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Methanolic extract of *Vernonia amygdalina* may protect against experimental benign prostatic hyperplasia in an albino Wistar rat model

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ABSTRACT

The effect of methanolic extract of Vernonia amygdalina (MEVA) on experimental benign prostatic hyperplasia (BPH) was investigated. Thirty male albino Wistar rats aged between 9 and 12 weeks were divided into 5 Groups of 6 rats each. Group I (standard control) received the basal diet, Group II was given 500 mg/kg bw MEVA dissolved in olive oil orally and a subcutaneous injection of an equivalent volume of olive oil (Hormone vehicle), Group III and IV received 500 mg/kg bw and 100 mg/kg bw of MEVA, respectively and subcutaneous injection of 9 mg/kg bw dihydrotestosterone and 0.9 mg/kg bw estradiol valerate every other day for 21 days. Group V received olive oil orally, and subcutaneous injection of 9 mg/kg bw and 0.9 mg/kg bw estradiol valerate. Serum prostate specific antigen (PSA) concentration (ng/ml) increased significantly in Group V (1.53 ± 1.02) in comparison to Group I (0.20± 0.26), Group II (0.13±0.23), Group III (0.23± 0.21) and Group IV (0.27± 0.12). There was also a significant increase ($p<0.05$) in relative prostate weight of Group V animals in relation to other groups. Testes weight in Group II, III and IV decreased significantly ($p<0.05$) in comparison to Group I and V. These findings suggest a positive protective effects of methanolic extract of Vernonia amygdalina against BPH, in a rat model.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a non-malignant neo-plastic enlargement of the prostate gland, characterized by symptoms such as decreased urinary flow, increased pressure during urination, residual urine in bladder, frequent urination especially at night and high incidence of lower urinary tract infection. It is age- related and its prevalence rate is as high as 40% and 90% in men aged 50-60 or 80-90 years, respectively [2]. Conventional therapies for BPH include watchful waiting, surgical treatment, and administration of 5α-reductase inhibitors and α-adrenergic receptor blockers. Recent research is focusing on the use of phytotherapeutic agents as alternative treatment option in the prevention and management of this

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disease [22]. *Vernonia amygdalina* is a popular African medicinal plant of the family *Asteraceae* [11]. The leaves of this herb are green with characteristic odor and bitter taste [21]. It is drought tolerant and grows throughout tropical Africa particularly in South Africa, Zimbabwe, and Nigeria [13]. Research evidence suggest various therapeutic potentials of different solvent extracts of *V. amygdalina* with antioxidant and tumor suppressing properties [10, 12, 18, 23]. The plant has also been shown to exhibit potential to manage many chronic and malignant diseases including diabetes and prostate disorders [7]. Some peptides found in the aqueous extract of this plant, called edotides, showed cell growth inhibitory effect in prostate cancer cell line (PC-3). A positive effect of aqueous extracts of *V amygdalina* in the management and treatment of BPH in human subjects has also been reported in the literature [11]. The present study, therefore, was aimed to investigate the effect of methanolic extract of *V. amygdalina* on benign prostatic hyperplasia in albino Wistar rat model.

MATERIALS AND METHODS

Collection of plant materials

Fresh mature leaves of *Vernonia amygdalina* were obtained from the Forestry Research Institute of Agriculture, Abia State, Nigeria and were botanically identified at the Forestry Department of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The leaves were air dried to a constant weight and milled into fine powder in an electric blender.

Extraction preparation and administration

An extraction column plugged at the base with glass wool was set up and flushed with 99.8% methanol. A weighed quantity (170g) of the milled *V. amygdalina* leaf sample was packed into the column and saturated with methanol and allowed to stand overnight to facilitate percolation of the milled leaves in the methanol medium. The column was covered to prevent evaporation of the solvent. It was eluted by successive addition of methanol and collection of extract into a pre-weighed beaker at a slow flow rate. The solvent was evaporated in a water bath at 40^{0}C to get dried extract. The percentage yield of dried extract obtained from the extraction was ~28g. Extract was formulated and administered as high and low dose. High dose contained 500 mg/kg bw methanolic extract while low dose contained 100 mg/kg bw extract.

