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STUDIES OF PROSTATE SPECIFIC ANTIGEN AND HISTOPATHOLOGY OF RATTUS NORVEGICUS WITH EXPERIMENTAL PROSTATE NEOPLASM

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ABSTRACT

The effects of Annona muricata fruit pulp extract have been observed which confirms its activity against experimentally induced non-cancerous tumor. The prostate gland provides the semen with vitamins and other nutrients thus, maintaining its vitality during the journey up the female reproductive tract. Diseases of the prostate gland, affect millions of people the world over, decreasing their quality of life. Benign prostate hyperplasia, BPH, is the medical term used to describe an enlarged prostate. It means a noncancerous enlargement of the prostate gland. Benign prostatic hyperplasia (BPH) is a progressive condition characterised by prostate enlargement accompanied by lower urinary tract symptoms. This study follows an experimental design in which a total of fifty five male wistar albino rats were used. These rats were grouped into five main groups, three of these groups were further sub-divided into three sub-groups of five rats each. The groups include; Hormone Control, HC, five rats, Vehicle Control, VC, five rats, Test Group, TG, which is further divided into three sub-groups of five ats each, TGa, TGb, and TGc, Vehicle + soursop, VS, which is further divided into three sub-groups of five rats each, VSa, VSb, and VSc and then Soursop Control, SC, which is further divided into three sub-groups of five rats each, bringing the total of groups and sub-groups to eleven groups. The sub-groups a, b, and c are to be administered soursop extract at graded doses of 500mg/kg. 1000mg/kg and 2000mg/kg respectively Tumor induction was done by administering Testosterone Propionate and Estradiol Valerate at 300pg:80µg/kg subcutaneously in the inguinal region of the rats on alternate days for 21 days. Induction caused a very significant increase in the serum level of prostate specific antigen (PSA) of the HC group above the normal range in comparism to that obtained in the VC sub-groups at $P \leq 0.05$; while the Annona muricata extracts at low dose (500mg/kg) reduced the PSA level, the normal and high dose (1000mg/kg) inhibited (prevented) any increase in the PSA level. In the histopathological study it would be noticed that in HC group, tissue sections were deranged from normal cytoarchitecture of healthy cells which improved in the test group, TGa, which received low dose,

(500mg/kg of soursop), however, the tissue sections of the group that received normal and high doses of the annona murcata extract (1000mg/kg) showed normal cytoarchitecture of prostate organs from these sub-group. The study clearly establish Annona muricata fruit pulp extract as a potential candidate in prevention of androgen dependent conditions like prostate neoplasm.

Key words: Prostate, Antigen, Histopathology, Rattus Norvegicus, Neoplasm, Experiment

INTRODUCTION

Annona muricataL. Belongs to the family of Annonaceae has a widespread pantropical distribution and has been proudly known as corossol. The plant is grown as a commercial herb crop for its 20-30 cm (7.9-12 in) long, prickly, green fruit, which can have a mass of up to 15 lb (6.8 kg) making it probably the second biggest annona after the junglesop. Away from its native area, some limited production occurs as far north as southern Florida within USDA Zone 10; however, these are mostly garden plantings for local consumption. It is also grown in parts of Southeast Asia and abundant on the Island of Mauritius. The soursop will reportedly fruit as a container specimen, even in temperate climates, if protected from cool temperatures (Wikipedia.org/wiki/soursop).

The fruit of Annona muricata Linn is found to be edible in Yunnan province of China (Chao-Ming et al., 1998) and their fruits are used commercially for the production of juice, candy and sherbets. Intensive chemical investigations of the leaves and seeds of this species have resulted in the isolation of a great number of acetogenins. The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties. *Annona muricata* is used in traditional medicine in tropical America and west India for their tranquilizing and sedative properties (Hasrat et al., 1997). It has also been used in some Africa countries including Nigeria for array of human ailment: especially for parasitic infection and cancers. The plant is generally used as antiparasitic, antispasmodic, astringent, anticancer, sedative, hypertensive, insecticide; piscide, vermifuge and for cough fever, pain and skin disease (Watt and Breyer-Brandwijk, 1962).

