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Anti-Inflammatory Potentials of Aqueous Soursop Pulp Extracts on Induced Benign Prostatic Hyperplasia in Adult Male Wistar Rats

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Abstract

Background*:** Benign Prostate Hyperplasia (BPH) is an age associated prostate gland enlargement in men that can lead to urination difficulty. The exact etiology of BPH is unknown. However, inflammation is a critical factor in the development of BPH. Soursop as a traditional medicine has anti-inflammatory properties. This study investigated the anti-inflammatory potentials of soursop pulp extracts on induced benign prostatic hyperplasia (BPH) in adult male wistar rats. ***Materials and methods: Twenty-four adult male rats grouped into five were used for the study. BPH was induced subcutaneously in the inguinal region with testosterone propionate 3mg/kg/day. Acute toxicity test was carried out to determine the safety of the extract. The rats in group A served as the control group. Rats in group B, C and D were administered 200mg, 400mg, and 600mg of aqueous soursop extract per kg body weight/day. Biochemical analyses of the protein specific antigen, albumin, white blood cell count, testosterone, and C-reactive protein were performed after the trial. Statistical analysis was done using SPSS version 23. ***Results:*** Aqueous soursop pulp extract significantly decreased ($p<0.05$) the protein specific antigen by 58.87%. The extract at 400mg resulted in a percentage decrease in white blood cell count by 35.37% and albumin by 31.23 %. The extract at 600mg significantly decreased the testosterone and C- Reactive Protein by 59.88% and 69.47% respectively. ***Conclusion*:** The inflammatory bio-markers (prostate specific antigen, albumin, white blood cell count, testosterone, and C-reactive protein) studied after treatment revealed that aqueous soursop pulp extract is promising in the management of benign prostate hyperplasia.

Keywords: Benign prostatic hyperplasia, inflammation, soursop pulp, extract, administered, bio-markers.

INTRODUCTION

Benign prostatic hyperplasia (BPH), is a histologic diagnosis that refers to the non-cancerous proliferation of smooth muscle and epithelial cells within the prostatic transition zone (Auffenberg *et al*., 2010). According to recent research, BPH's stroma or epithelial proliferation is influenced by chronic inflammation (Madersbacher *et al*., 2019). The prevalence of benign prostate hyperplasia (prostate enlargement) is increasing in both developed and developing countries. According to Nickel (2006), BPH affects more than 42% of men from age 51 to 60, 70% of men from 61-70 and as many as 90% of men in their 80s and above. As the population of ageing men increases, BPH has become an important topic of health concern (Ojewola *et al.*, 2016). Consequently, prostatic diseases are one of the major causes of enormous morbidity among men worldwide (Achukwu *et al*., 2017). BPH may also be involved in sexual functions and performance such as painful orgasms, pain in the testicles, difficulty achieving erection and painful ejaculation (Propert *et al*., 2016). In the

Furthermore, there is yet no medicinal cure for BPH and the related cancers (Doku, 2016). Management of benign prostate hyperplasia has been mainly to provide relief-treatment for the symptoms of the condition. Currently, there is widespread interest in developing phytotherapeutic agents for the management of benign prostate hyperplasia due to the perception that they are safer, more cost-effective and have fewer side effects than their conventional alternatives (Thompson *et al*., 2013).

Soursop, botanically known as *Annona muricata L*, a tropical fruit trees belonging to the Annonaceae family. The fruits are large, heart-shaped and dark green in color. *A. muricata* fruit consists of 80% water, 18% carbohydrates, 1% protein and small quantities of vitamins B_1, B_2, C and dietary fiber (Pier, 2014). The fruit also contains various significant minerals such as potassium, calcium, sodium, copper, iron and magnesium. Studies have linked *A. muricata* to a variety of anticancer effects including cytotoxicity and inhibition of proliferation on a variety of cancer cell (Rady *et al*., 2018). Due to its anti-proliferative effect on cancer cell, it is presumed that the plant can be used in the treatment of enlarged prostate. This study aimed to determine the anti-inflammatory effects of soursop pulp extracts on benign prostatic hyperplasia in male wistar rats.

