Brazilian Journal of Biological Sciences, 2017, v. 4, No. 7, p. 45-52.
ISSN 2358-2731
https://dx.doi.org/10.21472/bjbs.040706

Aqueous extract of bitter leaf *Vernonia amygdalina* Delile (Asterales: Asteraceae) ameliorates testosterone-induced benign prostatic hyperplasia (BPH) in Wistar rats

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Abstract. Benign prostatic hyperplasia (BPH) is an age-dependent condition involving prostate enlargement which may lead to lower urinary tract symptoms (LUTS). This study was designed to study the ameliorative effect of the aqueous extract of bitter leaf *Vernonia amygdalina* Delile (Asterales: Asteraceae) on testosterone-induced BPH in a rat model. Thirty adult male Wister rats were randomly divided into six groups (A to F), with BPH induced through a single subcutaneous injection of 5 mg/kg of testosterone propionate (TP) and *Vernonia amygdalina* administered in various doses through oral gavage for 14 days. Group A (control) was administered with distilled water only, group B with 5 mg/kg of TP only, group C with 5 g/mL of *V. amygdalina* only, group D with 5 mg/kg of TP and 5 g/mL of *V. amygdalina*, group E with 5 mg/kg of TP and 7.5 g/mL of *V. amygdalina* and group F with 5 mg/kg of TP and 10 g/ml of *V. amygdalina*. Results show that testosterone caused histological changes that are similar to the presentation of BPH in the prostate. Aqueous extract of *V. amygdalina* was observed to ameliorate testosterone-induced histological changes and prostatic parameters especially at higher concentration. This suggests that bitter leaf could be a candidate herb for the treatment of BPH.

Keywords: Benign prostatic hyperplasia; Testosterone; Bitter leaf; *Vernonia amygdalina*; Prostate gland.

Received
March 8, 2017

Accepted
June 24, 2017

Released
June 30, 2017

Open Acess
Full Text Article

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Introduction

Benign prostatic hyperplasia (BPH) is an age-dependent, non-malignant condition that is characterised by prostate enlargement, which may lead to a plethora of urinary problems known as lower urinary tract symptoms (LUTS) (Chan, 2011). Prostate enlargement occurs through the rapid increment of the smooth muscle and epithelial cells within the transition zone of the prostate which results in the compression of the urethra, thus resulting in restricted urine flow (Kapoor, 2012). BPH has significant effect on the quality of life and is common among men over 50 years of age (Chou, 2007). Its prevalence shows that between the ages of 50-60 years, 50% of men are affected while 90% of men above 80years old have this condition (Chan, 2011). It has also been observed that the chances of prostate cancer occurring in males increase with age similar to that of BPH (Suzuki, 2009).

Vernonia amygdalina Delile, commonly called bitter leaf belongs to the family Asteraceae, tribe Vernonieae and it is widely distributed in Nigeria (Hutchinson and Dalziel, 1963). The plant is a perennial shrub or small tree, usually cultivated for its leaf as vegetable, medicinal, traditional and domestic uses. The leaves have a very bitter taste, due to its chemical contents (Lasekan et al., 1998), which are responsible for its medicinal and anti-microbial properties (Rice-Evans et al., 1996; Okoh et al., 1995). Herbal preparations made from leaves of bitter leaf are used in curing ailments such as malaria, measles, dysentery, onchocerciasis, yellow fever, constipation, stomach pain, etc, while slender roots and stem branches of the plant are used as chewing stick that are very effective in dental care (Oluwalana and Adekunle, 1998). Herbal preparations of various forms are prepared from different parts of bitter leaf plant, but the easiest form is the fresh leaf extract, which is prepared by squeezing the leaves in water. The leaf extract is usually taken raw by people at an unregulated rate, depending on the severity of the ailment (Eisenberg et al., 1993). Bitter leaf is probably the most used medicinal plant in the genus *Vernonia*. Bitter leaf extracts may help suppress, delay or kill cancerous cell in many ways, such as induction of apoptosis as determined in cell culture and animal studies, enhance chemotherapy sensitivity, inhibition of the growth or growth signals of cancerous cells, suppression of metastasis of cancerous cells in the body by the inhibition of an anti-apoptotic transcription factors as demonstrated in animal studies and reduction of oestrogen level in the body by the suppression of aromatase activity (Erasto et al., 2006).