A neoplasm, which is also commonly referred to as a tumor, is an abnormal growth of tissues (Taylor J, 2000). This abnormal growth usuallv but does not always forms a mass (Williams and Wilkins, 2006).A neoplasm can be caused by an abnormal proliferation of tissues, which can be caused by genetic mutations.The World Health Organization (WHO) classifies neoplasms into four main groups: benign neoplasms (which may include Benign Prostate Hyperplasia, BPH), in situ neoplasms, malignant neoplasms, and neoplasms of uncertain or unknown behavior (WHO, 2014) Prostate Specific Antigen (PSA) is an androgen-regulated serine protease and member of the tissue kallikrein family of proteases (Yousef and Diamandis, 2001). It is produced primarily by prostate ductal and acinar epithelium and is secreted into the lumen, where its function is to cleave semenogelin I and II in the seminal coagulum (Lilja et al., 1987). However, its major relevance in oncology is as a biomarker to detect prostate cancer (PCa) and to assess responses to treatment.PSA is concentrated in prostatic tissue, and

serum PSA levels are normally very low. Disruption of the normal prostatic architecture such as by prostatic disease, inflammation, or trauma, allows greater amounts of PSA to enter the general circulation. Elevated serum PSA level has become an important marker of many prostatediseases including benign prostatic hyperplasia, prostatitis, and prostate cancer. Prostate weight may also serve as a means of studying the pathological impact of prostatitis, prostate cancer, as well as benign prostate hyperplasia (BPH) on the prostate organ. Transrectal Ultrasound (TRUS) and Direct Rectal Examination (DRE) are the two methods of prostate examination However, direct incision and harvesting and of the prostate organ maybe used in animal experiments.

The majority of the glandular tissue in prostate is located in the peripheral zone, and seminal fluid produced by these glands empties into 12 to 20 excretory ducts and then into the urethra. PSA is a major protein in seminal fluid, with a concentration of 0.5 to 2.0 mg/mL (Wang et al., 1981;Lovgren et al., 1999).It has a substrate specificity similar to chymotrypsin (Watt et al., 1986), and its major physiological substrates are semenogelin I and II (Lilja et al, 1987), the proteins that mediate gel formation in semen Prostate glands in humans consist of a single layer of secretory epithelial cells, which are surrounded by a continuous layer of basal cells and a basement membrane (Fig 2). PSA is produced by these secretory epithelial cells in the acini and ducts, and it is secreted directly into the lumen. A characteristic early feature of PCa is disruption of the basal cell layer and basement membrane, and this loss of the normal glandular architecture appears to allow PSA increased direct access to the peripheral circulation (Brawer et al.. 1989: Bostwick et al. 1994) PSA is pormally found at much lower levels in paraurethral and perianal glands, apocrine sweat glands, breast, thyroid, and placenta (Howarth et al; 1997; Lovgren et al; 1999).These sites do not normally contribute measurable levels of PSA into the circulation, as PSA production has also been reported in a variety of other cancers, including breast cancer. Its functions in these tissues are not yet clear, and its (Steven et al., 2003).

Studies in the early 1990s confirmed that serum total PSA could be used to identify patients with PCa (Catalona et al., 1991: Labrieet al., 1992; Brawer et al., 1992:Catalona et al., 1993).As a screening tool, serum PSA was clearly more sensitive than digital rectal examination, but it lacked specificity. When compared with prostatic acid phosphatase, serum PSA was demonstrated to be a more sensitive marker in PCa detection (although neither was highly specific) (Stamey et al., 1987).These findings have led to wide use of PSA testing for early detection of PCa, although the optimal approach to PSA testing remains uncertain.

