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Cucumis sativus (Curcubitaceae) inhibits prostate carcinoma cell growth and prevents the testosterone-induced benign prostatic hyperplasia in Wistar rat

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ABSTRACT

Pumpkin seeds are claimed to treat prostate tumour/cancer. The *in vitro* (ability to inhibit cell growth through MTT assay) and *in vivo* (ability to prevent testosterone-induced BPH in rats at the doses of 125, 250, 500 and 1000 mg/kg BW) of six edible pumpkin seeds found in Cameroonian were assessed. The endpoints were cell growth arrest, prostate mass and volume, prostatic epithelium height, prostatic proteins, prostate specific antigen (PSA) and inflammatory cytokines. *In vitro*, *C. sativus* seeds exhibited the most potent antiproliferative effects on DU145 and PC3 prostate cancer cells and its oil conserved almost all the effects of raw seeds. Further, it prevented the increased of prostate relative mass and volume, prostate epithelium height, PSA and testosterone dose-dependently compared to normal rats. This effect is thought to be mediated through antiandrogenic, estrogenic and anti-inflammatory activities, evidenced by a decreased in IL-1β, IL-6 and TNFα level. Overall, this results justify its traditional use.

1. Introduction

Cancer is a large family of diseases that involve abnormal cell growth with the potential to spread to other parts of the body (National Cancer Institute, 2018). With 1.4 million new cases and 375,000 deaths, prostate cancer was the fifth leading cause of cancer related deaths and the second most frequent cancer in men worldwide (Sung et al., 2021). Although not causing as many deaths, benign prostatic hyperplasia (BPH), remains a major public health challenge worldwide. It is the most common urogenic disease among men which greatly affects the lifestyle (Langan, 2019). The symptoms of BPH include but are not limited to urgency, frequency, nocturia, incomplete urination and weak urinary stream (McVary et al., 2011). Its etiology is not well understood, however, genetic factors, dietary, endogenous hormones (androgens and estrogens), inflammation and oxidative stress have been positively correlated with BPH (Devlin, 2021).

Several modern therapeutic strategies have been developed nowadays and 5α-reductase inhibitors such as finasteride and dutasteride are the most abundant in the market. Unfortunately, they have limitations such as dizziness, decreased libido, erectile dysfunction, hypotension, tachycardia and retrograde ejaculation (Yan et al., 2021). Hence, many patients rely to natural alternatives and functional foods offer wide range of health benefits (Singh and Kumar, 2023). The Cucurbitaceae is a large family distributed worldwide and gathering ~ 130 genera and 800 species including squash, gourds, melons and pumpkins (Sahayi and Shirali, 2018). Pumpkin seeds promote men fertility, prevent prostate

Abbreviations: ANOVA, Analysis of variance; ATCC, American Type Culture Collection; BPH, benign prostatic hyperplasia; BW, body weight; COX-2, cyclooxygenase-2; FBS, fetal bovine serum; DHT, dihydrotestosterone; HRP, horseradish peroxidase; IFN-γ, interferon; MTT, 3 [4,5-dimethylthiazol-2-yl] diphenyltetrazolium bromide; PSA, prostate specific antigen; SEM, standard error on mean; TNFα, Tumor Necrosing Factor alpha.

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related problems such as benign prostatic hyperplasia and prostate cancer and improve urinary dysfunction in overactive bladder syndrome (Paul et al., 2020). However, the pumpkins are distributed differently over the world and those of the unexplored countries attract the attention of scientists. In this line, this study sought the rational of using pumpkin seeds against benign prostatic hyperplasia.

2. Materials and methods

2.1. Substances

Finasteride (Chibroproscart), used as standard drug was obtained from Pierre Fabre® (Boulogne, France); Enanthate testosterone (Androtardyl) used to induced BPH was purchased from Bayer Pharma® (Berlin, Germany). The PSA ELISA kit was obtained from Cusabio Biotech Co. Ltd (Newark, Delaware, USA).

2.2. Plant material

2.2.1. Pumpkin seeds collection

Cucurbita maxima (CMx), *Cucumeropsis manii* (CMn), *Lagenaria siceraria* (LSi), *Cucurbita moschata* (CMs), *Cucumis sativus* (CSt) and *Cucumis melo* (CMl) seeds were harvested in Baleng and Bafang (West Region, Cameroon). They were authenticated by comparison with the botanical sample deposited at the Cameroon National Herbarium. The Table 1 depicts information on the selected pumpkin studied as well as their voucher.

2.2.2. Preparation of the different extracts

The dried seeds were crushed using an electronic grinding. Two (2) g of powder were mixed with 5 ml of distilled water. The paste obtained was wrapped in biodegradable sheets and then prepared in a stainless-steel pot for two hours. The obtained pistachio cake was then weighed and homogenized in distilled water for animal treatment.

Pumpkin seeds oil was extracted using an oil machine model YD-ZY-OIC purchased from RPC (Paris, France). For this to be done, 500 g of *Cucumis sativus* seeds have been extracted through the machine at low temperature to preserve all the nutritional properties. This process yielded 168.65 g of oil (33.73 %) and 317.1 g of cake (63.42 %).

The powder pumpkin seeds obtained as well as the cakes were administered to the animals after mixing with distilled water, while the oil was administered by dilution in corn oil.

2.3. Experimental organisms

2.3.1. Cell lines

DU145 and PC3, which are human androgen-independent prostate cancer cells and LNCaP, androgen-dependent prostate cancer cells, were purchased from the American Type Culture Collection (ATCC) (Wesel, Germany). The cells were cultured in RPMI 1640 medium containing 10 % fetal bovine serum (FBS), 1 % penicillin, 1 % glutamax and 20 mM HEPES-buffer (all from Gibco/Invitrogen, Karlsruhe, Germany). The cells were maintained at 37 °C in a humid atmosphere at 5 % CO_2. Subcultures from passages 5——22 were used. Before each experiment, cell viability was determined by trypan blue assay (Gibco/Invitrogen).

2.3.2. Animals

Male Wistar rats weighing ~ 250 g and aged ~ 2.5 months were obtained from the animal house of the Laboratory of Animal Physiology (University of Yaounde I, Cameroon). Animals were kept in the plastic cages and maintained in ambient temperature and humidity with a dark-light cycle of 12 h. The animals had free access to water and standard soy-free food consisting of: corn (36.5 %), bone flour (14.6 %), wheat (36.5 %), fish flour (4.9 %), crushed palm kernel (7.3 %), sodium chloride (0.3 %) and vitamin complex (Olivitazol® 0.02 %).

2.4. Experiments

2.4.1. MTT cell viability assay

Cell growth was estimated using MTT test (3 [4,5-dimethylthiazol-2-yl] diphenyltetrazolium bromide) (Roche Diagnostics, Penzberg, Germany). The cells (DU145, PC3 and LNCaP) were seeded in a 96-well plate at a density of 10,000 cells/well in 50 µL of RPMI-1640 medium. After 24 h, the cells were treated as follows:

- In the first experiment, all the six (6) pumpkin seeds have been tested at concentrations from 5 to 200 µg/mL.
- In the second experiment, oil and cake of *Curcumis sativus* have been tested at concentrations from 2.5 to 100 µg/mL.

In the both experiments, the control cells were incubated with vehicle (DMSO 0.01 % in RPMI-1640 medium). After 24 h, 48 h and 72 h of incubation, 10 µL of MTT (0.5 mg/mL in PBS) was added to each well and plates were incubated for an additional 4 h at 37 °C in the dark. The MTT was solubilized with 100 µL of a buffer containing 10 % SDS in 0.01 M HCl overnight. The absorbance was subsequently measured at

Table 1
Recapitulative information on the edible pumpkins of Cameroon.

Scientific name	Vernacular name	Parts used	Voucher	Phytochemical constituent	Biological activity	References
Cucurbita maxima Duchesne (CMx)	pɔɛ tùɔɛ̀	Leaves, flowers, seeds and fruit	45526/ HNC	Total phenols, flavonoids, total carotenoids, terpenoids, tannins	Antioxidant, antimicrobial, anticancer, anti-inflammatory and anti-diabetic	Wahid et al., 2021
*Cucurbita moschata*Duchesne (CMs)	pɔɛ mɛ̀lam	Seeds, pulp and fruit	25691/SRF CAM	Total phenols, flavonoids, tannins	Antioxidant, antiobesity, anti-diabetic, anticancer and antibacterial	Mindjou et al., 2021
*Lagenaria siceraria*Standl (LSi)	ndù tɔù	Leaves, fruits and seeds	48907/ HNC	Alkaloids, glycosides, steroids, tannins, saponins and flavonoids	Anticancer, anti-hyperlipidemic, anti-inflammatory, analgesic, antioxidant and antimicrobial	Aref et al., 2021
Cucumis sativus (CSt)	mbɔtɜmbùɔ	Seeds and leaves	42457/ HNC	Alkaloids, glycosides, steroids, tannins, saponins and flavonoids	Analgesic, anti-inflammatory, antioxidant antibacterial and cytotoxic	Wahid et al., 2021
*Cucumeropsis manii*Naud (CMn)	ndù nkaɔk	Seeds	42484/ HNC	Alkaloids, flavonoids, glycosides, steroids, and tannins	Antimicrobial, hypoglycemic, antioxidant and anti-atherogenic	Adepoju et al., 2021
Cucumis melo (CMl)	ndù	Aerial parts, fruit, peel and seeds		Tannins, alkaloids, saponins, flavonoids, glycosides and steroids	Antioxidant, hypolipidemic, hepatoprotective, anti-inflammatory, antiproliferative and antidiabetic	Malathi and Vadivelu, 2021

550 nm using a microplate ELISA reader (TECAN®, Crailsheim, Germany).