The plant, bitter leaf, is a multipurpose plant that has a lot of potential valuable uses yet to be harnessed by the rural population in Nigeria. The objective of this study is to investigate the effect of bitter leaf extract on hormone-induced BPH in rats.

Materials and methods

Chemical and reagent

Testosterone propionate injection was procured from Kuzak Pharmaceutical Store opposite Good Shepherd Hospitals, Anyigba, Kogi State, Nigeria.

Collection and preparation of bitter leaf extract

The plant *Vernonia amygdalina* (bitter leaf) was harvested from the Faculty of Agricultural Sciences Garden in Kogi State University, Anyigba. The gathered fresh *Vernonia* leafs were weighed in the histology laboratory, Department of Anatomy, and was grinded into powder before filter. The powder was further dissolved in 20 mL of distilled water, packaged in various containers and kept in the refrigerator at 4 °C.

Animals

Thirty (30) adult male Wistar rats weighing about 120 g-180 g were obtained from the Animal Unit of Physiology Department, Kogi State University. They were acclimatized for one week and exposed to free access of food and water *ad libithum* at standard laboratory condition of light and temperature. They were kept in a well partitioned, six-room wooden cage with iron mesh doors.

Experimental design

A total of 30 rats were divided into 6 groups ('A' to 'F') of 5 animals each by random selection. Group A was the placebo group, which was fed with vital feed in pellets and distilled water. Group B, apart from normal feeds, was administered with 5 mg/kg testosterone propionate (TP) only, aseptically by subcutaneous injection. Group C was given 1.5 mL bitter leaf only at a concentration of 5 g/mL. This was administered by oral gavage. Group D, E and F were given both testosterone (5 mg/kg) and 1.5 mL bitter leaf at 5 g/mL 7.5 g/mL and 10 g/mL, respectively. Animals' body weights were measured on the first day of the experiment (day 1), day 10 and on the last day (day 14).

Table 1. Treatment administration to experimented animals.

Group	A	B	C	D	E	F
Feed	Vital feed and distilled water	Vital feed and distilled water	Vital feed and distilled water	Vital feed and distilled water	Vital feed and distilled water	Vital feed and distilled water
Treatment	Placebo group	Testosterone propionate only	Bitter leaf only	Bitter leaf and testosterone	Bitter leaf and testosterone	Bitter leaf and testosterone
Testosterone administration	Nil	5 mg/kg	Nil	5 mg/kg	5 mg/kg	5 mg/kg
Bitter leaf administration	Nil	Nil	1.5 mL (100g)	1.5 mL (100g)	1.5 mL (150g)	1.5 mL (200g)
Concentration of bitter leaf	Nil	Nil	5 g/mL	5 g/mL	7.5 g/mL	10 g/mL

Animal sacrifice

All animals (group A-F) were sacrificed on the 14th day of the experiment by cervical dislocation. The prostate gland was harvested, carefully weighed in all the groups and immediately fixed in 10% formal saline as a preservative of choice for histological purpose. Blood sample was also collected by means of cardiac puncture.

Results

Weight changes in rats at the end of the experiment are presented in Table 2. Analysis of the weight changes (Table 3 e 4) shows that on day one, the control group (group A) had the lowest weight of 98.00±6.83 g while group E had the highest weight of 141.00±11.92 g. Group E animals were administered with 2 mL of testosterone and 7.5 g/mL of bitter leaf.

However, the weight of group E animals was not significantly different from those of groups D and F which received 5 g/mL and 10 g/mL of bitter leaf respectively. On the last day of the experiment (day 14), weights of the rats still followed the same trends, with group E animals having the highest weight of 163.50±9.81 g, followed by group D (150.75±19.28 g), which got 2 mL of testosterone and 5 g/mL of bitter leaf and group F (146.25±5.90 g), which got 2 mL of testosterone and 10 g/mL of bitter leaf. The control rats (group A) still had the least weight of 98.50±7.42 g on the last day. Weights of rats on day 14 across the groups are significantly different except between group B (128.75±5.25 g), which got 2 mL of testosterone only and group C (131.00±6.68 g), which got bitter leaf only.

