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Research Paper

Treatment of benign prostatic hyperplasia with *Croton membranaceus* in an experimental animal model

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ABSTRACT

Ethnopharmacological relevance: Croton membranaceus leaf extracts are used in the Bahamas to aromatize tobacco. In Nigeria it is used to improve digestion and in Ghana, the root extract is used for the treatment of benign prostatic hyperplasia (BPH). Despite claims of efficacy no data exists to support this. The aim of this study was to determine if *Croton membranaceus* aqueous root extract (*CMARE*) could attenuate the development of BPH in an animal model.

Materials and methods: Fifty (50) adult male Sprague-Dawley rats weighing 200–250 g were randomly divided into 5 groups. Group 1 served as the control and received normal saline p.o. Groups 2–5 were castrated and injected with 5 mg/kg b.wt. testosterone propionate subcutaneously for 28 days. Group 2 (model group) had no further treatment. Group 3 was simultaneously given 0.5 mg/kg b.wt. finasteride p.o. throughout. Groups 4 and 5 received 30 mg/kg b.wt. [low dose (LD)] and 300 mg/kg b.wt. [high dose (HD)] *CMARE*, respectively, for 28 days. Rats were sacrificed at the end of the study and all prostate organs harvested. Wet weights, volumes and prostatic index (PI) were determined. Tissues were histologically examined. Serum prostate specific antigen (PSA) and dihydrotestosterone (DHT) levels were determined.

Results: Prostate volume of the control group was $0.67 \pm 0.23\ cm^3$. The model, finasteride, CMARE LD and HD groups had the following volumes: 0.92 ± 0.12, 0.84 ± 0.16, 0.79 ± 0.16 and $0.80 \pm 0.19\ cm^3$, respectively. Only the model group showed significant statistical differences with the control ($p=0.007$). PI for control, model, finasteride, LD and HD groups was as follows: 0.19 ± 0.04, 0.30 ± 0.04, 0.25 ± 0.04, 0.21 ± 0.05 and 0.22 ± 0.05. No statistical differences between the control PI and the CMARE treated groups were observed. Histologically, the model group had massive growth of columnar stromal and epithelial cells. CMARE and finasteride attenuated this growth with a resultant thin layer of stromal and epithelial cells similar to the control. PSA levels were significantly lower in the treatment groups.

Conclusion: CMARE reduces stromal and epithelial cell growth, and subsequently shrinks enlarged prostate. This is the first scientific proof validating the anecdotal evidence of CMARE efficacy in the management of BPH.

1. Introduction

Benign prostatic hyperplasia (BPH) and its related prostate cancer are an enlargement of the prostate gland from progressive hyperplasia or abnormal growth of cells of glandular epithelial and stromal cells (Dhirgra and Bhagwat, 2011; Roehrborn, 2012). Commonly experienced symptoms include frequent urination during the day and night, incomplete emptying of the bladder, weak urine stream, inability to delay urination, incontinence and painful or bloody urination (Eisenberg et al., 1998). These symptoms can cause significant bother and impact on quality of life. Prostate cancer in some instances metastasizes to other organs of the body especially the bone and therapeutic options are often limited. Though the underlying etiology of BPH and its related cancer are yet to be fully understood, risk factors such as age, diet,

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race, androgens and estrogens have been implicated in both conditions (Ross and Henderson, 1994; Wu et al., 1995).

BPH occurs in 88% of autopsies in men of 80 years and beyond, with compatible symptomatology reported in nearly 50% of men aged, or older than 50 years in the general population (Napalkov et al., 1995). Cancer of the prostate has been documented as the most frequently diagnosed cancer and the third most common cause of cancer-related deaths among men in developed countries (Jemal et al., 2011). The incidence and mortality rate of prostate cancer has been found to be 60% and 2.4 times higher in African American men than White men (American Cancer Society, 2007–2008). Several published findings indicate high prevalence of BPH and its related cancer in West Africans, with most of the data obtained from Nigeria and Ghana (Gueye et al., 2003; Klufio, 2004; Odedina et al., 2006). The prevalence of enlarged prostate in Ghanaian men detected by digital rectal examination was found to be higher than previously reported in American men (Chokkalingam et al., 2012). Wiredu and Armah (2006) reported that 17.35% of male cancer deaths in Ghana was due to prostate cancer. A recent study in prostate cancer patients referred to the National Center for Radiotherapy (Ghana) revealed a third of these patients presented with metastatic disease, suggesting the need for earlier detection and curative therapy (Yamoah et al., 2013).