2.4.2. Testosterone-induced BPH in rat

Rats were acclimatized for 2 weeks and divided into several groups of 5 rats each. Testosterone enanthate was administered at a dose of 3 mg/kg *s.c.*, reported as optimal dose to induce benign prostatic hyperplasia rats (Zingué *et al.*, 2020). Three (3) different experiments were performed but in all of them, the normal rats (NOR) and the negative (BPH) control groups received the vehicle (distilled water), while the positive control (FINAS) group received the finasteride at a dose of 5 mg/kg BW as reported by Njamen *et al.* (2020) and Zingue et al. (2020). The test groups vary in function of the experiment as follows:

- For the experiment dealing with the comparative effects of the six selected edible pumpkin seeds 12 test groups (n = 5) were randomly distributed and treated at two concentrations (500 and 1000 mg/kg BW) each seed;
- For the experiment dealing with the comparative effects between the raw and cooked *Curcumis sativus* (who showed promising effects in the previous experiment), 8 test groups were treated either with raw or cooked *Curcumis sativus* seeds at doses of 125, 250, 500 and 1000 mg/kg BW (doses extrapolated from the daily amount (10 g/day) recommended by the German commission and framed);
- For the experiment dealing with the assessment of the effects of *Curcumis sativus* seeds oil 4 test groups were treated with oil at 42, 85 and 170 mg/kg BW [doses extrapolated from the optimal dose (500 mg/kg) by applying the percentage of oil yield, which has been framed]. One group received the *C. sativus* raw seed extract at the dose of 500 mg/kg.

All treatments were done once daily around 8:00 a.m. by gavage for 28 days. At the end of all treatments, rats were sacrificed by decapitation under light anesthesia with Diazepam (10 mg/kg) and ketamine (50 mg/kg). Blood was immediately collected in dry tubes and centrifuged at 3000 rpm for 15 min. The collected supernatants were stored at −20 °C for biochemical analyses. The ventral prostates were removed, weighed and a part was immediately fixed in 10 % formalin for histological analysis. The other part was weighed and ground with the teflon-glass potter on ice in sodium phosphate buffer (0.1 M; pH 7.5) to obtain a final 20 % homogenate. After centrifugation at 3000 rpm for 15 min at 4 °C, the collected supernatant was stored at −20 °C for the determination of total protein in the prostate. The prostate wet weight was calculated as the prostate weight/body weight ratio as described by Nahata and Dixit (2012). The size of the prostate was measured using a 1 mm precision sliding caliper (IGAGING®) and prostate volumes were calculated using the formula of Rahul *et al.* (2014): volume = length × weight × height × $\pi/6$.

2.4.3. Evaluation of the anti-androgenic activity of C. Sativus seed oil

The anti-androgenic property of *C. sativus* was evaluated following the OECD guideline 441 (2009). Thus, 36 six-week-old rats were acclimatized and then castrated, while 6 rats underwent sham-operation. After one week of hormone depletion, the rats were divided into 6 groups of 6 animals each and randomized as follows: a normal control group receiving vehicle (distilled water) *per os* and corn oil subcutaneously. The negative control group (CAST) received distilled water *per os* and testosterone *s.c.*; while the positive control (FLU) received flutamide as standard at a dose of 3 mg/kg BW per day *per os* and testosterone *s.c.* (0.4 mg/kg bw/day). The four test groups received *C. sativus* seed oil at doses of 42.5, 85 and 170 mg/kg BW as well as the total crude extract (500 mg/kg BW) *per os* and testosterone *s.c.*. The treatment lasted for 10 days, after which the animals were sacrificed by decapitation and androgen-dependent organs (prostate, seminal vesicles, glans and Tyson's glans) were immediately removed and weighed.

2.4.4. Evaluation of estrogenic activity of c. Sativus seeds oil

Estrogenic activity was evaluated as a possible mode of action of *C. sativus*, using a modified protocol based on Jasem and Tawfeek (2020). Thus, 45 male rats that were 2 months old were acclimatized and divided into 8 groups of 5 animals each treated as follows: the first group received the vehicle (corn oil); the second group was treated with a pure antiestrogen (Fulvestrant) at a dose of 300 μg/kg BW; the third group received estradiol valerate (E_2V) at 400 μg/kg; the fourth group was co-treated with E_2V and letrozole at the fore mentioned doses. The last four groups received *C. sativus* seed oil (CSt-oil) at doses of 42.5, 85 and 170 mg/kg, respectively, or the total *C. sativus* seed extract (CSt) at a dose of 500 mg/kg, concomitantly with E_2V and letrozole at the fore mentioned doses. The treatment was *per os* and lasted for 30 days, after which, the animals were sacrificed and androgen-dependent organs (prostate, epididymis, seminal vesicles, testes, deferent canal and penis) were removed and weighed.

2.5. Evaluation of biochemical parameters

2.5.1. Total protein level

Total protein in prostate tissue was evaluated by Bradford methods (Bradford, 1976). In an acidic environment, the hydrophobic amino acid residues of the proteins react with Coomassie Blue G250. This reaction leads to a change color reaction which turns blue and absorbs at 595 nm. The intensity of the coloration is proportional to the total of protein residues contained in the homogenate.

2.5.2. PSA activity

The serum level of prostate specific antigen (PSA), the main biomarker of prostate disease, was measured by an ELISA method described by Nilsson et al (1997). The PSA ELISA is a non-competitive solid phase immunoassay based on the direct sandwich technique. Calibrators, controls and samples were incubated together with a biotinylated anti-PSA monoclonal antibody and a horseradish peroxidase (HRP)-labeled anti-PSA monoclonal antibody in streptavidin-coated microtiter strips. Color intensity was determined in the microtiter plate spectrophotometer at 620 nm.

2.5.3. Serum level of IL-1β, IL-6 and TNF-α

They were measure by the quantitative sandwich enzyme immunoassay technique. Antibody specific for Tumor Necrosing Factor alpha (TNF alpha), interleukin 1 beta (IL-1 beta) and interleukin 6 (IL-6) has been pre-coated into a microplate, then standards and samples were pipetted into the wells. The IL-1β/ IL-6/TNFα present in sample bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-1β/ IL-6/TNFα has been added to the wells. After washing, avidin conjugated HRP was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added into each wells and color develops was proportional to the amount of IL-1β/ IL-6/TNFα bound in the initial step. The color development is stopped and the intensity of the color is measured through a multyreader ELISA ("*Lumineux MAGpix Analyzer*", XMAP Technology, USA).

2.6. Histological analysis

The prostates were fixed in 10 % neutral formalin and embedded in paraffin wax, sectioned into 5-μm slices and stained with hematoxylin and eosin stain. Microphotographs were captured at 400 × magnification using complete Zeiss equipment consisting of a microscope Axioskop 40 connected to a computer. The images were analyzed using MRGrab1.0 and Axio Vision 3.1 software, all provided by Zeiss (Hallbermoos, Germany).

2.7. Statistical analysis

Results were expressed as mean ± standard error on the mean (SEM). Means were compared by analysis of variance (ANOVA) followed by Dunnet's posttest using Sigma plot software version 11. The difference was considered significant at $p < 0.05$.

