Effect of *Alliumcepa* (Onion) bulb and *Annonamuricata* (Soursop) pulp juice on body weight, prostate weight, selected hormones and prostate-specific antigen (PSA) in testosterone and oestradiol-induced benign prostatic hyperplasia albino rats.

ABSTRACT

Aim: This study investigated the effect of soursop pulp and onion bulb juice samples on body weight, prostate weight (PW), percentage prostate increase inhibition (PPII), dihydrotestosterone (DHT), testosterone (T), prolactin and PSA levels intestosterone and estradiol-induced BPH in albino rats.

Study design: The simple random design.

Place and Duration of Study: Dave Research House, Federal University of Technology, Owerri, Nigeria, between May 2022 and July 2022.

Methodology: Fifty-four (54) male albino rats were randomly distributed into 9 groups (A-I) of 6 rats each, with B to I induced with 10 mg/kg b.w of exogenous hormones (1:1) every 2 days. They were treated as follows: A (normal control), B (BPH control), C (standard control), D (10 ml/kg b.w soursop), E (10 ml/kg b.w onion), F (10 ml/kg b.w, 1:1 soursop and onion), G (20 ml/kg b.w soursop), H (20 ml/kg b.w onion), and I (20 ml/kg b.w, 1:1 soursop and onion), for 28 days. The rats were weighed weekly. At the end of the treatment, they were sacrificed, prostate and serum samples collected.

Results: The results showed that at low dose, the body weights of the rats were maintained. The single samples at low dose improved PW and PPII better. DHT and T levels reduced in all test groups, and the standard control. The single samples reduced DHT levels with increase in concentration, while the soursop juice and combination samples decreased testosterone levels with increase in concentration. Prolactin levels significantly (p<0.05) reduced in the test groups and standard control when compared with the BPH control. While, PSA levels also decreased in the test groups, but were not significantly (p>0.05) different from the controls.

Conclusion: The study suggested that soursop pulp and onion bulb juices exerted ameliorative effects on BPH, especially individually, and at the low dose studied. Hence, their consumption by older males should be encouraged.

Keywords: Alliumcepa bulb,Annonamuricata pulp, Benign prostatic hyperplasia, Hormones, Juices

1. INTRODUCTION

Benign prostatic hyperplasia (BPH), also referred to as benign prostatic hypertrophy [1], is a disease condition that is common in older men. It is a progressive condition identified by an enlarged prostate accompanied by lower urinary tract symptoms (LUTS) [2]. According to Lukacz*etal*. [3] and Lepor [4], LUTS can be grouped into two; filling/irritative symptoms (nocturia, dysuria, and increased urinary urgency and frequency) and obstructive symptoms in voiding (poor stream, hesitancy, incomplete voiding, terminal dribbling, and overflow incontinence). The pathophysiology of BPH is still not fully understood, and several pathways/mechanisms appear to be implicated in the onset and progression of BPH. Although, aging is the primary mechanism involved, research has demonstrated the critical involvement of hormonal changes/alterations, metabolic syndrome, and inflammation [5]. Prostate weight increases with age [6], while DHT, testosterone and prolactin has been noted to increase prostate volume [7][8].

During the treatment of BPH, the chemotherapeutic agents used in are mainly the alpha blockers and the 5 α-reductase inhibitors. The alpha blockers relax prostate and bladder neck muscles allowing easy flow of urine, while the 5 α-reductase inhibitors stop further enlargement or even causes shrinking of prostate. These drugs have side effects. The alpha blockers produce dizziness, fainting,

headaches, light-headedness, low blood pressure and occasionally, retrograde ejaculation, while the 5 α-reductase inhibitors produce low libido, erectile dysfunction, as well as, retrograde ejaculation, as side effects. More than 42 % of men between the ages of 51 to 60, 70 % of men between 61-70 years of age and as many as 90 % of men from 80 and above are affected by BPH [9]. LUTS adversely affects the quality of life of BPH patients. BPH still has no known medicinal cure. The primary goal of BPH management has been to provide relief-treatment for the symptoms of the condition. However, it is very common for the condition to reoccur once treated. Hence, there is pressing need to discover and develop phytotherapeutic agents with curative effect on BPH and related diseases.