MATERIALS AND METHODS

Procurement and preparation of samples

Fresh soursop fruits were plucked from a farmland located at 1 Mbonu Ojike Street, University of Nigeria, Nsukka, Enugu State. They were identified in the Harbarium Center of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Fresh ripped soursop fruits were washed, allowed to drain in a colander, cut open with a knife to scoop out the pulp and the seeds were handpicked. Fifty grams of the pulp was blended with 100mls of water in a household blender into a creamy liquid. The content was sieved with 1-mm mesh to obtain the extract.

Animal procurement

Twenty-four adult male Albino rats were procured from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The metabolic cages were washed with detergent and clean water, disinfected and left to air dry. The rats were housed individually at standard environmental conditions of temperature (27 +2^{o}C), humidity (55 +10%) with 12-hour light and dark cycle. All the rats were fed water and rat chow ad libitum for 4 days for acclimatization. After this, the rats were allotted to five groups of six rats each on the basis of body weight with difference in weights within each group not exceeding 5g.

Induction of BPH

Testosterone propionate (3 mg/kg body weight) was used to inject subcutaneously in the inguinal region for 7 days to induce proliferation. This proliferative stimulation with testosterone propionate resulted in the development of benign prostatic hyperplasia in rats (Shin *et al*., 2012). Successful induction of BPH was ascertained by testing prostate specific antigen (PSA) level of the rats on the 9th day.

Experimental Design

The study was conducted for thirty-four days consisting of four days of acclimatization, seven days for inducement of benign prostatic hyperplasia (BPH), two days for establishment of prostatic enlargement and twenty-one days on experimental diets. The male Wistar rats after acclimatization for a period of four days were rested overnight and evenly distributed into five groups of six rats each. The aqueous soursop pulp and leaf extract were fed to the rat groups in different concentrations. Group A containing 6 Prostate enlarged rats that were not treated, group B containing 6 Prostate enlarged rats fed with 200mg of aqueous soursop pulp extract /kg body weight daily, group C containing 6 Prostate enlarged rats fed with 400mg of aqueous soursop pulp extract / kg body weight daily, group D containing about 6 rats fed with 600mg of aqueous soursop pulp extract / kg body weight daily. All the rats received rat chow and water ad libitum for the period of the study. The route of administration of the soursop extracts was oral through the use of cannula.

Blood Sample Collection

Blood were collected twice for the determination of the presence of prostatic enlargement prognosis and inflammatory markers parameters; after BPH induction and after treatment (experiment). The rats were fasted overnight for ten hours. After fasting, the media cantus of the eye was punctured. Then drops of blood that appeared on the skin surface were collected in a capillary tube while a little pressure was applied to stop the bleeding. Blood was collected into EDTA and non-EDTA bottles and centrifuged at 3500 rpm for 10 minutes and the clear serum and plasma aspirated off respectively for biochemical evaluation.

Inflammatory bio-marker analysis

PSA was determined by using the quantitative sandwich enzyme immunoassay technique Antibody specific for PSA according to Nandecha (2010) method. Albumin was determined with Bromocresol green (BCG) method by Doumas *et al.*, (1971). Testosterone was determined using enzyme linked immunosorbent assay (ELISA) kit. White blood cell count was done using standard technique as describe by Ramnik (2003). C-reactive protein (CRP) was determined using Mark Wener (2007) Nephelometry method.

Acute toxicity (LD_{50}) test

The LD_{50} of the aqueous soursop pulp extracts were tested to determine the safety of the extract according to the guidelines set by Lorke (1983).

Statistical analysis

All data obtained were subjected to statistical analysis using SPSS version 23.0. All values obtained were expressed as means and standard deviation and data were analysed using analysis of variance (ANOVA) for separation of mean. Duncan Multiple range test was used to determine which experimental diet was significantly better in relation to the parameters tested at 5% probability level ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

Acute Toxicity Results

The acute toxicity result showed that there was no toxicity/death observed for the soursop pulp at doses less than or equal to 1000mg/kg body weight after 24 hours. However, the animal survived at higher doses of 1600mg/kg body weight while at 2900 and 5000mg/kg body weight death was observed. This implies that at extremely high doses, the sample can be toxic. This implies that the sample is safe for consumption and is non-toxic up to dose of 1000mg/kg body weight.