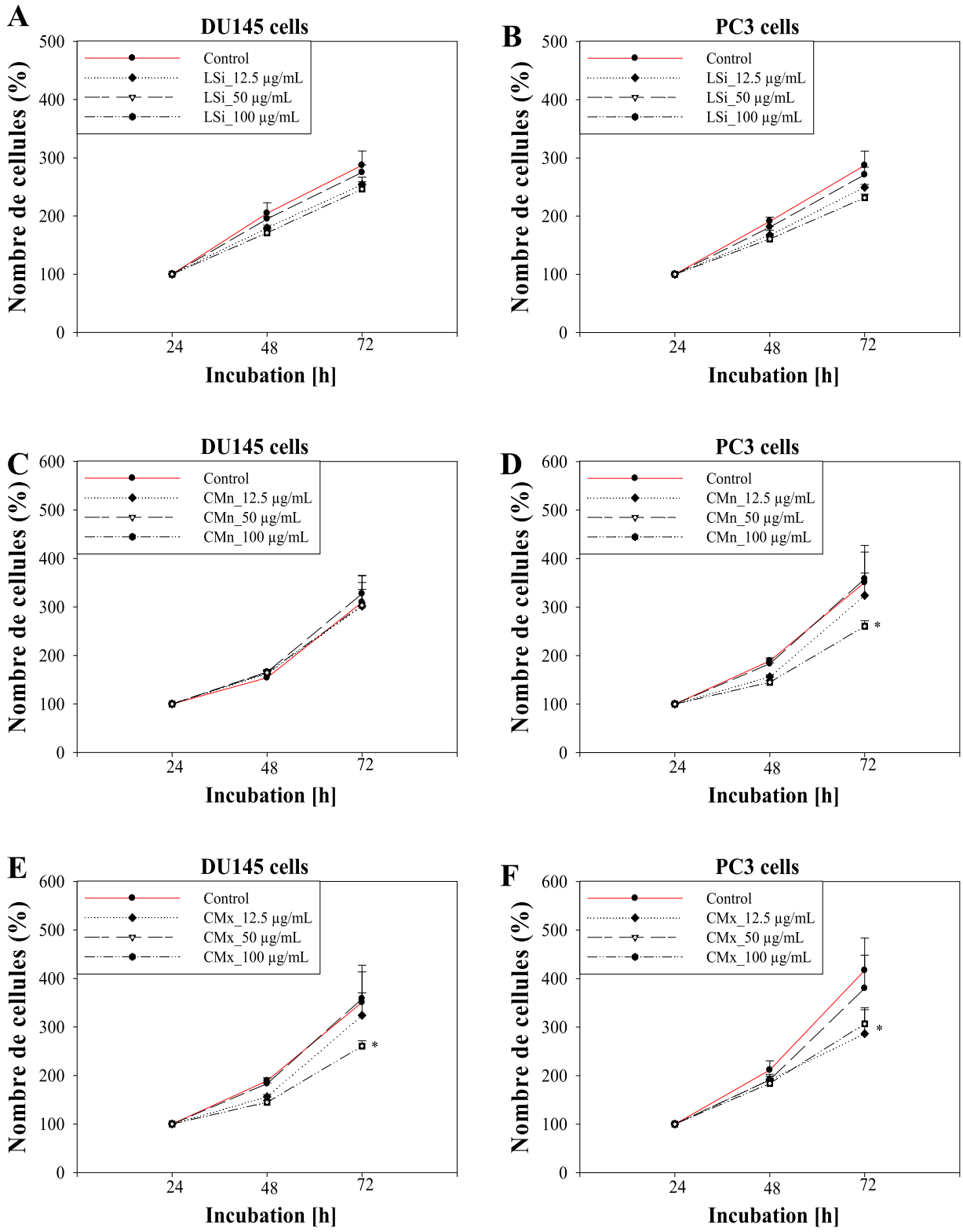

Fig. 1. Cell growth of *Lagenaria siceraria*-LSi (A & B), *Cucumeropsis manii*-CMn (C & D), *Cucurbita maxima*-CMx (E & F), *Cucurbita moschata*-CMs (G & H), *Cucumis melo*-CMl (I & J) and *Cucumis sativus*-CSt (K & L) on DU145 and PC3 cells, respectively. Cells were treated with concentrations range from 12.5 to 200 µg/mL and incubated for 24, 48 and 72 h. Control cells remained untreated. *$p < 0.05$ and *$p < 0.01$ versus controls.

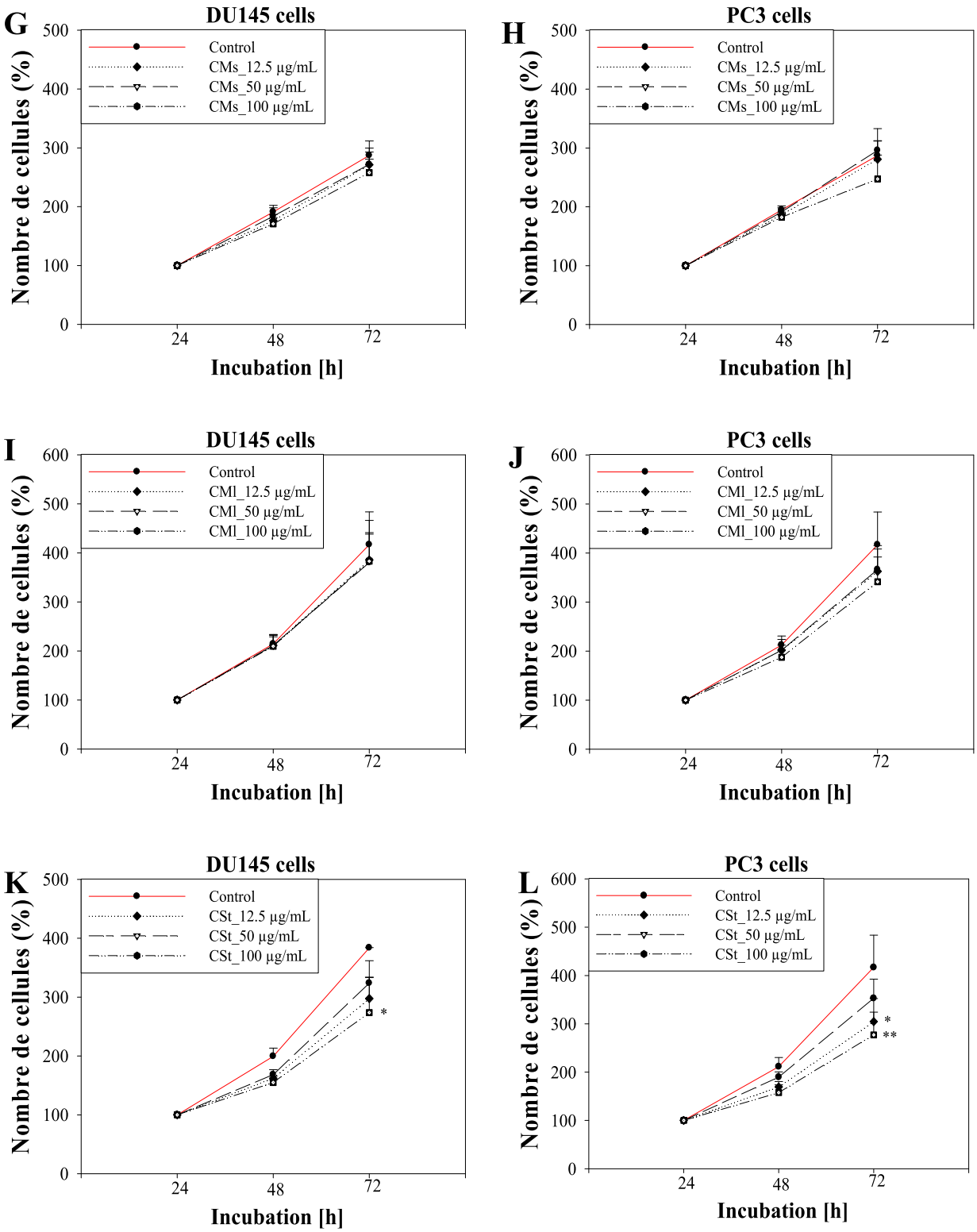

Fig. 1. (*continued*).

3. Results

3.1. Effects of selected pumpkin seeds on cell growth

The Fig. 1 depicts the effects of 3 (*Lagenaria siceraria*-LSi, *Cucumeropsis manii*-CMn, *Cucurbita maxima*-CMx) out of the six selected pumpkin seeds on DU145 and PC3 cells growth after 24, 48 and 72 h. It can be observed that CMx exhibited significant inhibition of the both cancer cell lines ($p < 0.05$) at 100 µg/mL in DU145 cells and 50 and 100 µg/mL in PC3 cells.

The Fig. 2 presents the effects of the others selected pumpkin seeds (*Cucurbita moschata*- CMs, *Cucumis melo*-CMl; *Cucumis sativus*-CSt). CMs

