exposure of prostate epithelia to ongoing oxidative stress molecules can trigger the otherwise inactive transcription factor NF-κB [33], a key inflammatory transcriptional regulator. As a consequence, by the TNF-α/AP-1 transduction pathway and the NF-κB-Inducing Kinase (NIK) transduction pathway, the local production of proinflammatory cytokines [34] is determined by triggering apoptotic pathway which limits uncontrolled cell proliferation. In this regard, TR10/P3795 exerted a significant reduction of MDA and inflammatory markers unlike finasteride which had already been demonstrated not to affect protein oxidation in the prostate [35]. This is likely to be advocated for by the total phenolic content of a selected extract from pumpkin in its composition. Indeed, it has been shown that they maintain an effective radical scavenging activity from as low as 0.16 mg/ml *in vitro* concentration [36]. Such anti-oxidant, anti-inflammatory and pro-apoptotic properties have been also demonstrated for two other main components of the present formula, i.e., pomegranate and serenoa [37-39]. In this setting, the triggered inflammatory cascade has not simply an ancillary meaning but a definite pathogenetic role of disease progression till potential cancerous transformation [40,41].

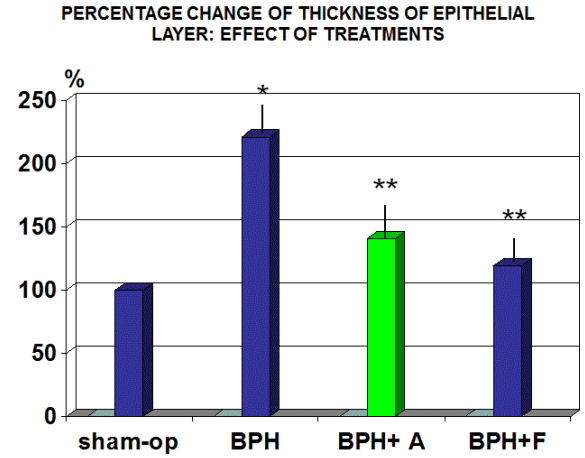

Figure 5: Percentage change of thickness of epithelial layer: effect of treatments. *p<0.01 vs. sham-op group; **p<0.05 vs. untreated BPH model.

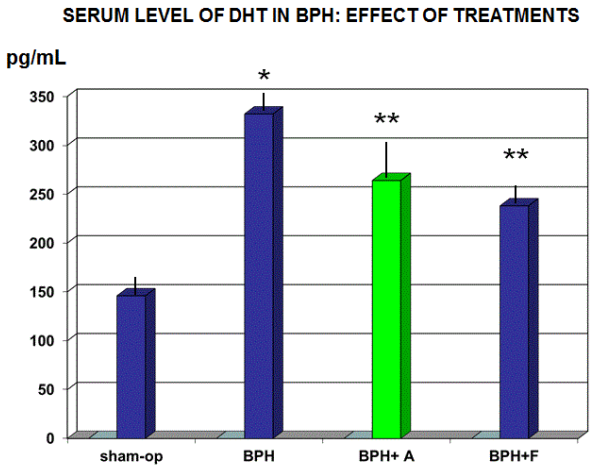

Figure 6: Effect of with phytocompound and finasteride treatment on serum level of DHT in BPH model rats. *p<0.01 vs. sham-op group; **p<0.05 vs. untreated BPH model.

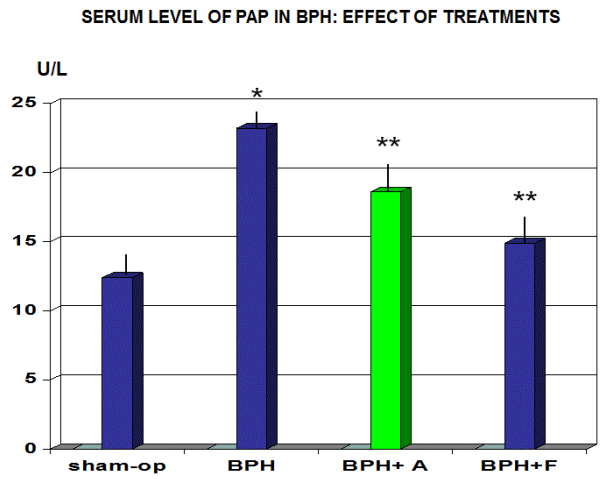

Figure 7: PAP serum level in BPH and modification induced by treatment with Phytocompound and Finasteride. *p<0.001 vs. sham-op group; **p<0.05 vs. untreated BPH model.