The impact of prostate weight (PW) has been a topic of analysis for radical prostatectomy for quite some time. Recently, Yong et al.,2010, from Duke University, observed in a population that larger prostates were associated with longer operative times (OT) and this effect was maintained independently of cumulative robotic experience (Yong et al., 2010). Moreover, other large robotic series, including the current series, have not observed such time-related outcomes (Msezane et al., 2007; Zom et al., 2007). Possible explanations of the discordance include the lack of overcoming the learning curve and other patient related factors (degree of nerve sparing, performance of a pelvic lymph node dissection and pelvic anatomy) Prostate weight however does appear to impact pathological outcomes. Zorn et al.,

2007,previously published on a transperitoneal series case which were stratified by PW similar to the current study. While age and PSA were significantly higher in larger prostates, no significant differences in OT, blood loss, transfusion rate, hospital stay or complications were observed (Zorn et al., 2007). The objective return of baseline and subjective sexual and urinary function, as determined by validated questionnaire scores, was not affected by the PW. Pathologically, the overall rate of positive surgical margins (PSM) was significantly different among the groups demonstrating a trend of increasing PSM with a lower PW.

A neoplasm can be benign, potentially malignant (pre-cancer), or malignant (cancer).

Benign neoplasms include uterine fibroids and melanocytic nevi (skin moles). They are circumscribed and localized and do not transform into cancer.

Potentially malignant neoplasms include carcinoma in situ. They do not invade and destroy but, given enough time, will transform into cancer.

Malignant ncoplasms are commonly called cancer. They invade and destroy the surrounding tissue, may form metastases and eventually kill the host.

Secondary neoplasm refers to any of a class of cancerous tumor that is either a metastatic offshoot of a primary tumor, or an apparently unrelated tumor that increases in frequency following certain cancer treatments such as chemotherapy or radiotherapy.

A neoplasm can be caused by an abnormal proliferation of tissues, which can be caused by genetic mutations. Not all types of neoplasms cause a tumorous overgrowth of tissues, however (such as leukemia or carcinoma in situ). Recently, tumor growth has been studied usine mathematics and quantum mechanics. Vascular tumors are thus looked at as being amalgams of a solid skeleton formed by sticky cells and an organic liquid filling the spaces in which cells can grow (Ambrosi and Mollica, 2002). Under this type of model, mechanical stresses and strains can be dealt with and their influence on the growth of the tumor and the surrounding tissue and vasculature elucidated. Recent findings from experiments that use this model show that active growth of the tumor is restricted to the outer edges of the tumor, and that stiffening of the underlying normal tissue inhibits tumor growth as well (Volokh, 2006) Benign conditions that are not associated with an abnormal proliferation of tissue (such as sebaceous cysts) can also present as tumors, however, but have no malignant potential. Breast cysts (as occur commonly during pregnancy and at other times) are not examples, as are other encapsulated glandular swellings (thyroid, adrenal gland, and pancreas).

MATERIALS AND METHODS

Materials

Hormones: Estradiol Valerate (EDV) and testosterone propionate (TP) bought from a German owned company, sigma Aldrich Ltd, Germany were administered subcutaneously into the rear flank of the inguinal region of a certain group of the rats. Typical for this model the palpable tumors were present in the rats visible to touch within ten (10) days of induction and were allowed to grow for a total of twenty one (21) days prior to euthanasia and of the

tumor harvest. When harvested tumor tissue were fixed in 10% neutral buffered formalin for 24hrs.

Plants : The fruits, annona muricata, soursop popularly called pears in Nigeria bought from anyogba central market, anyiogba, the seat of kogi state university.

Reagents : distilled water**,** Chloroform **,**10% buffered phosphate, buffered formalin**,** Olive oil (goya) and Monod ELISA kit.

Apparatus : these include

Apparatus/Equipments	Specification
Avon stainless plate (small size)	Chinese made
Beakers	Pyrex
Electric blender	Made by Binatone
Electric weighing balance	PB3002-S (mettler)
Foil papers (wapper)	Compay (silver)
Measuring cylinder	Pyrex
Micropipette	Rayto Rx-450
Microplate reader	Rayto RT-2100C
Microplate washer	Rayto RT-2600C
Spatular	Chinese made Sieve
Surgical gloves	Seward
Syringes/needles	Johel
Steam bath	998I

METHODS

Study design: This study follows an experimental design in which a total of fifty five male wistar albino rats were used.