Animal housing/handling

A total of 30 male albino wistar rats aged 9-12 weeks were purchased from the animal breeding unit of University of Nigeria, Nsukka. They were housed in standard steel cages with plastic base and acclimatized for 7 days under humid tropical condition in the animal house of College of Natural and Applied Science, Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State. The rats were exposed to 12 hrs light/dark cycle and were given free access to clean tap water and commercial rat chow, purchased from Vital Feeds Nigeria Ltd.

Animal treatment and experimental design

Hormonal induction of BPH was carried out using a slight modification of the method described earlier. The rats were divided into 5 groups of 6 rats each. Group I served as standard control group and received only normal rat feed. Group II (MEVA control group) received 500 mg/kg bw MEVA and subcutaneous injection of olive oil (hormone vehicle) in place of hormone. Group III (high dose group) received 500 mg/kg bw MEVA and subcutaneous injection of 9mg/kg b.w. dihydrotestosterone and 0.9 mg/kg bw estradiol velerate. Group IV (low dose group) received 100 mg/kg bw MEVA and subcutaneous injection of the 9 mg/kg bw dihydrotestosterone and 0.9 mg/kg bw estradiol velerate. Group V (hormone control group) received olive oil (MEVA vehicle) in place of MEVA and subcutaneous injection of the 9 mg/kg bw dihydrotestosterone and 0.9 mg/kg bw estradiol velerate. The treatment lasted for 21 days in all the groups at the end of which all the rats were dazed and bled by cardiac puncture. Vital organs were excised and weighed. Relative organ weights were calculated as the ratio of organ weight to the body weight of each animal on the day of sacrifice. Ethical

guidelines for animal care and handling in biological experiments were strictly adhered to as proposed by NIH Publication. The Board of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike approved the protocol used in this study.

Prostate specific antigen (PSA) assay

PSA concentration in serum was determined using PSA kit supplied by Sytron Bioresearch, Inc. The principle of the method is based on the Microwell Enzyme Linked Immuno Assay as out lined earlier [4]. Briefly, a working dilution of washing buffer was prepared by mixing 30 ml of buffer in 270 ml of distilled water. Microwell representing standard, controls and samples were placed in the well holder. A volume of 50 µl of each reference standards (0.0, 1.0, 2.5, 5.0, 12.5, 25.0, 50.0), control (level I and level 2) and test samples were dispensed into the appropriate wells. A volume of 50 µl of enzyme conjugate was added to all the wells except substrate blank before rocking the wells for about 20 sec. The wells were sealed with a sealant film and incubated at 37^{0}C for 60 min. The sealant film was removed and the incubation mixture decanted before washing the wells with diluted washing buffer 5 times. The wells were properly dried by firmly tapping with a clean soft paper. A drop of substrate reagent A and substrate reagent B was added to all the wells including substrate blank and gently rocked for 20 sec before incubating at room temperature for 15 min. A drop of stop solution was added to each well and gently mixed together. The absorbance of each well was read at 450 nm against substrate blank.

Statistical analysis

Descriptive statistics analysis was performed on the data generated using a computer software (SPSS version 17) and the significance of differences between the means were compared at $p<0.05$.