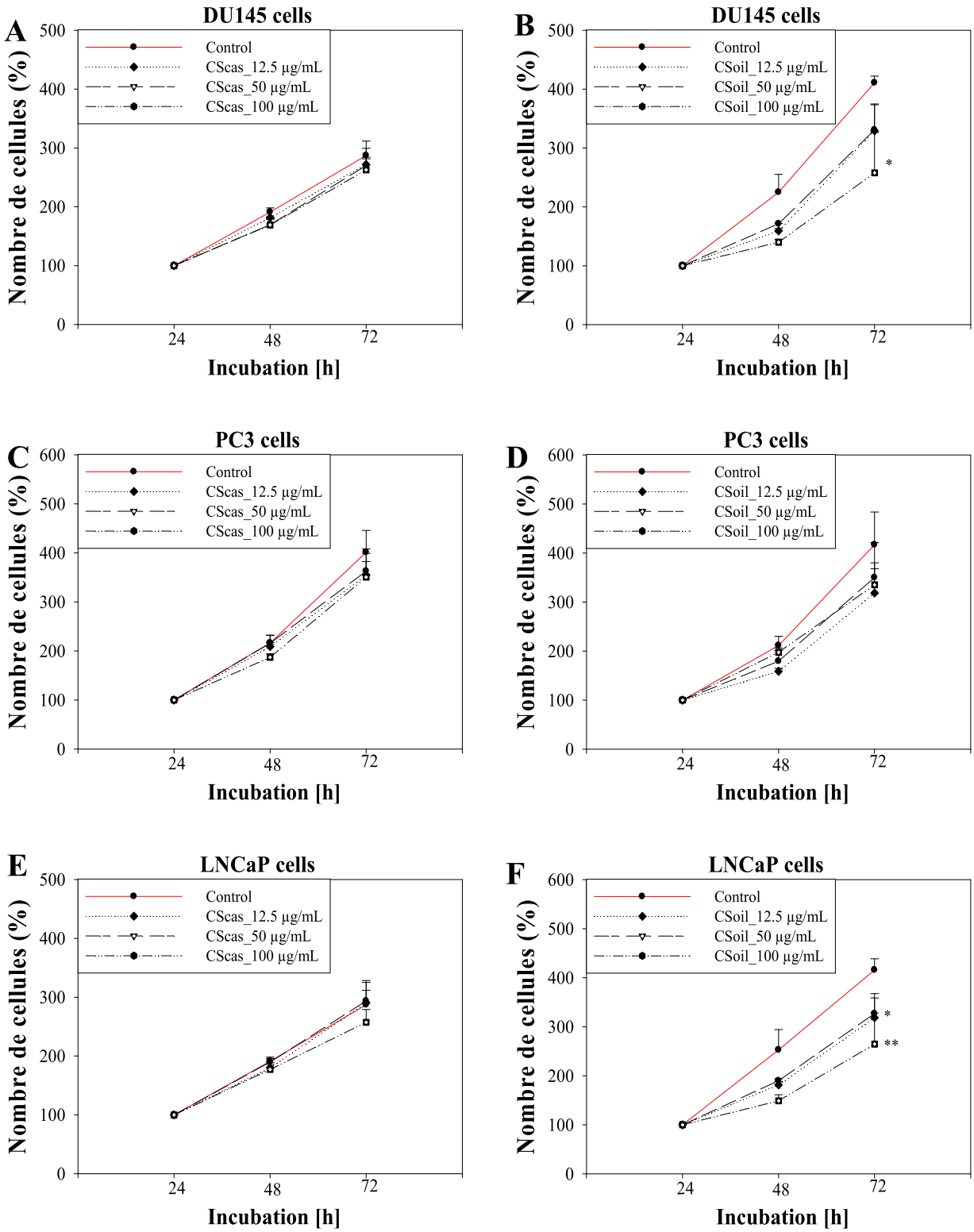

Fig. 2. Cell growth of *Cucumis sativus*-CSt cake and oil on DU145 (A & B), PC3 (C & D) and LNCaP (E & F) cells. Cells were treated with concentrations range from 12.5 to 200 µg/mL and incubated for 24, 48 and 72 h. Control cells remained untreated. *$p < 0.05$ and *$p < 0.01$ versus controls.

and CMl as well as LSi (Fig. 1) did not change the growth of the DU145 and PC3 cells. The *Cucumis sativus* (CSt) seeds time-depend and concentration depend fashion inhibit the both DU145 ($p < 0.05$ at 100 µg/mL) and PC3 cells ($p < 0.05$ at 50 µg/mL and $p < 0.01$ at 100 µg/mL).

Out of the six samples, *Cucumis sativus* (CSt) exhibited the most potent antiproloferative effects *in vitro*.

3.2. Comparative effects of the six selected pumpkin seeds on BPH induced in rat

A 28-day administration of testosterone induced significant increase in the prostate index ($p < 0.01$), prostatic epithelium height ($p < 0.001$) and prostate cells diameter ($p < 0.01$) (Table 2). The finasteride

Table 2
Protective effects of the six selected pumpkin seeds from Cameroon against HBP induced in rats.

Groups	Prostate wet weight (mg/kg BW)	Prostate growth inhibition (%)	Prostate volume (mm^3)	Total protein level (mg/mL)	Epithelium height (μm)
NOR	1616 ± 99	–	292 ± 14	85 ± 1.4	2.4 ± 0.1
BPH	2436 ± 71##	–	332 ± 14	88 ± 0.8	5.8 ± 0.1###
FINAS	1889 ± 30#	54.4 %	202 ± 20**	75 ± 1.2***	3.5 ± 0.1***
LSi 500	2377 ± 258	7.21 %	325 ± 15	89 ± 1.9	4.3 ± 0.1***
LSi 1000	2385 ± 280	6.22 %	248 ± 13	89 ± 0.1	4.3 ± 0.1***
CMn 500	2451 ± 168	0.0 %	313 ± 20	86 ± 3.3	4.6 ± 0.2*
CMn 1000	2390 ± 240	5.5 %	292 ± 33	85 ± 2.8	6.8 ± 0.3
CMx 500	2324 ± 122	13.6 %	260 ± 38	78 ± 4.4*	4.4 ± 0.2**
CMx 1000	2210 ± 187	27.5 %	249 ± 20	84 ± 1.4	4.8 ± 0.1*
CMs 500	2192 ± 129	29.8 %	264 ± 18	83 ± 4.4	5.5 ± 0.3
CMs 1000	2493 ± 224	0.0 %	271 ± 43	80 ± 0.9	5 ± 0.3
CMl 500	2454 ± 68	2.1 %	327 ± 5	69 ± 2.7***	5.2 ± 0.3
CMl 1000	2418 ± 163	2.1 %	257 ± 12	66 ± 1.6***	5.6 ± 0.4
CSt 500	2029 ± 96	49.5 %	216 ± 14**	74 ± 1.3***	4.4 ± 0.1**
CSt 1000	2272 ± 49	20.3 %	224 ± 17*	86 ± 1.0	4.3 ± 0.2***

NOR = Normal rats that received distilled water; BPH = Rats treated with testosterone enanthate (3 mg/kg) and distilled water for 28 days; FINAS = Rats co-treated with testosterone enanthate (3 mg/kg) and finasteride (5 mg/kg); LSi = Rats co-treated with testosterone enanthate (3 mg/kg) and *Lagenaria siceraria* at 500 and 1000 mg/kg; CMn = Rats co-treated with testosterone enanthate (3 mg/kg) and *Cucumeropsis mannii* at 500 and 1000 mg/kg; CMx = Rats co-treated with testosterone enanthate (3 mg/kg) and *Cucurbita maxima* at 500 and 1000 mg/kg; CMs = Rats co-treated with testosterone enanthate and *Cucurbita moschata* at 500 and 1000 mg/kg; CMl = Rats co-treated with testosterone enanthate and *Cucumis melo* at 500 and 1000 mg/kg; CSt = Rats co-treated with testosterone enanthate (3 mg/kg) and *Cucumis sativus* at 500 and 1000 mg/kg. ## $p < 0.01$ and ### $p < 0.001$: significant difference *v.s.* normal. *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$: significant difference *v.s.* negative control (BPH).

protected against the testosterone-induced the increased in prostate index ($p < 0.05$), prostate volume ($p < 0.01$), prostatic epithelium height ($p < 0.001$), prostate cells diameter ($p < 0.01$) and protein levels in prostate ($p < 0.001$). Concerning the selected pumpkin seeds, *Lagenaria siceraria* (LSi) at the 500 ($p < 0.001$) and 1000 mg/kg ($p < 0.001$); *Cucumeropsis mannii* (CMn) at 500 mg/kg ($p < 0.001$); *Cucurbita maxima* (CMx) at 500 ($p < 0.01$) and 1000 mg/kg ($p < 0.05$) and *Cucumis sativus* at 500 ($p < 0.01$) and 1000 mg/kg ($p < 0.001$) significantly decrease the prostatic epithelium height. Protein levels in prostate was significantly decreased and *Cucumeropsis mannii* (CMn) at the dose of 500 mg/kg ($p < 0.05$); *Cucumis melo* (CMl) at both 500 and 1000 mg/kg ($p < 0.001$) and *Cucumis sativus* at 500 mg/kg ($p < 0.001$).

Amongst all the tested pumpkin seeds, *Cucumis sativus* extract showed the most potent effect at the dose of 500 mg/kg (Table 2); evidenced by a reduction of prostate index (from 2436 ± 71 in BPH *v.s.* 2029 ± 96; non-significant), prostate volume (from 332 ± 14 in BPH *v.s.* 216 ± 14 mm^3; $p < 0.01$), total protein level (from 88 ± 0.8 in BPH *v.s.* 74 ± 1.3 mg/mL; $p < 0.001$) and epithelium height (from 5.8 ± 0.1 BPH *v.s.* 4.4 ± 0.1 μm; $p < 0.001$). The following sample was then retained for further experiments.

3.3. Effect of raw and cooked C. Sativus seeds on BPH

As expected, the 28-day testosterone administration induced a significant increase in the relative prostate weight ($p < 0.001$), prostate volume ($p < 0.001$), total protein level in prostate ($p < 0.01$) and prostatic epithelial height compared to normal rats (Table 3). Raw and cook *C. sativus* seeds significantly decreased ($p < 0.05$; $p < 0.01$; $p < 0.001$) these parameters compared with the negative control group mostly at 250 and 500 mg/kg BW. Finasteride at 5 mg/kg BW significantly ($p < 0.001$) prevented the testosterone-induced relative prostate weight, prostate volume, total protein level in prostate and prostatic epithelial height as compared to BPH rats (Table 3).