Settings: Fifty five male Wister Rats, 2-3 weeks of age were obtained from Abdul Rats Venture, Staff Quarters, Kogi State University, Anyigba. Their weights were taken to be between 150 to 200 grams and they were maintained in an individually ventilated caging system with sawdust bedding which changed in three davs for proner sanity and hygiene of the animals. Free access was provided to drinking pure water obtained from the nearby tap Water or borehole system within the university and feed bought from Kogi State University Second Gate. The animals inhabited their new environment for a period of two (2) weeks before the experiment kicked off.

Preparation of Plants Extract

A naturally softened and ripened fruits of Annona Muricata were gently whashed and the exocap (the greenish rough rough and soft back) that was not needed for this research was gently removed, the whitish soft multiple seed contained endocarp were placed in a cleaned and dried container, moderate water was added and squeezed gently to release the multiple seed into the solution, the seeds were carefully removed, the jelly-like solution left was blended and the homogenous mixture of water and the soursop was filtered using sieve. The filtered solutions was moderately poured into a small size stainless plate up to one quarter level, water bath or steam bath was prepared and the plates containing the solution were water bathed until the water in the solution was dehydrated completely. The disappearance of the original whitish colour and the appearance of the dark

brown colour confirmed its readiness to be used for the tumor induction.

Prostate and Blood Sample collection

The rate were taken in cages into the laboratory, they were euthanized; a tissue paper was soaked with chloroform, put in the bottom portion of the desiccators and the rats were kept in the upper portion of the desiccators and covered for some minutes, no fresh air was allowed so that the rats were deprived of oxygen after sometimes they were found very inactive, dull and weak. They were brought out and placed side way on a carbon paper attached to the flat and smooth 60x40m wooden board, one student held the forelimb, another held its hind limb while another student slaughtered the rat. The blood is collected into a clean sterilized EDTA container and covered the rat's diaphragm were dissected and the prostates were located. harvest and fixed in a big EDTA container containing 10% neutral buffered formalin for 24hrs which prevent decay The prostate harvested was weighed on the electrical weighing balance and the weight were emblemed on the bottle with masking tape and the container of the blood of that particular rats was also marked same with the prostate for easy identification. The blood collected were centrifuged at 4000r/min for 10 minutes, the clear fluid at the ton of the blood plasma (serum) was collected carefully into another container and kept for further studies and analysis

Animal grouping/Experimental Design

The animals used for this work were stratified into five strata as shown below;

1. Soursop control: this strata was made of fifteen rats and it was further sub stratified into three strata namely, ab, and c. and each of these had five rats. The rats in "a" took 500mgkg^{-1} of the soursop extract, "b" rats took 1000mgkg^{1} 'of the soursop extract and finally, "c" the rats here took 2000mgkg^{-1} of the soursop extract.

2. Vehicle control: This stratum had only five rats and were induced with only the vehicle, olive oil daily for the 21 days induction exercise

3. Hormone control: This stratum contained five rats and they were given tumor inducer in alternated days for the 21 inducing days, they were control or placebo to the experiment.

4. Vehicle + soursop SV: This stratum had fifteen rats also and it was further sub-grouped into a, b, and c with each having five rats. A rat in each group was induced with Olive oil and subsequently treated with soursopextract of 500mgkg^{-1}, 1000 mgkg^{-1} and 2000 mgkg^{-1} respectively at alternate days in the 21 days of induction exercise

5. Inducer + soursop (test group): like those groups given soursop only and soursop + olive oil, this group also had fifteen rats and was divided into a,b, and c with five rats in each group, "a" was induced with Benign Prostatic Hyperplasia (BPH) using the inducer S, "b" with BPH using inducer SS and "C" with BPH with inducer and treated with soursop at 500 mgkg^{-1}, 1000 mgkg and 2000 mgkg^{-1} respectively.

Prostate weight determination

After dissection and the prostates were harvested, the empty petridish was placed on the weighing balanced that was plugged to the electric source and zeroed, the weight of petridish was cleared, the prostates were now transferred onto the zeroed balance in the petridish and the weight noted.