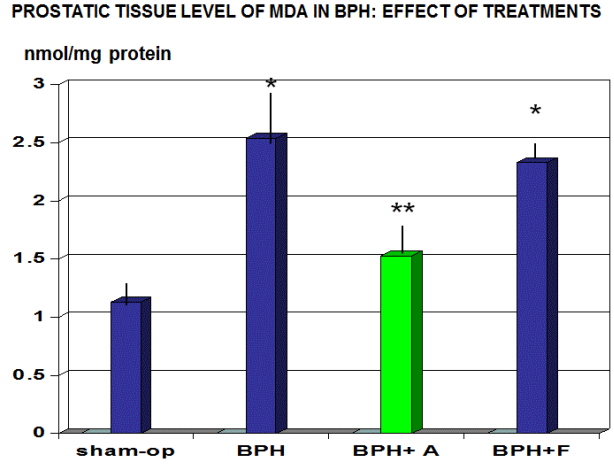

Figure 8: Prostatic tissue level of MDA in BPH experimental model: effect of treatments with phytocompound and finasteride. *p<0.01 vs. sham-op group; **p<0.05 vs. finasteride-treated rats. This latter group did not show any significant difference as compared to untreated BPH model.

Thus, the proven anti-inflammatory properties of specific serenoa, pomegranate and pumpkin extracts [37-39,42] which are not directly shared by finasteride, come to be all the more relevant. This has been confirmed last month also in an obese mouse model [43] further paving the way to a wider systemic metabolic understanding of the disease [44-47]. In favour of such integrated phyto-pharmaceutic approach, it is worthwhile reporting that components such as specific pumking seed, red clover isoflavones, serenoa and pomegranate extracts have been shown to have antiandrogenic activity by dose-dependently blocking the binding of DHT besides am anti-aromatase and anti-5-α-reductase Type II action [38,48-51]. It goes without saying that inflammation per se exerts an endocrine disrupting effect at the prostate level by negatively affecting tissue estrogen metabolism [52], this being, together with antioxidant property, one of the suspected hormone receptor-independent anti-mutagenic mechanisms of some phytocompounds [53].

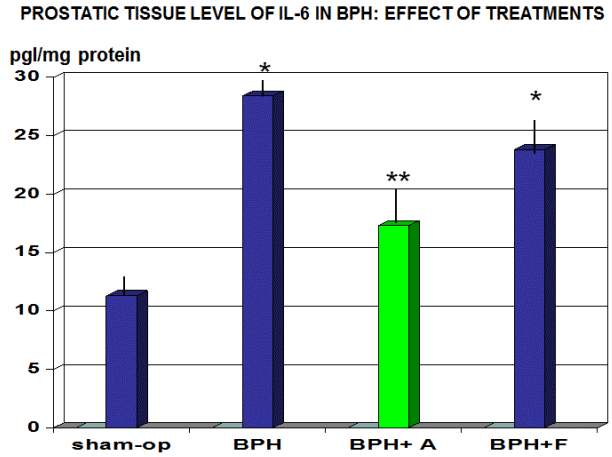

Figure 9: Prostatic tissue level of IL-6 in BPH: effect of treatments with phytocompound and Finasteride. *p<0.001 vs. sham-op group; **p<0.05 vs. finasteride-treated rats. This latter group did not show any significant difference as compared to untreated BPH model.