The raw *C. sativus* seeds extract exhibit potent effects than the cooked one with the optimal effects at the dose of 500 mg/kg which significantly inhibit the testosterone induced the increased in the prostate weight (from 3690.8 ± 127.0 mg/kg in BPH *v.s.* 2659.6 ± 178.9 mg/kg), prostate volume (from 925.2 ± 56.5 mm^3 in BPH *v.s.* 387.9 ± 54.3 mm^3), total protein level in prostate (from 108.7 ± 4.4 mg/mL in BPH *v.s.* 98.2 ± 0.4 mg/mL) and prostatic epithelial height (from 10.6 ± 0.4 μm v.s. 9.1 ± 0.1 μm) as compared to BPH rats.

3.4. Comparative effects of oil and cake from C. Sativus seeds on cell growth

The Fig. 3 shows that the cake of *C. sativus* seeds failed to inhibit the prostate cancer growth *in vitro*. Interesting the oil of *C. sativus* seeds conserved the quasi-total effects of the raw seeds on prostate cancer cells, materialized by the time- and concentration-dependent inhibition of DU145, PC3 and LNCaP cells with best effect at 100 μg/mL.

Base on this result, only the oil of *C. sativus* seeds has been further tested on BPH in rat.

Table 3
Protective effects of raw and cooked *C. sativus* seeds against BPH induced in rat.

Groups	Prostate wet weight (mg/kg BW)	Prostate volume (mm^3)	Proteins in prostate (mg/mL)	Prostate epithelium height (μm)
NOR	1690.8 ± 54.4	454.8 ± 87.9	95.4 ± 2.2	6.9 ± 0.4
BPH	3690.8 ± 127.0###	925.2 ± 56.5###	108.7 ± 4.4##	10.6 ± 0.4###
FINAS	2298.2 ± 177.7***	423 ± 48.1***	86.6 ± 2.3***	7.9 ± 0.4***
CSt-raw 125	2929.0 ± 314.8	451.9 ± 94.7***	113.1 ± 3.9	9.4 ± 0.1
CSt-raw 250	2991.6 ± 218.3	512.7 ± 72.2**	109.1 ± 4.4	8.6 ± 0.1**
CSt-raw 500	2659.6 ± 178.9**	387.9 ± 54.3***	98.2 ± 0.4*	9.1 ± 0.1*
CSt-raw 1000	3188.1 ± 169.8	456.2 ± 46.7***	87.9 ± 1.2***	9.9 ± 0.2
CSt-cook 125	3249.3 ± 345.1	662.8 ± 74.1	94.1 ± 2.8***	9.9 ± 0.6
CSt-cook 250	3004.6 ± 110.9	536.2 ± 35.2**	91.7 ± 2.1**	9.1 ± 0.2*
CSt-cook 500	3040.3 ± 163.4	681.1 ± 60.7	90.5 ± 2.1***	9.7 ± 0.2
CSt-cook 1000	2832.8 ± 269.8*	564 ± 67.1**	94.1 ± 1.9**	9.9 ± 0.3

NOR = Normal rats that received distilled water; BPH = Rats treated with testosterone enanthate (3 mg/kg) and that received distilled water for 28 days; FINAS = Rats co-treated with testosterone enanthate (3 mg/kg) and finasteride (5 mg/kg); CSt-raw = Rats co-treated with testosterone enanthate (3 mg/kg) and raw *Cucumis sativus* seeds. CSt-cooked = Rats co-treated with testosterone enanthate (3 mg/kg) and cooked *Cucumis sativus* seeds. ###$p < 0.001$; ##$p < 0.01$: significant difference *v.s.* normal. *$p < 0.05$; **$p < 0.01$: significant difference *v.s.* BPH.

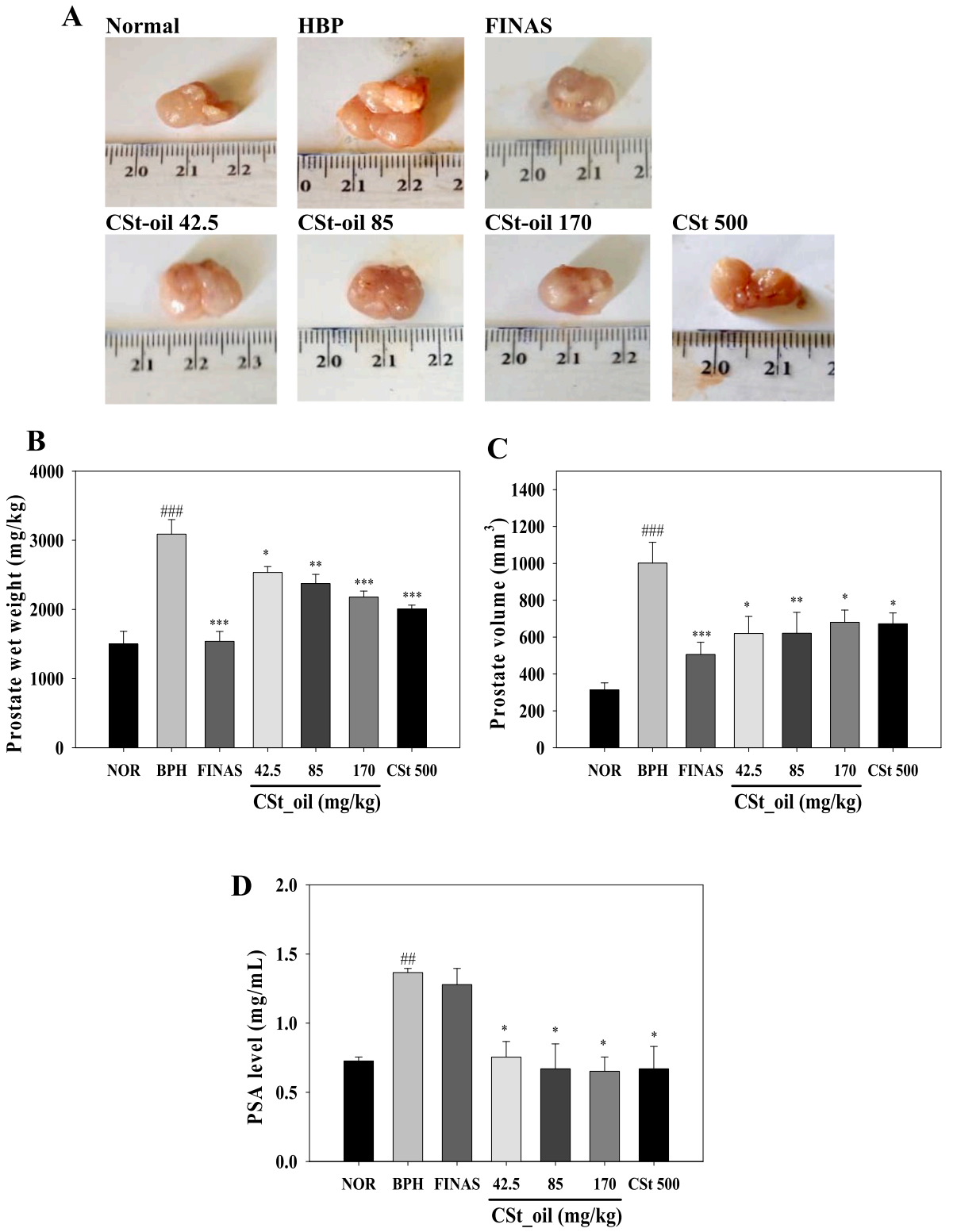

Fig. 3. Protective effects of *C. sativus* seed oil on morphology (A), prostate relative weight (B), prostate volume (C) and serum PSA level (D) after 28 days of treatment. Each bar represents the mean ± SEM (n = 5). NOR = Normal rats that received distilled water; BPH = Rats treated with testosterone enanthate (3 mg/kg) and received distilled water; FINAS = Rats co-treated with testosterone enanthate (3 mg/kg) and finasteride (5 mg/kg); CSt-oil = Rats co-treated with testosterone enanthate (3 mg/kg) and *C. sativus* seed oil at 85, 170, and 340 mg/kg; CSt 500 = Rats co-treated with testosterone enanthate (3 mg/kg) and crude *C. sativus* raw seed at 500 mg/kg BW. ###$p < 0.001$: significant difference versus normal. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$: significant difference versus negative control (BPH).

3.5. Effect of oil from C. Sativus seeds in BPH

3.5.1. Effect on relative mass, volume and prostatic epithelium height

Testosterone has induced significant ($p < 0.001$) increase in the relative prostate weight and prostate volume in BPH rats compared to normal rats (Fig. 4). Finasteride significantly (at least $p < 0.001$) decreased these parameters compared to BPH group. *C. sativus* seeds oil induced a significant dose-dependent decreased in prostate relative mass ($p < 0.05$) and prostate volume ($p < 0.05$) at all the tested doses with optimal effects at dose of 170 mg/kg. The effects recorded in all prostate parameters following oil treatment are similar to that observed with the raw *C. sativus* seeds at 500 mg/kg.

3.5.2. Effect on PSA level

The Fig. 4D shows the effects of *C. sativus* seed oil on prostate specific antigen (PSA) levels after 28 days of treatment. A 28-day consecutive administration of testosterone resulted in a significant increase in PSA levels ($p < 0.01$) compared to the normal control group. Finasteride did not change the PSA level. Both oil from *C. sativus* and raw seeds extract significantly ($p < 0.01$) prevented the testosterone-induced increased in the PSA at all tested doses compared to BPH rats.