RESULTS AND DISCUSSION

Effect of MEVA on prostrate specific antigen (PSA) concentration in the experimental animals is shown in (Fig 1). A significant increase ($p<0.05$) in PSA value was observed in group V animals in comparison to groups III, II and I. However, there was no significant difference ($p>0.05$) between PSA value of group V, and group IV (low dose test group). Also there was no significant difference between the two control groups (group I and II) and the high dose test group. Mean relative prostate weight (Fig 2) increased significantly in group V in relation to all the other groups. Mean relative prostate weight was lowest in group II. Group I (standard control) had a significantly higher mean relative testes weight (Fig. 3) compared to all the other groups. However, the mean relative testes weight of all the other groups were not significantly different Cummulative results of our study have indicated that methanolic extract of *V. amygdalina* leaves

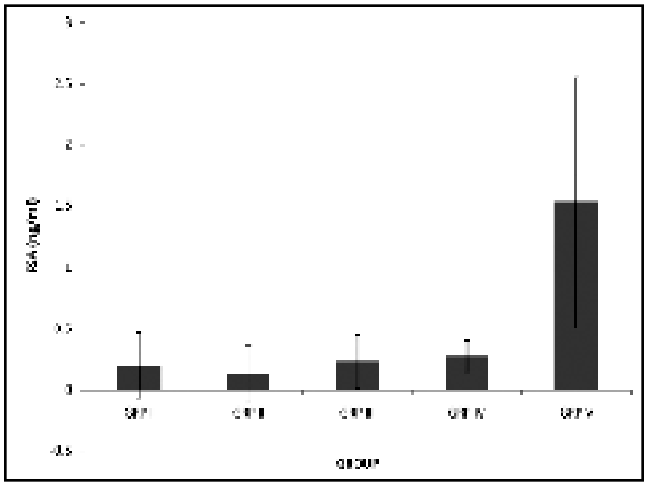

Figure 1: Serum prostate specific antigen concentration of control and test animals administered with MEVA along with hormonal induction.

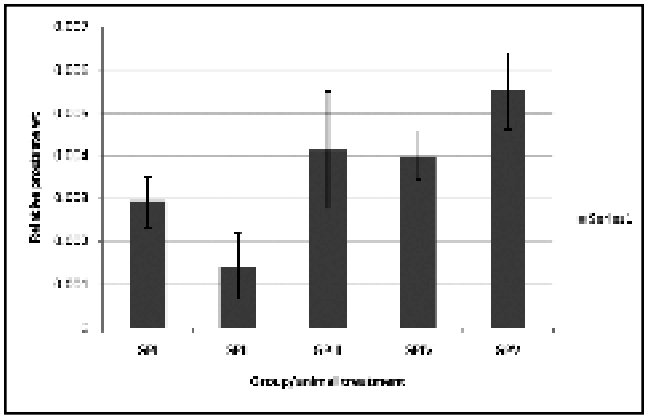

Figure 2: Mean relative prostate weight of control and test animals administered with MEVA along with hormonal induction.

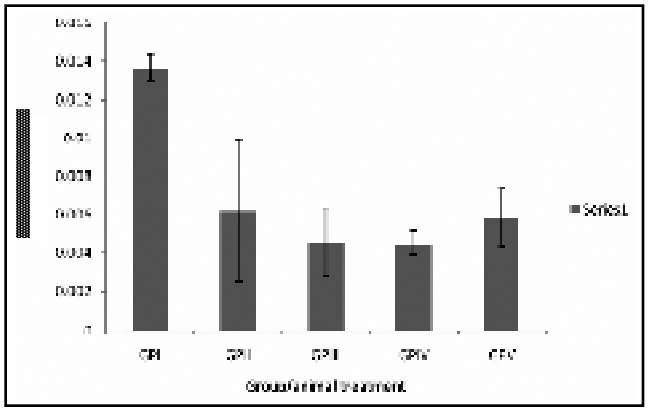

Fig 3: Mean relative testes weight of control and test animals administered with MEVA along with hormonal induction