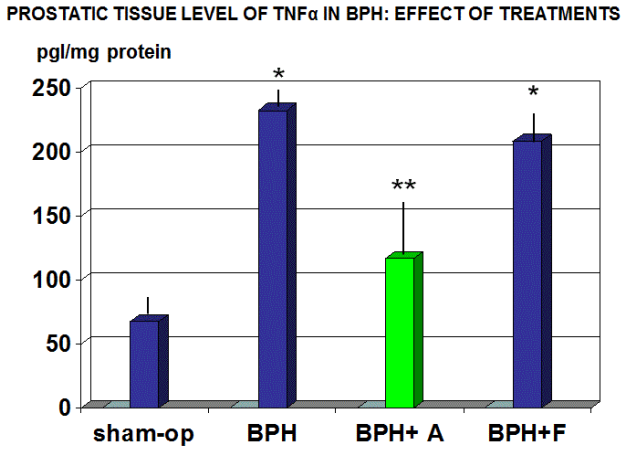

Figure 10: Prostatic tissue level of TNFα in BPH: effect of treatments with phytocompound and finasteride. *p<0.001 vs. sham-op group; **p<0.05 vs. finasteride-treated rats. This latter group did not show any significant difference as compared to untreated BPH model.

The very high dosage of arginine into pumpkin seed extract may be a further distinctive feature of the potency of the formula since it has been shown in rats that via a nitric oxide-mediated mechanism, this significantly improves in-bladder pressure and bladder volume adaptation [54]. While there is a growing evidence in clinics of the rationale use of phytocompounds for prostate health [55-57], the present experimental work using a formula combining a number of the most effective phytochemicals, offers new hints for its larger use either

as sole therapeutic approach in prevention management, an alternative to chemicals when appropriate and as a potential adjuvant weapon during current chemical treatments.

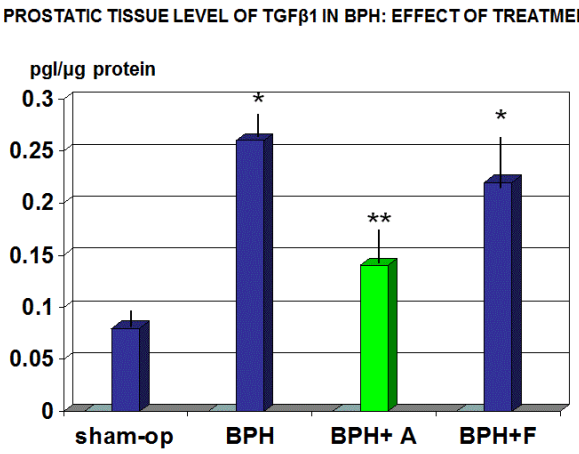

Figure 11: Tissue level of TGFβ1 in ventral pancreas in BPH: comparison between the effect of phytocompound and Finasteride. *p<0.001 vs. sham-op group; **p<0.05 vs. finasteride-treated rats. This latter group did not show any significant difference as compared to untreated BPH model.

Finally, although there are some assumptions on the role of fish oil in prostate health, there is a paucity of scientific evidence [58,59], moreover, it has been reported the possible deleterious role of -3 PUFAs alpha-linolenic acid in the development of prostate cancer [60]. Some of our group has tried in-house preliminary studies with a EPA-rich marine compound (Caviarlieri) with no results whatsoever and also have tested to no avail Celergen, a further fish-lipoprotein derivative with anti-inflammatory properties *in vitro* studies but which has indeed no published *in vivo* or clinical prove so far.

Conclusion

From this study, it appeared that either TR10/P3795- and finasteride-treated groups showed a significant comparable reduction of all typical morphometric and functional parameters seen in BPH, (volume, weight and weight/body weight ration and urinary output) together with a significant decrease of serum level of DHT and PAP. However, only TR10/P3795 brought about a partial but significant decrease of MDA, IL-6, TNFα and TGFβ1 (p<0.05 vs. sham and vs. finasteride). Taken overall and considering the multifactorial aetiology of BPH, the data from this experimental model show the promising larger spectrum of mechanisms of action of the tested poly-phytocompound.

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