Prostate specific antigen (PSA) of the rats

Treatment with aqueous soursop pulp extract significantly decreased the PSA levels of the rats in treated groups when compared to the control group A (Table 1). Highest percentage decrease in PSA level occurred with 400mg of the test sample. Testosterone propionate induced BPH indicates the ameliorative impact of testosterone propionate induced BPH (Onyegeme-Okerenta *et al*., 2022). As a result of inhibiting 5-reductase, a decrease in PSA is linked to a reduction in BPH (Sing et al., 1991). The fact that the PSA level in the negative control group remained high indicates that the observed decline in PSA in treated rats was due to the role of aqueous soursop pulp in reducing the effect of PSA which is one of the factors that plays a crucial role in higher chance of developing BPH. This corroborates the findings of Ogbu *et al*., (2020) who discovered acetogenins rich fraction isolated from soursop plant reduced the levels of PSA in induced BPH rats.

Table 1: Mean prostate specific antigen (PSA) levels of the rats (miu/ml)

Groups	Baseline	End Value	End value – baseline	% diff.
A	1.53 + 0.28 ^{b}	2.32 + 0.32 ^{b}	0.79	51.63↑
B	1.35 + 0.42 ^{b}	0.71 + 0.01 ^{a}	-0.64	47.41↓
C	1.30 + 0.42 ^{b}	0.61 + 0.06 ^{a}	-0.69	58.87↓
D	1.08 + 0.17 ^{b}	0.68 + 0.13 ^{a}	-0.40	37.04↓

Values are expressed as Mean + SD of each rat group (n=6). Mean values with different letters as superscripts across the column are considered significant at $p<0.05$.
Baseline= after experiment, End value= after the study.
Group A =Induced and not treated.
Group B = Induced rats fed 200mg/kg bwt of aqueous soursop pulp extract.
Group C= Induced rats fed 400mg/kg bwt of aqueous soursop pulp extract.
Group D= Induced rats fed 600mg/kg bwt of aqueous soursop pulp extract.

White blood cell count of the rats

White blood cells (WBCs) are considered a valid biomarker of inflammation. Aqueous soursop pulp extract showed a reduction in WBC count of the rats. Percentage decline in WBC was observed with administered doses of soursop pulp extract with maximum reduction (35.73%) in group treated with 400mg/kg body weight (Table 2). Elevated levels of WBC have been linked to several chronic diseases (Libby *et al*., 2016). This was observed in control group A. The ability of the test groups to show a decrease in white blood cell count could be attributed to the anti-inflammatory properties of the extract.

Table 2: Mean total white blood cell count of the rats (x10 mm^3)

Group	Baseline	End value	End value -baseline)	% diff
A	16633.33 + 524.09^{b}	18016.67+1431.67^{c}	1383.34	8.32↑
B	16166.67 + 1458.31^{b}	12183.33+1062.86 ^{b}	-3991.61	24.69↓
C	16650.00 + 926.82^{b}	10700.00+389.87 ^{a}	-5950.00	35.73↓
D	13680.00 + 458.3^{b}	10575.00+289.87 ^{a}	-3105.00	22.70↓

Values are expressed as Mean + SD of each rat group (n=6). Mean values with different letters as superscripts across the column are considered significant at $p<0.05$.
Baseline= after experiment, End value= after the study.
Group A =Induced and not treated.
Group B = Induced rats fed 200mg/kg bwt of aqueous soursop pulp extract.
Group C= Induced rats fed 400mg/kg bwt of aqueous soursop pulp extract.
Group D= Induced rats fed 600mg/kg bwt of aqueous soursop pulp extract.

Albumin levels of the rats

Albumin concentration reflects the nutritional status of the experimental rats. The control group showed an increase in albumin level (hypalbuminaemia) in Table 3. Treatment with aqueous soursop pulp extract significantly decreased the albumin levels of the rats in treated groups when compared to the control group A. Highest percentage decrease in albumin level occurred with 400mg/kg body weight while the lowest percentage decrease was observed in 600mg/kg body weight of the test sample. Studies have shown that there is always a significant

decrease in albumin concentration in BPH condition, (Kappor, 2012). On the other hand, this study shows that the albumin concentration was properly managed in rats fed with the extracts.