Hiatopathological Analysis of the Prostates

Tissues make up organ (prostate), the histopathological analysis of prostate is simply the lysis of the organ, prostate into tissues for further studies or detection of disease conditions such as cancer of the prostate.

Procedures

1. Tissue processing: this took place in the tissue processor, it includes 6 major steps as shown below;
   i. Dissecting a sacrificed rat
   ii. Harvesting of the prostates
   iii. Preservation of the prostates in a fixative e.g. formalin
   iv. Dehydration using alcohol
   v. Dealcoholination with toluene
   vi. Infiltration and embedding with paraffin wax.
2. Tissue sectioning: this is done in tissue sectioning machine (microturn), tissues are sectioned at 5-micron thickness.
3. Heat fixing of the sectioned tissue by the microscope slide.
4. Staining: this is done with dye e.g. xylene and dipping in alcohol, it is rinsed in water: it is further dipped in hematoxylin and water rinsed again.
5. Differentiation: dipping of the slide into the acid alcohol and rinsed with water, then dipped in ammonium water that is stained with Eosin and then in alcohol.
6. The slide is dipped in xylene for 10 minutes, removed and allowed to dry in the air.

RESULTS

Results of the histopathological analysis.

Group one, Hormone Control (HC)

Figure 3.1a. Tissue section showing enlargement of cells at the fibro muscular and prostatic urethra, as indicated by the arrows. This particular prostate section is deranged of the normal one
Staining uptake H & E
Magnification x200

Figure 3.1b
Tissue section showing atypical cell inflammation as indicated by the arrows, The prostate is abnormal as revealed by this section.
Staining uptake H& E
Magnification x200

Group two, Test Group (TG a(500/KG))

Figure 3.2a. Tissue section showing an arrangement of fibrin and inflammation, at fibro muscular stroma as denoted by the arrows. The section reflects an abnormal cytoarchitecture although hyperplesia is not clear.

Staining uptake H & E

Magnification x200

Figure 3.2b.
Tissue section showing the infiltration of atypical epithelia cells in the stroma as indicated by arrows, indicative of an unhealthy prostate.
Staining uptake H & E
Magnification x200

Group three, Test Group (TG b (1000mg/kg))

Figure 3.3a.

Tissue section showing a normal cytoarchitecture of prostate. This section is indicative of a healthy prostate.

Staining uptake H & E

Magnification x200

Figure 3.3b.
Tissue section showing a normal cytoarchitecture of prostate. Indicative of healthy prostate.
Staining uptake H & E
Magnification x200

Group four, Test Group (TG c(2000mg/kg))

Figure 3.4a.
Tissue section showing a normal cytoarchitecture of prostate. Indicative of a healthy prostate.
Staining uptake H & E
Magnification x200

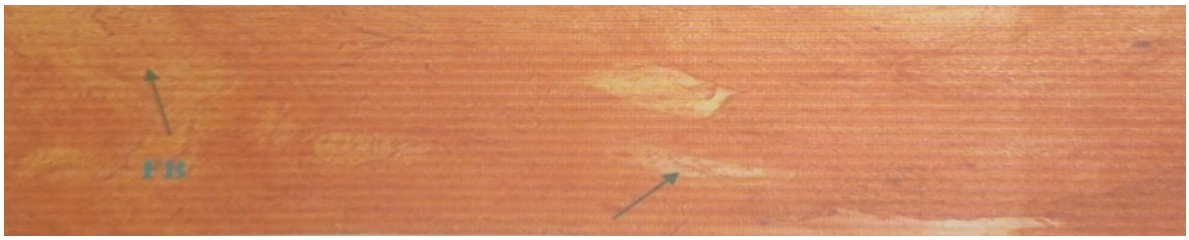

Figure 3.4b.