Table 2. Weight changes in Wistar rats.

	Group A	Group B Testosterone only	Group C Bitter leaf only	Group D Testosterone+ Bitter leaf	Group E Testosterone+ Bitter leaf	Group F Testosterone+ Bitter leaf
Day 1	97	112	123	157	157	139
	101	110	148	136	136	121
	105	126	126	129	129	150
	89	114	118	142	142	115
	92	113	119	144	143	113
Day 10	100	126	126	130	158	138
	89	124	125	170	178	150
	107	129	134	164	157	146
	98	136	139	139	141	151
	102	135	133	131	151	140
Day 14	105	138	128	162	145	135
	116	125	122	131	150	156
	121	123	140	166	163	134
	102	125	125	166	145	142
	105	124	124	164	150	151

Table 3. Weight of prostate gland across groups.

Group A		Group B		Group C		Group D		Group E		Group F	
A/W	P/W	A/W	P/W	A/W	P/W	A/W	P/W	A/W	P/W	A/W	P/W
105	1.12	138	0.41	128	0.16	162	0.91	145	0.50	135	0.32
116	0.12	125	0.51	122	0.16	131	0.62	150	0.39	156	0.43
121	0.13	123	0.49	140	0.17	166	0.49	163	0.51	134	0.32
102	0.12	125	0.51	125	0.16	166	0.46	145	0.50	142	0.42
105	0.12	124	0.52	124	0.16	164	0.46	150	0.32	151	0.40
110	0.12	127	0.36	128	0.16	158	0.59	151	0.44	144	0.38

Group A = Placebo, Group B = Testosterone, Group C = Bitter leaf only, Group D = Testosterone + Bitter leaf, Group E = Testosterone+Bitter leaf, Group F = Testosterone + Bitter leaf, A/W = Animal weight, P/O = Prostate organ.

Table 4. Effect of bitter leaf on prostatic parameters.

Groups	Animal weight at day 1 (g)	Animal weight at day 14 (g)	Mean prostate specific antigen (PSA) (mg/mL)	Prostrate weight (g)
A	98.00±6.83^{c}	98.50±7.42^{d}	0.912±0.02^{c}	0.122±0.004^{c}
B	115.75±7.14^{b}	128.75±5.25^{c}	0.360±0.01^{f}	0.488±0.449^{b}
C	117.00±9.49^{b}	131.00±6.68^{c}	0.419±0.02^{e}	0.162±0.045^{c}
D	128.8±13.25^{ab}	150.75±19.28^{ab}	1.471±0.43^{a}	0.588±0.192^{a}
E	141.00±11.92^{a}	163.50±9.81^{a}	1.169±0.02^{b}	0.444±0.085^{b}
F	131.25±16.13^{ab}	146.25±5.90^{b}	0.431±0.20^{d}	0.378±0.540^{b}
LSD	6.14	9.24	0.19	0.08

A - Control, B - Testosterone, C - Bitter Leaf, D - Testosterone (2 mL) + Bitter Leaf (5 g/mL), E - Testosterone (2 mL) + Bitter Leaf (7.5 g/mL), F - Testosterone (2 mL) + Bitter Leaf (10 g/mL), LSD-Least significant difference.

The prostate specific antigen (PSA) values when analysed show that group D rats had the highest PSA of 1.471±0.43 mg/mL, which was significantly higher than the rest. This is followed by group E rats (1.169±0.02 mg/mL) while group B had the least PSA of 0.360±0.01 mg/mL. Similarly, group D rats showed the highest prostate weight of 0.588±0.192 g which was significantly different ($p < 0.05$) from the other groups. Following after group D in terms of prostate weight are group B (0.488±0.449 g), group E (0.444±0.085 g) and group F (0.378±0.540 g).