3.5.3. Effects on cytokines levels

The Table 4 displays the effects of *C. sativus* oil on some pro-inflammatory cytokines. Testosterone resulted in a significant ($p < 0.01$) increase in the levels of TNF alpha, IL-1 beta and IL-6 compared to the normal rats. Just like finasteride, *C. sativus* seed oil as well as the raw seeds extract, significantly ($p < 0.001$) reduced the serum levels of TNFα ($p < 0.05$), IL-1β ($p < 0.01$) and IL-6 ($p < 0.01$) mainly at the dose of 170 mg/kg and 500 mg/kg BW, respectively compared to BPH rats.

3.5.4. Effects on histo-architecture of the prostate

The cross-sections of the prostates stained by the classical H&E stain (Fig. 5) showed that *C. sativus* seeds oil in the light of finasteride reduced the prostatic epithelium height at a simple layer such as in normal rats. The measurement of the prostatic epithelium height showed that testosterone-induced a significant ($p < 0.001$) increase in prostatic epithelium height. Finasteride ($p < 0.001$), oil at all tested dose ($p < 0.01$ at 42.5 mg/kg and $p < 0.001$ at 80 and 170 mg/kg BW) and raw extract ($p < 0.001$) significantly diminished the prostatic epithelium height compared to BPH rat.

3.6. Anti-androgenic effect of C. Sativus

Fig. 5 illustrates the anti-androgenic effects of *C. sativus* seed oil on the relative weights of the prostate (Fig. 5A), seminal vesicles (Fig. 5B), glans (Fig. 5C) and Tyson glans (Fig. 5D). Castration resulted in a significant reduction ($p < 0.05$) in the relative weight of the prostate, seminal vesicles and glans compared to the non-castrated group (SHAM) after 17 days. Administering testosterone to castrated animals at 0.4 mg/kg for 10 days resulted in a significant ($p < 0.001$) increase in the relative weight of these parameters, as well as that of Tyson glan. Similar to flutamide (3 mg/kg), *C. sativus* seed oil significantly reduced the relative weight of the prostate ($p < 0.05$; $p < 0.01$), seminal vesicles ($p < 0.01$), glans ($p < 0.05$) and Tyson glan ($p < 0.001$) compared to the testosterone-only group. The total seed extract significantly reduced only the weight of the seminal vesicle ($p < 0.01$) and Tyson's glans ($p < 0.001$).

3.7. Estrogenic effect C. Sativus

The estrogen-potentiating effects of *C. sativus* oil and total seed extract on the relative weights of the testes, prostate, seminal vesicles, epididymis, deferens ducts and penis are shown in Table 5. The results show that fulvestrant significantly reduced the relative weights of the prostate ($p < 0.05$) and seminal vesicles ($p < 0.01$) compared to the normal control. Letrozole produced a non-significant reduction in these parameters compared to the non-castrated rats after 28 days of treatment. Letrozole co-administered with estradiol valerate (E_2V) significantly reduced prostate weight ($p < 0.05$) compared with the normal control. *C. sativus* seed oil and raw extract potentiated the effects of estrogen by significantly reducing the relative weights of the testes ($p < 0.05$), prostate ($p < 0.05$; $p < 0.01$), deferens ducts ($p < 0.05$; $p < 0.01$), and penis ($p < 0.05$) compared to the LETRO group.

4. Discussion

Prostate cancer and benign prostatic hyperplasia are linked by the uncontrolled proliferation of cells. To scientifically verify the claim on the beneficial effects of selected pumpkin seeds, they were first assessed on aggressive prostate cancer DU145 and PC-3 cells through a well characterize MTT assay, which measure the metabolic activity of cells. *C. sativus* seed extract inhibitd DU145 and PC3 cells in a concentration-dependant manner mainly at 100 μg/mL. These results are in line with various reports, which showed the antiproliferative and anticancer effects of pumpkin seeds (Malathi and Vadivelu, 2021; Aref et al., 2021). BPH is a common male disease caused by excessive proliferation of the prostate gland and muscle tissue with age under the influence of androgens in particular testosterone (Mobley et al., 2015). Once released from its plasma carrier protein, testosterone enters into the prostate cell and is metabolized in dihydrotestosterone (DHT) by 5α-reductase. DHT bind androgen receptors and induces growth and differentiation of the prostate cells; which in turn cause hyperplasia of stromal and epithelial cells and BPH (Seo et al., 2021). Alternatively, in eldery men, abundant adipocytes increase the amount aromatase which in turn increase the rate of conversion of androgens to estrogens. These later would act via estrogen receptors in prostate and activate the proliferation of stromal and epithelial cell autocrine and paracrine pathways (Devlin, 2021). In this study, the testosterone administration induced a significant increase in the prostate relative weight, prostate volume and prostate epithelium height compared to normal rats, confirming the set up of BPH (Nahata and Dixit, 2012). In fact, prostate weight and prostate volume are crucial indicators for the diagnosis of BPH and for the screening of potential beneficial compounds. This BPH rat model is widely used by the scientific community (Zingué *et al.*, 2020). As expected, the finasteride, a selective inhibitor of type II 5-α reductase, used as standard in this study significantly counteracted the effects of testosterone after 28 days of co-treatment (Evans *et al.*, 2005). Except for *Cucumeropsis mannii* and *Cucurbita moschata* seeds who failed to change the prostate parameters, the other exhibited protective effects against the testosterone-induced prostate hyperplasia, suggesting antiproliferative effects. The best effect was observed with *C. sativus* seeds. This might be due to its high content in cucurbitacins, which are well known antiproliferative (Wahid and Khan, 2021). It is worth noting that all the six pumpkins were tested at the same doses, which were extrapolated from the daily recommended amount of 10 g/day for human consumption as recommended by the German commission. Throughout this study, no signs of toxicity were observed, confirming the pumpkin's status as food.

Raw and cooked *C. sativus* seeds mainly at the doses of 250 and 500 mg/kg significantly reduced relative prostate weight mass and prostate volume as compared to BPH rat. It is well known that the intensity or the probability of occurrence of the effects is proportional to the quantity of drug present at the site of action. To generate a detectable effect, the drug must be able to reach the site of action and be present in sufficient quantity to interact with the target receptor or enzyme (Brunel *et al.*, 2021). Moreover, the raw extract was the most active. This observation is consistent with observation of Olufeko et al. (2020), when evaluating the effects of the cooking on chemical and phytochemical compositions of raw and cooked melon as well as walnut seeds demonstrated that except for the flavonoids, cooking reduced tannin, steroid, terpenoids, alkaloids and phenol content in melon seeds. Polyphenols including flavonoids are known to have antiproliferative activity on BPH via the