Medicinal plants are one of the major source of complementary and alternative medicine. Phytotherapeutic agents are widely perceived to be more cost-effective, safer and have fewer side effects compared with conventional therapy for the management of ailments. This belief is responsible for the growing interest in their development [10][11]. *Alliumcepa* Linn (Onion) is the most cultivated member of the genus *Allium*, native to Europe, Asia, North America and Africa. After tomatoes, it is the second most significant horticultural crop, with current production being around 44 million tonnes, worldwide. Onion is a common ingredient in many dishes. It is increasingly consumed due to its flavour and health benefits. Onions are rich in flavonoids and the alk(en)yl cysteine sulphoxides (ACSOs), which are beneficial to human health. The two flavonoid subgroups; anthocyanins and flavanols such as quercetin and its derivatives, found in onion play major role in its antioxidant activity [12]. Research has reported that compounds from onion possess anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic and antibiotic effects [13].

Annonamuricata L. (soursop or graviola) is a member of the Annonaceae family comprising about 130 genera and 2300 species [14]. It is native to the warmest tropical areas in South and North America and now widely distributed throughout tropical and subtropical parts of the world, including India, Malaysia and Nigeria [15]. *A*. *muricata* is an erect evergreen, terrestrial tree that can grow to a height of 5-8m, featuring an open roundish canopy with large, glossy, dark green leaves. During fruiting season, the tree produces edible fruit that are large, heart shaped and green in colour, with diameter ranging between 15- 20cm [16]. In herbal therapy, phytochemicals from soursop plant have a long history of use. Cancer, bacterial or parasitic infections, fever, high blood sugar, high blood pressure, inflammation, and anxiety, are a few of the conditions they are used to treat. Various scientific research results have shown that soursop extract contains antibacterial, antiprotozoan, anti-inflammatory, antioxidant, and anticancer effects [17].

This study reports the effect of *Annonamuricata* (Soursop) pulp and *Alliumcepa* (Onion) bulb juice samples on body weight, prostate weight (PW), percentage prostate increase inhibition (PPII), and serum levels of DHT, testosterone, prolactin and prostate-specific antigen (PSA) intestosterone and estradiol-induced BPH in albino rats.

2. METHODOLOGY

2.1 Sample Collection, Identification and Preparation

Bulbs of common onion and fresh ripe fruits of soursop were purchased at Relief Market, Owerri, Nigeria. They were identified by Dr. C. M. Duru, a taxonomist in the Department of Biology, Federal University of Technology, Owerri, Nigeria, with authentication numbers; FHI 110177 (*Annonamuricata* L.) and FHI 107561 (*Alliumcepa* L.). They were cleaned and washed with clean running water. The soursop fruit was cut open to collect the pulp, and remove all the seeds. The onion was cut into smaller pieces. The onion and soursop pulp were blended separately. They were then filtered with muslin cloth and the filtrates collected into separate specimen bottles for use and stored at 4 ^{o}C every other day as these samples were prepared every 2 days.

2.2 Animal Handling

A total of 54 male albino rats of Wistar strain were used for this study. The rats were acclimatized in different cages for 14 days. The simple random sampling technique was used in the grouping. The animals were maintained under standard environmental conditions (12 h light and 12 h dark cycle, uniform temperature of 28 ± 5 °C), with free access to food (Vital Feed Finisher Pellets, Ibadan) and

water. All investigations were conducted in accordance with the accepted principles for animal care and use [18].

2.3 Induction of Benign Prostatic Hyperplasia

The BPH was induced by administration of 10 mg/kg b.w of exogenous testosterone (CAS 57-85-2) and oestradiol (CAS 979-32-8) in the ratio of 1:1 intraperitoneally (every 2 days) for four weeks. The steroids were diluted in olive oil as solvent [19].