Therapeutic options such as radical prostatectomy, radiation, hormonal therapy, alpha 1-adrenoreceptor blockers and 5 alpha-reductase inhibitors are available for BPH and its cancers (Di Silverio et al., 1993). However, the adverse effects often associated with these options such as impotence, decreased libido, orthostatic hypotension, fatigue, dizziness, and the associated high cost of these options have underpinned the increased shift towards search and usage of "safer" natural plant extracts for the management of these conditions (Buck, 1996; Madersbacher et al., 2005; Hutchison et al., 2007). Africa abounds with vast medicinal plants which are employed in the management of several diseases. *Croton membranaceus* Mull. Arg. (Euphorbiaceae) which grows wildly often along rivers in West Africa i.e. Nigeria, Niger and Ghana has been of keen interest in recent phytochemical and pharmacological research, due to its medicinal potential (Aboagye, 1997). In Ghana, the aqueous root extract of *Croton membranaceus* has been used in the management of BPH and prostate cancers for decades (Mshana et al., 2000). Recent pharmacological studies revealed that it is generally nontoxic (Asare et al., 2011), possessed antimicrobial activity, and demonstrated cytotoxic effects against human prostate cells (Bayor et al., 2009), anti-atherogenic, cardio protective and inhibitory effects on proliferation of prostate cells (Afriyie et al., 2013, 2014). Despite its *in vitro* and *in vivo* prostate growth inhibitory effects, and anectodal reports of its ability to improve the quality of life of BPH and prostate cancer patients, there has been no *in vivo* study on its possible protective effects against BPH. Hence, the aim of the study was to investigate the curative effectiveness of the aqueous root extract of *Croton membranaceus* in a testosterone-induced BPH model in castrated rats.

2. Materials and methods

2.1. Extraction

The roots of *Croton membranaceus* were harvested in December 2012 and authenticated by the Center for Scientific Research into Plant Medicine (CSRPM), Mampong, Akwapim. The sample of the plant was deposited at the herbarium of CSRPM with a voucher specimen number CSRP 2110. The aqueous root extract was obtained as previously described (Afriyie et al., 2013). The freeze-dried extract was weighed and stored in a sealed container in a refrigerator at a temperature of 5 ± 3 °C until use.

2.2. HPLC determination of CMARE

Different batches of extract were monitored by chromatographic fingerprint. Samples were analyzed on a Shimadzu HPLC system (Kyoto, Japan). Ultimate XB-C_{18} column (150×4.6 mm^2, 5 um) the absorbance was measured at 208 nm. The mobile phase solvent A was water and solvent B acetonitrile (ACE) at a flow rate of 0.5 ml/min and an injection volume of 10 uL. The gradient procedure used was as follows: 10–10% ACE at 0–5 min, 10–85% ACE at 5–60 min and 85–85% ACE for 60–65 min. Based on the fingerprint as shown in Fig. 1, an optimum and reproducible procedure for preparing CMARE was established.

2.3. Animal experimentation

Fifty (50) specific pathogen free (SPF) grade adult male Sprague-Dawley (S-D) rats weighing between 200 and 250 g were purchased from Shanghai Si-Lai-Ke Experimental Ltd. (Shanghai, China) for the study. The rats were acclimatized to laboratory environment (21–23 °C) with a 12 h light-darkness cycle for 7 days prior to experimentation. The rats had access to standard laboratory diet and water *ad libitum*. All experimental procedures were carried out in accordance with international ethical guidelines and the National Institutes of Health Guide for the care and use of Laboratory

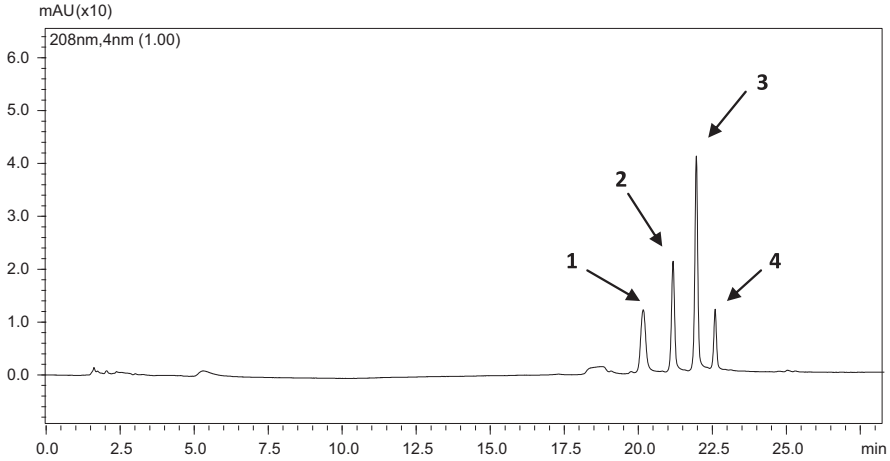

Fig. 1. The Ultimate XB-C18 column (150×4.6 mm^2, 5 μm) on a Shimadzu HPLC system (Kyoto, Japan) was used. Absorbance was set at 208 nm. The mobile phase solvent A was water and solvent B acetonitrile (ACE) with a flow rate of 0.5 ml/min and an injection volume of 10 μL. The gradient was set at 10–10% ACE (0–5 min), 10–85% ACE (5–60 min) and 85–85% ACE (60–65 min). The chromatographic profile of CMARE produced four (4) major peaks (1–4) having area ratios of 1.7:1.8:3.2:1.