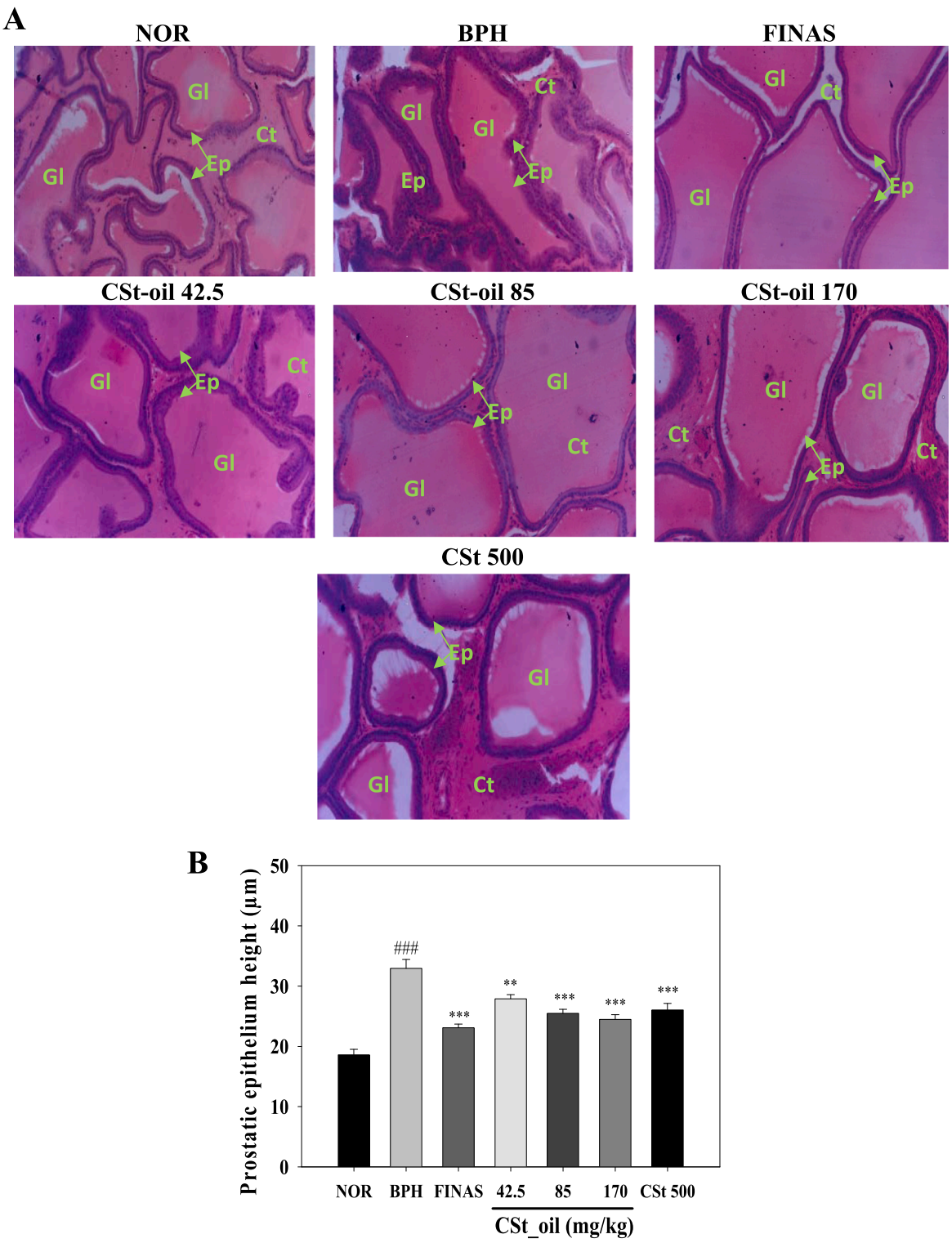

Fig. 4. H&E 100 × photomicrographs of prostate section (A) and prostatic epithelium height (B) in BPH rats treated with *C. sativus* seeds oil. Each bar represents the mean ± SEM (n = 5). NOR = Normal rats that received distilled water; BPH = Rats treated with testosterone enanthate (3 mg/kg) and received distilled water; FINAS = Rats co-treated with testosterone enanthate (3 mg/kg) and finasteride (5 mg/kg); CSt-oil = Rats co-treated with testosterone enanthate (3 mg/kg) and *C. sativus* seed oil at 85, 170, and 340 mg/kg; CSt 500 = Rats co-treated with testosterone enanthate (3 mg/kg) and crude *C. sativus* raw seed at 500 mg/kg BW. ###$p < 0.001$: significant versus normal. **$p < 0.01$; ***$p < 0.001$: significant versus negative control (BPH). Ep = epithelium; Bl = blood vessels; Ct = connective tissue; Gl = glandular lumen.

Table 4
Protective effects oil from *C. sativus* seeds in some inflammatory cytokines against BPH in rat.

Group	Concentration level in serum (ρg/ml)		
	TNFα	IL1β	IL-6
NOR	142.4 ± 9.6	727.9 ± 50.7	155.1 ± 2.2
BPH	221.6 ± 6.4##	870.9 ± 10.3##	184.8 ± 8.8##
FINAS	142.4 ± 6.4**	677.5 ± 4.8***	148.1 ± 3.1
CS-oil 42.5	248.1 ± 16.1	832.5 ± 25.4	176.2 ± 3.5
CS-oil 85	179.2 ± 20.1	749.2 ± 26.8*	167.8 ± 3.2*
CS-oil 170	175.7 ± 8.7*	753.6 ± 7.4**	156.1 ± 1.6**
CS 500	148.1 ± 5.2**	696.5 ± 10.3***	157.6 ± 1.2***

NOR = Normal rats receiving distilled water; BPH = Rats given testosterone enanthate (3 mg/kg) and distilled water for 28 days; FNS = Rats given testosterone enanthate and treated with finasteride (5 mg/kg); CS-oil = Rats given testosterone enanthate and treated with *Cucumis sativus* oil; CS = Rats given testosterone enanthate and treated with *Cucumis sativus*. PGI = Prostate growth inhibition. ### $p < 0.001$; ## $p < 0.01$: significant difference from normal. * $p < 0.05$; ** $p < 0.01$: significant difference from negative control.

inhibition of 5α-reductase isoenzymes (Mitsunari et al., 2021). *In vitro*, the oil better prevented the prostate cancer cells growth than the cake from *C. sativus* raw seeds. The difference effect could be due to the variation in the relative composition of bioactive ingredients in the oil and in the cakes; oil exhibited the quasi-totality effects of the raw seeds extract. *In vivo* oil significantly decreased the prostate wet weight, prostate volume and prostatic epithelium height compared to untreated BPH rats. These results are in line with those of Gossel-Williams et al. (2006) who showed that the oil extracted from raw *C. sativus* as well as *C. pepo* inhibit prostate hyperplasia through the inhibition of 5α-reductase. Although we did not assess the effect of *C. sativus* oil on 5α-reductase, we can hypothesize than it induces part of its effect through this pathway. Studies have suggested that the actions of pumpkin seed oil may also be attributed to its content on phytosterols, which are known to interfere with the actions of dihydrotestosterone (Carbin et al., 1990).

PSA is a glycoprotein produced through the phosphorylation of DHT in the prostatic stromal cells (Kim *et al.*, 2017; Eleazu et al., 2021) showed that a 28-day consecutive administration of testosterone at 3 mg/kg BW to induce BPH *s.c.* in rats is characterized by an increase of the serum PSA level. Concomitant with this observation, the testosterone induced significant increase in the PSA level and finastéride as well as *C. sativus* seeds oil at all tested doses reduced the serum PSA level, strengthen their anti-proliferative effects on the prostate. Prostate cell proliferation in BPH has been shown exacerbate under chronic inflammation. Inflammation is a physiological response, initiated by the synthesis of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12, and IFN-γ) by macrophages in response to inflammatory injury. Inflammatory cytokines amplify the inflammatory response by activating the expression of various genes like cyclooxygenase-2 (COX-2) by

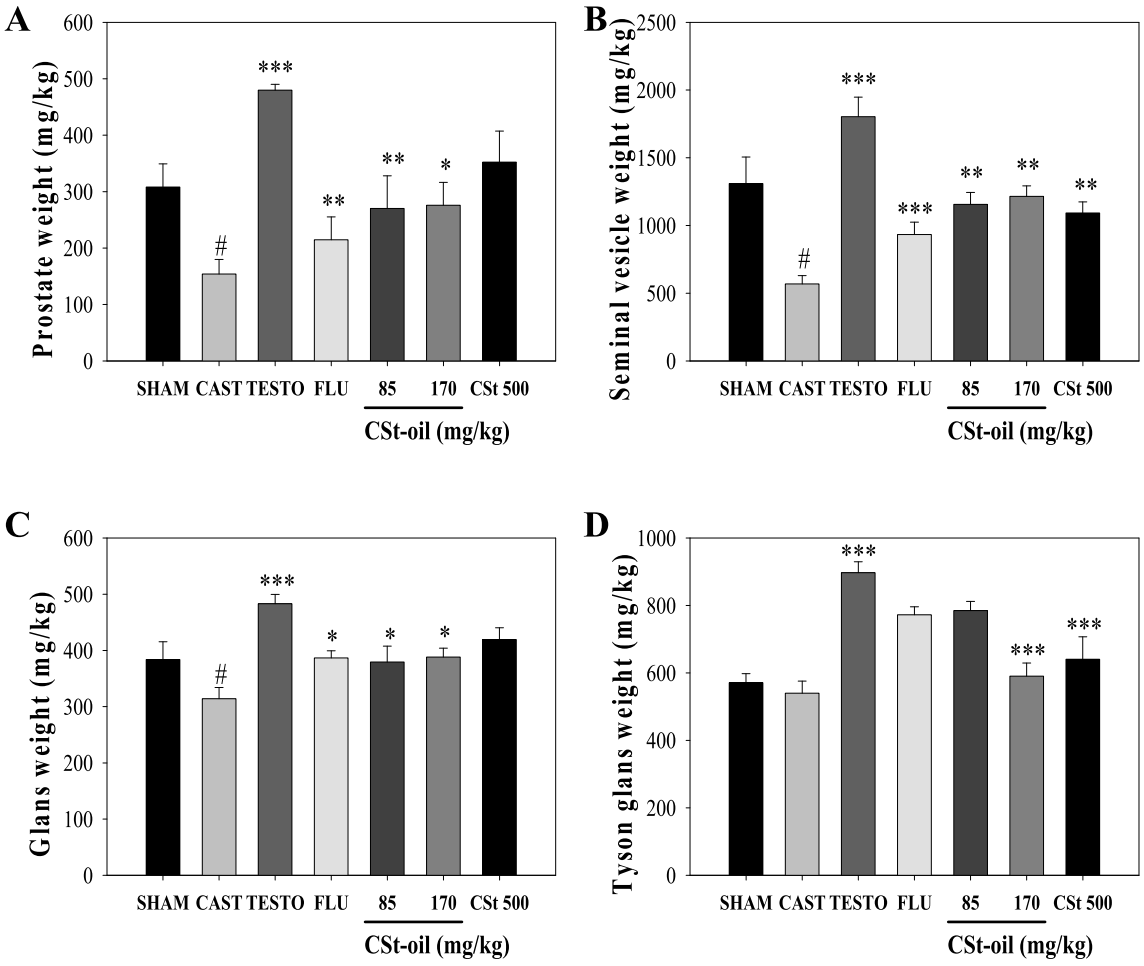

Fig. 5. The effects of *C. sativus* seed oil on the relative weights of prostate (A), seminal vesicles (B), glans (C) and tyson glans (D). SHAM = sham-operated rats receiving corn oil; CAST = castrated rats receiving corn oil for 10 days; TESTO = castrated rats receiving testosterone at 0.4 mg/kg; FLU = castrated rats co-treated with testosterone (0.4 mg/kg) and flutamide (3 mg/kg); CSt-oil = castrated rats co-treated with testosterone (0.4 mg/kg) and *C. sativus* seed oil at 85 and 170 mg/kg (most potent doses in the previous experiments); CSt = castrated rats co-treated with testosterone (0.4 mg/kg) and *C. sativus* seed crude extract at 500 mg/kg. (n = 5). #$p < 0.05$: significant difference versus normal control; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$: significant difference versus negative control (CAST).