2.4 Animal Grouping and Treatment Administration

Chart 1 :The grouping and treatment of the experimental animals were as follows

Groups	Number of rats	Treatment for 28 days
A (normal control)	6	Water and feed
B (BPH control)	6	BPH induced rats + solvent. (Untreated)
C (standard control)	6	BPH induced rats + Finasteride (standard drugs - CAS 98319-26-7) at 10 mg/kg bw through gavage.
D	6	BPH induced rats + 10 ml/kg bw of soursop juice orally and feed (10 ml/kg AM)
E	6	BPH induced rats + 10 ml/kg bw of onion juice orally and feed (10 ml/kg AC)
F	6	BPH induced rats + 1:1 combination of soursop and onion juices (10 ml/kg bw orally) and feed (10 ml/kg AMAC)
G	6	BPH induced rats + 20 ml/kg bw of soursop juice orally and feed (20 ml/kg AM)
H	6	BPH induced rats + 20 ml/kg bw of onion juice orally and feed (20 ml/kg AC)
I	6	BPH induced rats + 1:1 combination of soursop and onion juices (20 ml/kg bw orally) and feed (20 ml/kg AMAC)

Key:

AM - *Annonamuricata*

AC – *Alliumcepa*

AMAC - *Annonamuricata* and *Alliumcepa*

Treatments were administered daily for 28 d. At the end of the treatment, the rats were fasted overnight and euthanized by cervical dislocation. Blood was collected by ocular puncture into plain sample tubes and allowed to clot. Serum was separated within one hour of blood clotting with a Pasteur pipette after centrifugation (centrifuge, MSLZL09) at 3000 x g for 20 min. the serum samples were used for biochemical tests. The prostates were excised and rinsed in ice-cold 1.15 % KCl, blotted and weighed using an electronic weighing balance (SUN-224CL-220 Gm) [20].

2.5 Determination of Percentage Change in Body Weight

The body weights of the rats were measured using an electronic weighing balance (SUN-224CL-220 Gm) and recorded before commencement of experiment, and subsequently once a week till sacrificed [21].

The percentage change in weight (per week) was calculated as follows:

Final body weight – Initial body weight x 100 / Final body weight

2.6 Calculation of Percentage Prostate Increase Inhibition (PPII)

Percentage prostate increase inhibition = 100 – [(T – C)/(B – C) x 100]

Where C, B, and T are the prostate weights of the normal control, BPH control, and treatment group [22].

2.7 Biochemical Tests

Prostate Specific Antigen (PSA) level was measured using AccubindImmunoenzymometric assay (TYPE 3) test kit. Prolactin level was measured using Accubind, Immunoenzymometric sequential assay (TYPE 4) test kit. Testosterone level was measured using Accubind, Competitive Enzyme Immunoassay (TYPE 7) test kit. DHT was measured using enzyme-linked immunosorbent assay (ELISA) kits.

2.8 Statistical Analysis

Data were analyzed based on one-way analysis of variance (ANOVA) with Statistical Product and Service Solutions (SPSS) version 23. Results are expressed as mean ± SD and presented in tables. Differences between means were considered to be significant at *P* =.05 using Duncan multiple range test (DMRT).

3. RESULTS AND DISCUSSION

Table 1: Effect of *Annona muricata* pulp and *Allium cepa* bulb juices on body weight of albino rats with testosterone and oestradiol-induced BPH

Group	Body weight per week (g)				
	Initial weight	Week 1	Week 2	Week 3	Final weight (Wk 4)
NC	177.13 ± 19.13	196.31 ± 22.65 (9.70 ± 3.30^{c} %)	218.15 ± 26.18 (9.98 ± 1.18^{b} %)	235.18 ± 31.03 (7.13 ± 2.19^{d} %)	244.60 ± 47.56 (3.10 ± 6.47^{ab} %)
BPH	198.56 ± 18.26	200.04 ± 17.03 (0.78 ± 0.69^{a} %)	197.92 ± 13.50 (-1.00 ± 1.75^{a} %)	189.68 ± 20.61 (-4.67 ± 5.22^{a} %)	199.84 ± 15.48 (5.23 ± 3.28^{b} %)
SC	159.37 ± 1.98	166.52 ± 3.71 (4.24 ± 3.33^{abc} %)	167.23 ± 8.40 (0.18 ± 7.39^{a} %)	175.54 ± 8.38 (4.73 ± 1.59^{cd} %)	185.41 ± 9.70 (5.30 ± 1.51^{b} %)
10 ml/kg AM	148.47 ± 6.80	158.13 ± 5.21 (6.13 ± 1.67^{abc} %)	162.93 ± 3.20 (2.96 ± 1.94^{a} %)	168.55 ± 2.42(3.32 ± 2.42^{bcd} %)	176.42 ± 4.99 (4.40 ± 3.41^{ab} %)
10 ml/kg AC	140.26 ± 17.45	153.75 ± 17.52 (8.85 ± 1.04^{bc} %)	161.30 ± 16.61 (4.75 ± 1.16^{ab} %)	162.23 ± 18.73 (0.47 ± 1.56^{abcd} %)	165.46 ± 20.21 (1.89 ± 0.77^{ab} %)
10 ml/kg AMAC	151.68 ± 13.57	159.98 ± 19.32	167.43 ± 13.63	170.65 ± 11.97	176.06 ± 15.92