Histological analysis shows that animals in group A (placebo) as well as group C appeared to have normal histological features of the prostate. Group B showed glandular hyperplasia and nodulas which are papilated and thrown into folds in foci. These are typical features of BPH. Group D with low dose of 5g/ml of bitter leaf was observed to present with the most severe degeneration of prostate tissue, whereas group E showed moderate hypertrophy of the glands with inflammatory cell infiltrates. Group F showed unremarkable glandular epithelia and stroma of the prostate.

Discussion

BPH affects ageing men and there is need to find alternative therapies, especially in the face of an increasing ageing population that may be averse to surgery. Plant and dietary sources are potential elements in the management of BPH as shown in this study. Aqueous extract of bitter leaf was administered in three regimes; low, intermediate and high dose which corresponds to 5g/ml (group D), 7.5g/ml (group E) and 10g/ml (group F) of aqueous extract of bitter leaf. Results indicate the highest PSA level was recorded in animals that got low dose of bitter leaf. This suggests that aqueous extract of bitter leaf might have some effect on PSA levels in rats. Although PSA levels may not be an indicator for the development of BPH, high PSA levels in man might be indicative of prostate cancer (Onyechi et. al., 2012).

Structurally, the most severe histological damage to the prostate gland, sequel to testosterone-induced BPH, was witnessed in animals taking low dose of bitter leaf. Degeneration of glands, epithelia and stroma of prostate tissue was massive in the low dose group, whereas the