Table 5
Antiestrogenic effect of *C. sativus* on androgeno-dépendent organs.

Groups	Testicles	Prostate	Seminale vesicles	Epididyms	Deferent ducts	Penis
NOR	14.50 ± 0.55	0.85 ± 0.15	3.33 ± 0.45	3.86 ± 0.14	0.67 ± 0.04	1.11 ± 0.05
FASLO	12.56 ± 0.93	0.32 ± 0.06#	1.56 ± 0.24##	3.28 ± 0.20	0.61 ± 0.04	0.96 ± 0.08
LETRO	14.36 ± 0.35	0.65 ± 0.03	2.91 ± 0.36	3.55 ± 0.06	0.65 ± 0.05	1.06 ± 0.03
LETRO + E2V	11.97 ± 0.73	0.33 ± 0.05*	2.20 ± 0.49	3.51 ± 0.14	0.62 ± 0.07	1.03 ± 0.03
LETRO + E2V + CS-h 42.5	9.21 ± 1.61*	0.34 ± 0.07	3.13 ± 0.43	3.61 ± 0.28	0.31 ± 0.06**	0.70 ± 0.05
LETRO + E2V + CS-h 85	10.30 ± 1.25	0.37 ± 0.06	1.73 ± 0.36	2.65 ± 0.42	0.50 ± 0.07	0.95 ± 0.1*
LETRO + E2V + CS-h 170	9.75 ± 1.02*	0.35 ± 0.07*	0.85 ± 0.07**	3.05 ± 0.11	0.36 ± 0.03**	0.76 ± 0.06*
LETRO + E2V + CS-500	8.73 ± 2.27*	0.21 ± 0.02**	2.24 ± 0.57	3.20 ± 0.18	0.40 ± 0.09*	0.74 ± 0.08*

NOR = normal rats treated with corn oil; FASLO = normal rats treated with fulvestrant at 300 μg/150 g; LETRO = normal rats treated with letrozole at 0.5 mg/kg; LETRO + E2V = normal rats treated with letrozole (0.5 mg/kg) and estradiol valerate (400 μg/kg); LETRO + E2V + CSt-oil = normal rats treated with letrozole (0.5 mg/kg), estradiol valerate (400 μg/kg) and *C. sativus* seed oil. *C sativus* seed oil at 42.5, 85 and 170 mg/kg; LETRO + E2V + CSt-500 = normal rats co-treated with letrozole (0.5 mg/kg), estradiol valerate (400 μg/kg) and *C. sativus* total crude seed extract at 500 mg/kg. (n = 5). #$p < 0.05$; ##$p < 0.01$: significant difference versus normal control. *$p < 0.05$; **$p < 0.01$: significant difference versus negative control (LETRO).

transcription factors (Mitsunari et al., 2021). Immune infiltrating cells are activated in BPH condition by the production of various inflammatory cytokines including TNF-α, IL-1β and IL-6. These cytokines contribute to prostate enlargement and growth of epithelial cells as reported by Rho *et al*. 2020. Similarly, in this study an up-regulation of TNF-α, IL-1β and IL-6 in BPH rat compared to the normal rats. *C. sativus seeds* oil significantly reduced the testosterone-induced up-regulation of TNF-α, IL-1β and IL-6 cytokines. This result is on accordance with the observation of El-Sherbiny et al. (2021), who demonstrated that diacerin inhibited the above-mentioned inflammatory cytokine on testosterone-induced BPH in rat. *C. sativus* seeds oil contains a high amount of cucurbitacins A, B, C, D, E and I, which are potent anti-inflammatory agents acting by inhibition of the cyclooxygenase (COX) enzymes (Wahid et al., 2021).

The etiology of BPH is not well understood, although various causes such as anti-inflammatory, antioxidant, androgenic, and estrogenic factors have been implicated. To assess the anti-androgenic and estrogenic potential of *C. sativus* oil, it was evaluated according to OECD protocols. The administration of testosterone to castrated animals led to a significant increase in the relative weights of the prostate, seminal vesicles, glans, and Tyson glans. These findings align with a study by Kim et al. (2002), which investigated the anti-androgenic effects of bisphenol-A in immature castrated rats. Both *C. sativus* and flutamide, when administered concurrently with testosterone to castrated rats, significantly reduced the relative mass of these organs, suggesting an anti-androgenic effect (OECD, 2009). Specifically, the increase in prostate weight is dependent on DHT (Ajayi and Abraham, 2018), and *C. sativus,* through its phytoconstituents such as delta-sterols, may inhibit 5-reductase (Heim et al., 2018). Moreover, the administration of fulvestrant (a pure anti-estrogen) and letrozole (an aromatase inhibitor) to normal adult rats resulted in a reduction in the relative weights of the prostate and seminal vesicles compared to untreated normal animals. These results support the findings of Jasem and Tawfeek (2020), who demonstrated that letrozole administration induced a decrease in the weights of androgen-dependent organs in adult rats. In the etiology of BPH, estrogens promote inflammation and hyperplasia by binding to ERα (Ajayi and Abraham, 2018). Fulvestrant, an estrogen receptor antagonist, inhibits estrogen dimerization and translocation while increasing estrogen degradation (Carlson, 2005). The co-administration of letrozole with estrogens led to a significant reduction in the relative weight of the prostate and a non-significant reduction in the weights of other androgen-dependent organs. Estrogens are known to inhibit the growth of male reproductive organs and spermatogenesis through negative feedback on the hypothalamic-pituitary complex (Schulster et al., 2016). By binding to ERβ, estrogens induce antiproliferative and pro-apoptotic effects in the prostate by inhibiting apoptosis, increasing oxidative stress, and decreasing testosterone levels (Ajayi and Abraham, 2018). Concurrent administration of *C. sativus* seed oil with estrogen and letrozole resulted in a decrease in the weights of androgen-dependent organs compared to normal animals. These findings are consistent with those of Weber et al. (2001), who demonstrated that the administration of phytoestrogens, particularly soybean phytoestrogens, to male rats significantly reduced prostate weight. In fact, Kuhnle et al. (2009) found that cucurbits contain an average of 12.5 μg of phytoestrogens per 100 g. *C. sativus* phytoestrogens preferentially bind to ERβ, inducing the observed antiproliferative effects in this study (Karsli-Ceppioglu et al., 2015).

Conclusion

Out of the six Cameroonian edible pumpkin seeds tested *in vitro* on prostate cancer cells growth and *in vivo* on testosterone-induced BPH *C. sativus* seeds was the most potent. Furthermore raw *C. sativus* seeds at 500 mg/kg and its oil at 170 mg/kg significantly reduced prostate weight, prostate volume, prostate epithelial height, total protein level and PSA. *C. sativus* seed oil appears to gather the overall bioactive ingredients presents in seeds, and it seems to act through anti-inflammatory, anti-androgenic and weak estrogenic activities. In sum *C. sativus* seeds has antiproliferative effects prostate; which could justify its use by Cameroonian male against prostate ailments.

Authors' contributions

ZS, NTD, BRA and ND designed the study. BYB, GT and ZS performed the *in vitro* study. BYB, FRU, NBC and ZS performed the *in vivo* part of the study. BYB and ZS drafted the paper. All authors have revised and approved the final manuscript.

Ethic statement

The experiments in animals were performed following the recommendation of the Joint Institutional Review Board Animal & Human Bioethics of the Faculty of Science (University of Yaounde 1) reference # BTC-JIRB2021-010, which adopted the directives established by the European Union on the care of animals (EEC Council 86/609).

CRediT authorship contribution statement

Berlise Yengwa Bakam: Writing – original draft, Methodology, Investigation, Data curation. **Romeo Urich Fosso:** Methodology, Investigation. **Timothy Grein:** Methodology, Investigation, Data curation. **Derek Tantoh Ndinteh:** Writing – review & editing, Writing – original draft, Supervision. **Sebastian Maxeiner:** Validation, Data curation. **Stéphane Zingue:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Roman A Blaheta:** Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Dieudonne Njamen:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Ethics approval and consent to participate

Housing of animals and all experiments were approved by the Cameroon Institutional National Ethic Committee, which adopted all procedures recommended by the European Union on the protection of animals used for scientific purposes.

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