Table 3: Mean albumin levels of the rats (mg/dl)

Group	Baseline	End value	End value –baseline	% diff
A	6.36 + 0.33 ^{b}	8.52+0.62 ^{d}	2.16	33.96↑
B	5.82 + 0.85 ^{b}	5.14 + 0.47^{c}	-0.68	11.68↓
C	5.89 + 0.46 ^{b}	4.05 + 0.79 ^{b}	-1.84	31.23↓
D	5.86 + 0.29 ^{b}	5.25 + 0.55 ^{b}	-0.61	10.40↓

Values are expressed as Mean + SD of each rat group (n=6). Mean values with different letters as superscripts across the column are considered significant at p<0.05.
Baseline= after experiment, End value= after the study,
Group A =Induced and not treated.
Group B = Induced rats fed 200mg/kg bwt of aqueous soursop pulp extract.
Group C= Induced rats fed 400mg/kg bwt of aqueous soursop pulp extract.
Group D= Induced rats fed 600mg/kg bwt of aqueous soursop pulp extract.

Testosterone level of the rats

Treatment with aqueous soursop pulp extract significantly lowered the testosterone level of the rats in treated groups when compared to the control group B (Table 4). Highest percentage decrease was observed in 600mg/kg body weight of the test sample. The decline in testosterone levels in treated groups suggested that its phytochemical composition of soursop plant may reduce the effect of the pituitary gland and testosterone synthesis (Yakubu *et al*., 2017).

Table 4: Mean testosterone levels of the rats (mg/dl)

Group	Baseline	End value	End value –baseline	% diff
A	0.88+0.03^{b}	2.47+0.45^{c}	1.59	180.68↑
B	1.54+0.31^{b}	0.96+0.08^{b}	-0.58	37.66↓
C	1.52+0.25^{b}	0.77+0.18^{b}	-0.75	49.34↓
D	8.15+0.81^{ab}	3.27+0.61^{b}	-4.88	59.88↓

Values are expressed as Mean + SD of each rat group (n=6). Mean values with different letters as superscripts across the column are considered significant at p<0.05.
Baseline= after experiment, End value= after the study.
Group A =Induced and not treated.
Group B = Induced rats fed 200mg/kg bwt of aqueous soursop pulp extract.
Group C= Induced rats fed 400mg/kg bwt of aqueous soursop pulp extract.
Group D= Induced rats fed 600mg/kg bwt of aqueous soursop pulp extract.

Table 5 shows the C-Reactive Protein (CRP) levels of the rats after induction and after treatment. CRP is a non-specific marker of inflammation and it may reflect the presence of inflammation in urologic pathways that could lead to BPH (Nnatuanya *et al*., 2022). Treatment with aqueous soursop pulp extract significantly lowered the CRP level of the rats in the treated groups when compared to the control group A. Highest percentage decrease was observed in 600mg/kg body weight of the test sample. This indicates that CRP was controlled because its decrease could be related to an effective management of the implication of BPH in the treated animals.

Table 5: Mean C- reactive protein (CRP) levels of the rats (µg/Ml)

Group	Baseline)	End value	End value-baseline	Percentage diff (%)
A	8.44 + 0.21^{b}	8.89 + 0.55^{d}	0.45	5.33↑
B	8.89 + 0.90^{b}	4.34 + 0.21^{c}	-4.55	51.18↓
C	8.54 + 0.90^{b}	2.89 + 0.40^{c}	-5.65	66.16↓
D	8.55 + 0.70^{b}	2.61 + 0.53^{c}	-5.94	69.47↓

Values are expressed as Mean + SD of each rat group (n=6). Mean values with different letters as superscripts across the column are considered significant at p<0.05.
Baseline= after experiment, End value= after the study.
Group A =Induced and not treated.
Group B = Induced rats fed 200mg/kg bwt of aqueous soursop pulp extract.
Group C= Induced rats fed 400mg/kg bwt of aqueous soursop pulp extract.
Group D= Induced rats fed 600mg/kg bwt of aqueous soursop pulp extract.

CONCLUSION

The findings of this study indicates that aqueous soursop pulp extract have beneficial effect on the inflammatory bio-markers of enlarged prostate. The ability of the extracts to have ameliorative effect on induced prostate enlargement makes them potentially useful in the management of BPH. Because the majority of previous studies were focused on the biological activities of the leaf extract, additional studies are required to isolate the active components responsible for the observed effects in the fruit extract.

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Declaration of Conflict of Interests

The authors declared no potential conflicts of interests with respect to the research.

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