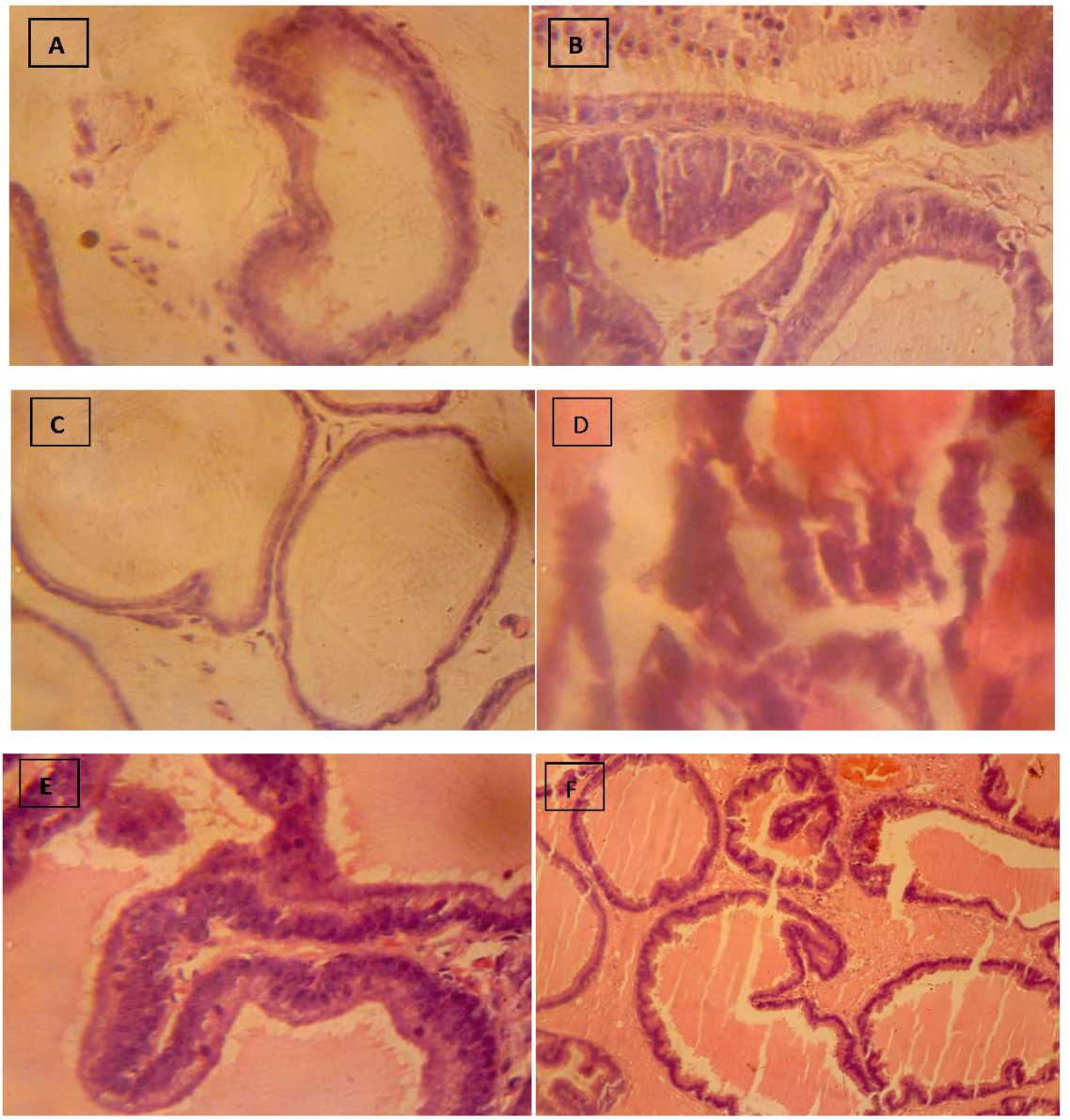

Figure 1. Histology of prostate gland. Prostate gland showing in group (A) normal glandular histology, group (B) glandular hyperplasia and nodulas accompanied by atrophy of the intervening fibromuscular stroma of the glands, group (C) normal histology, group (D) degenerated and hypertrophic structure, group (E) moderate hypertrophy with inflammatory cell infiltrate and group (F) moderate hypertrophy. H & E stain. Magnification 40x.

intermediate group showed moderate hypertrophy of the glands with inflammatory cell infiltrates, an indication of recovery. The high dose group showed unremarkable glandular epithelia and stroma distortion and prostate weight in this group is lower than the those of intermediate and low dose groups, the former being the highest. This observation suggests that bitter leaf could have ameliorating effect on BPH in a dose-dependent manner. This is consistent with previous reports that found bitter leaf to have (Onyechi et. al., 2012). Bitter leaf contains a number of compounds, which include proteins, fibre, carbohydrate,

chlorophyll, hemicellulose, ascorbic acid, carotenoid, calcium, iron, phosphorus, potassium, sulphur, sodium, manganese, copper, zinc, magnesium and selenium, stigmastane-type saponins, steroidal saponins, sesquiterpene lactones, flavonoids, terpenese, coumarins, phenolic acids, lignans, xanthones, anthraquinones and peptides (Okoli et al., 2007; Eleyinmi et al., 2008). Although the particular compound that may be responsible for the effect of the aqueous extract of bitter leaf on BPH remains unknown, we speculate that phytoestrogens might have played a role. Phytoestrogens have been said to be beneficial in the management of BPH due to their affinity for estrogen beta receptor (Abraham and Ajayi, 2014) and they are present in bitter leaf in form of lignans and flavonoids. Moreover, sesquiterpene lactones contained in bitter leaf may suppress aromatase activity thereby reducing the level of oestrogen in the body (Erasto et al., 2006). Apart from the beneficial effects bitter leaf has on the prostate, as shown by this study, the plant has also been shown to significant antiparasitic, antibacterial, platelet anti-aggregating and cytotoxic activity (Ijeh and Ejike, 2011). Hence, *Vernonia amygdalina* has a lot of medicinal uses.

Although surgery remains the most effective treatment for cases of severe symptoms BPH, pharmacological treatment can also be used to treat or manage severe symptoms cases (Dhingra and Bhagwat, 2011). Aqueous extract of bitter leaf may therefore complement standard pharmacological treatment and possibly used as prophylactic.

Conclusion

Bitter leaf is beneficial for the treatment of enlarged prostrate (BPH) in rat, especially at high concentration. Hence, hormone-induced prostate enlargement in adult male may be reduced by oral administration of fresh bitter leaf extract. It is recommended that prophylactic use of bitter leaf for BPH be studied as well as clinical trials, to validate the efficacy of bitter leaf extract in the treatment of enlarge prostate in man.

Conflicts of interest

Authors declare that they have no conflict of interests.

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