possessed marked protective effect against BPH that is consistent with an earler report [11] wherein it was indicated that bioactive components of this herb may be present in its methanolic fraction. Symptoms of BPH are known to arise due to static and a dynamic components. The static component represents hyperplasia as a result of the activities of androgens and estrogens [14]. The effect of androgens on the prostate is mediated by testosterone and the more potent prostatic androgen, dihydrotestosterone [15, 25]. The conversion of testosterone to dihydrotestosterone in the peripheral tissue by 5α-reductase activity is thought to be the key event of BPH [17]. This contention is supported by two main evidences, viz; the reduction in prostate size following treatment with 5α-reductase inhibitors in BPH patients [1] and pseudohermaphroditism of 5α-reductase deficiency or androgen receptor abnormalities associated with lack of prostate development in fetal and adult life [9]. This implies that methanolic extract of *V. amygdalina* may confer protection against BPH since there was significant reduction in relative prostate weight of all the groups treated with MEVA. But we speculate that this effect of MEVA on relative prostate weight may not be through the 5α-reductase axis inhibition since exogenous hormone was used, thus bypassing the need for 5α-reductase activity. The reduction in PSA values of all the groups treated with MEVA was yet another indication of the protective effect of MEVA against BPH since cellular proliferation (hyperplasia) was accompanied by increased cellular secretion. This is especially true when high PSA concentration was used as a biochemical marker and clinical diagnosis of the presence of BPH. These observation also suggested that the reduction in PSA could have resulted from reduced prostatic cell proliferation in the groups treated with MEVA. We believe that MEVA could have produced this effect through the estrogen receptor axis. Estrogens have been reported to exhibit dual action on prostrate glands in males. Estrogen can have a direct and indirect effect on epithelial cell differentiation and proliferation [6, 8, 9] and thus, playing a role in the static component of BPH. It has also been reported that androgens plus estrogen can produce greater prostatic growth than androgens alone [5]. Although, estrogens acting locally through estrogen receptor alpha (ERα) within the prostatic stroma, can also stimulate aberrant epithelial cell differentiation and proliferation, leading to squamous metaplasia [19, 20]. In another study was suggested that anti-proliferative action of estrogen occurs through activation of epithelial ERβ [24]. Thus it is likely that observed effect of MEVA in the present study were mediated through estrogen receptors. This possibility is supported by the findings of an earlier study in which ERβ was shown to be a key factor in the regulation of prostatic epithelial proliferation and growth [16]. It is therefore possible that MEVA in our study may have acted preferentially stimulating ERβ over ERα. This, however, need to be further investigated.

The reduction in relative testes weight of all the groups treated with MEVA observed by us may have resulted from feedback inhibition of testosterone secretion in the leydig cells of the testis since exogenous hormone was administered. Androgens are required for normal growth and functional activities of the human prostate. In men, the major circulating androgen is testosterone T. Testes produce more than 95% and the adrenal gland less than 5% of this sex steroid [5]. In both the tissues the δ5 synthetic pathway which results in androstenedione and testosterone production is the predominant pathway, whereas the δ4 pathway leading to the synthesis of dehydroepiandrosterone (DHEA) and

androstenediol is the minor route [5]. Once synthesized, most of DHEA is inactivated via sulfation while a small fraction of this weak androgen is converted to androstenedione and then to testosterone in the peripheral tissues and in the prostate [3]. Testicular steroidogenesis in the Leydig cells is regulated primarily by the gonadotropin LH, whereas the adrenal androgen production is under the control of ACTH. Therefore, inhibition of endogenous testosterone synthesis by exogenous administration of hormone is resulted in decreased testes weight in this study as organ size is related to its secretory activity. Although this inhibition may have resulted from exogenous hormone administration through feedback inhibition, it is possible that MEVA may have also contributed to this as group II animals without hormonal induction showed a marked decrease in relative testes weight when compared with Group I standard control. Also, the two test groups (group III and group IV) showed more decrease in the relative testes weight when compared to group V (Fig 3).

CONCLUSION

These findings of present investigation clearly suggest that methanolic extract of *V. amygdalina* have chemo-protective effect against BPH. However, with the current global interest in phytotherapeutc management of BPH, more research is needed on this subject to unveil the bioactive component(s) of *V. amygdalina* responsible for the observed effects and also the possible mechanisms through which their actions are mediated.

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