		(4.88 ± 5.22^{abc} %)	(4.62 ± 4.74^{ab} %)	(1.92 ± 2.70^{abcd} %)	(2.93 ± 3.33^{ab} %)
20 ml/kg AM	163.18 ± 9.27	171.36 ± 5.73 (4.83 ± 2.27^{abc} %)	172.70 ± 7.93 (0.73 ± 1.27^{a} %)	168.80 ± 10.42 (-2.44 ± 4.70^{ab} %)	165.88 ± 16.48(-2.06 ± 5.18^{a} %)
20 ml/kg AC	179.80 ± 26.23	188.88 ± 22.33 (4.99 ± 3.05^{abc} %)	185.65 ± 14.00 (-1.54 ± 4.71^{a} %)	180.57 ± 3.00 (-2.75 ± 6.01^{ab} %)	189.69 ± 9.98 (4.69 ± 3.41^{ab} %)
20 ml/kg AMAC	177.95 ± 17.18	184.29 ± 12.48 (3.54 ± 3.54^{ab} %)	182.58 ± 13.84 (-0.98 ± 1.27^{a} %)	182.13 ± 10.75 (-0.18 ± 1.86^{abc} %)	187.81 ± 7.26 (3.07 ± 2.54^{ab} %)

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 2: Effect of juices of *Annona muricata* pulp and *Allium cepa* bulb on prostate weight and percentage prostate increase inhibition of albino rats with testosterone and oestradiol-induced BPH

Group	Prostate weight (g)	Percentage prostate increase inhibition (%)
NC	2.18 ± 0.26^{a}	-
BPH	3.31 ± 0.35^{c}	-
SC	2.53 ± 0.21^{ab}	69.32 ± 40.15^{b}
10mg/kg AM	2.85 ± 0.66^{bc}	41.00 ± 35.33^{ab}
10mg/kg AC	2.77 ± 0.13^{abc}	48.08 ± 24.44^{ab}
10mg/kg AMAC	2.98 ± 0.07^{bc}	29.79 ± 23.15^{ab}
20mg/kg AM	3.25 ± 0.22^{c}	5.61± 28.81^{a}
20mg/kg AC	2.95 ± 0.58^{bc}	32.45± 34.25^{ab}
20mg/kg AMAC	3.00 ± 0.07^{bc}	27.73± 24.50^{ab}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 3: Effect of *Annona muricata* pulp and *Allium cepa* bulb juices on serum levels of dihydrotestosterone, testosterone, prolactin and PSA of albino rats with testosterone and oestradiol-induced BPH

Group	DHT (ng/ml)	Testosterone (ng/ml)	Prolactin (ng/ml)	PSA (ng/ml)
NC	8.02 ± 0.20^{bc}	2.30 ± 0.40^{a}	1.00 ± 0.30^{a}	0.20 ± 0.00^{ab}
BPH	10.50 ± 0.60^{d}	10.20 ± 0.70^{f}	1.80 ± 0.10^{b}	0.40 ± 0.10^{c}
SC	8.70 ± 0.50^{c}	6.40 ± 0.20^{b}	1.00 ± 0.10^{a}	0.40 ± 0.20^{c}
10mg/kg AM	9.10 ± 0.10^{c}	9.80 ± 0.50^{f}	0.90 ± 0.30^{a}	0.30 ± 0.10^{bc}
10mg/kg AC	8.10 ± 0.80^{bc}	7.30 ± 0.60^{c}	1.10 ± 0.10^{a}	0.30 ± 0.00^{bc}
10mg/kg AMAC	7.10 ± 1.20^{b}	8.30 ± 0.20^{de}	1.00 ± 0.30^{a}	0.40 ± 0.10^{c}
20mg/kg AM	5.30 ± 0.80^{a}	9.60 ± 0.10^{f}	0.90 ± 0.00^{a}	0.20 ± 0.10^{ab}

20mg/kg AC	7.20 ± 0.30^{b}	8.70 ± 0.00^{e}	1.00 ± 0.01^{a}	0.10 ± 0.00^{a}
20mg/kg AMAC	8.40 ± 0.20^{c}	7.90 ± 0.10^{cd}	0.80 ± 0.20^{a}	0.20 ± 0.10^{ab}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

The body weights of experimental animals determined weekly, is usually used as a general index of overall health [23]. For the body weight (table 1), the results indicated increase in the body weights of all the groups in week 1. It was noticed that there was a reduction in the body weight of albino rats in Group B (BPH control), and the high dose groups H (20ml /kg AC) and I (20 ml/kg AMAC) in week 2. Further reduction in the body weights of the experimental animals in these groups and Group G (20 ml/kg AC-high dose) was observed in week 3. While in week 4, an increase in the body weights of the experimental animals in all the groups was observed, except for Group G where there was further decrease in body weight. This indicated that at the low dose (10 ml/kg b.w) of the test samples; soursop juice, onion juice, and combination of soursop and onion juice, the body weights of the animal models increased steadily throughout the weeks of the study, in the same manner observed in the normal and standard controls (Groups A and C, respectively). The weight gain in the affected groups throughout weeks can be easily noticed with the positive values of percentage change in body weights per week. Hence, at low doses, the samples maintained the body weights of the albino rats.

In Table 2, the results showed that the PWs reduced in all test groups. Lower values were observed in the groups treated with low doses of the test samples, meaning that increasing the dosage of the test sample did not reduce the PWs any further. The results of PPII indicated that the low dose onion juice (10 ml/kg AC) showed better PPII among the test groups, followed by low dose soursop juice (10 ml/kg AM). Although, all the PPII values from the test groups were not significantly different from the standard control, except for that of high dose soursop juice (20 ml/kg AM). The results of this study agreed with the findings of Ogbu *etal*. [24], which stated that actogenin-rich fraction of *Annonamuricata* leaves extract reduced PW in treated rats.

Table 3 shows the results of the effect of juice samples of *Annonamuricata* pulp and *Alliumcepa* bulb on serum DHT, testosterone, prolactin and PSA levels of BPH induced albino rats. DHT and testosterone are androgens crucial for the growth and development of the internal organs of the human male reproductive system, and BPH [25][26][27]. The 5-α reductase enzyme catalyzes the conversion of testosterone to DHT. Due to its high affinity to the androgen receptors, DHT is the most potent androgen that promotes the growth of the epithelial and stromal cells of the prostate [7][28][29].

PSA is predominantly produced in the prostate gland. Benign prostatic hyperplasia is an age-related condition, hence, its occurrence increases with increase in age, as the prostate volume increases with age. This increase in prostate volume with age leads to increase in PSA levels [30]. Although, PSA values greater than 4.0 ng/ml is considered abnormal in men above 60 years of age, yearly increase in PSA levels greater than 0.35 ng/ml is alarming and requires further testing [31]. Prolactin has also been implicated in development of BPH, as it targets the prostate through interactions with the prolactin receptors expressed in the prostate [32]. Chronic hyperprolactinemia induces enlargement and inflammation of the lateral rat prostate [33]. High estrogen and prolactin levels also increase DHT levels and stimulate growth of prostate cells [8].

From the results of this study, a reduction in DHT and testosterone levels were observed in all test groups, same as the Standard control. It was also noted that the individual samples caused a reduction DHT levels with increase in concentration, while the Soursop juice and combination samples decreased testosterone levels with increase in concentration. The results for serum prolactin levels showed a significant ($p<0.05$) reduction in both the test groups and Standard control when compared with the Disease control. While, there was also an observed decrease in serum PSA levels in the test groups, however, all the values obtained for both tests and controls did not vary significantly ($p>0.05$). The increased values obtained in the BPH control further reiterated the discoveries of other researchers. The ability of soursop and onions in ameliorating BPH as observed in this study agreed with the findings of Hong *etal*. [34] on stem bark extracts of soursop and Elberry*etal*. [35] on red onions scales extract.

4. CONCLUSION

The results of this study suggested that soursop pulp and onion bulb juice exerts ameliorative effects on BPH. Better effects were achieved with the use of single samples and at the low dose studied.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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