Tissue section showing a normal fibro muscular stroma and the normal concretion of the prostate gland as denoted by the arrows. FB indicates fibrins and the other arrow shows concretion.
Staining uptake H & E
Magnification x200

Group five, Vehicle Control (VC)

Figure 3.5a.
Tissue section showing a normal cytoarchitecture of the prostate tissue prostate. Staining uptake H & E
Magnification x200

Figure 3.5b.
Tissue section is a representative of a normal and healthy prostate.
Staining uptake H & E
Magnification x200

GROUP SIX, Vehicle and Sourson (VSa (500mg/kg)

Figure 3.6a.
Tissue shows a normal cytoarchitecture which is indicative of a healthy prostate.
Staining uptake H & E
Magnification x200

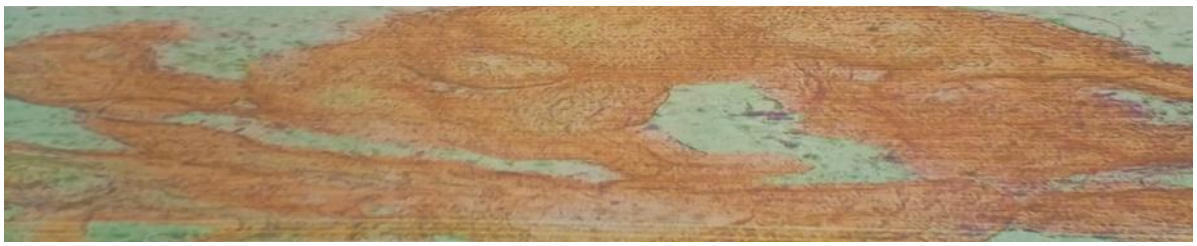

Figure 3.6b.
Tissue section shows a normal and healthy prostate by cytoarchitecture.
Staining uptake H & E
Magnification x200

GROUP SEVEN, Vehicle and Sour-sop (VS a (1000mg/kg))

Figure 3.7a.
Tissue section showing normal orostate devoid of any inflammations or infiltration. This signifies a possible healthy prostate organ.
Staining uptake H & E
Magnification x200

Figure 3.7b.
Tissue section showing histologically normal prostate. An indication that the prostate organ may be healthy.
Staining uptake H & E
Magnification x200

GROUP EIGHT, Vehicle and Sour-sop sop (VSa (2000mg/kg))

Figure 3.8a.
Tissue section showing normal histological prostate section as expected of a healthy prostate.
Staining uptake H & E
Magnification x200

Figure 3.8b.
Tissue section reveals arrangement of fibrins and infiltration of atypical epithelia cells. This is deranged of the normal cytoarchitecture of the healthy prostate.
Staining uptake H & E
Magnification x200

Group nine, Soursop Control (SC a (500/kg))

Figure 3.9a.
The section showing no distortion of epithelia arrangement as indicated in the prostatic urethra by the arrows.
Staining uptake H & E
Magnification x200

Figure 3.9b.
Tissue section showing a normal arrangement of epithelia cells and the concretion.
Staining uptake H & E
Magnification x200

GROUP TEN, Sour-sop Control (SCa (1000mg/kg/)

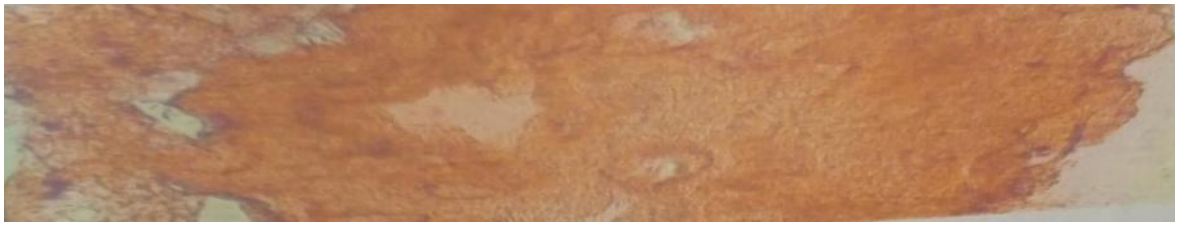

Figure 3.10a
Section showing a normal cytoarchitecture of the prostate tissue, an indication of a healthy prostate organ.
Staining uptake H & E
Magnification x200

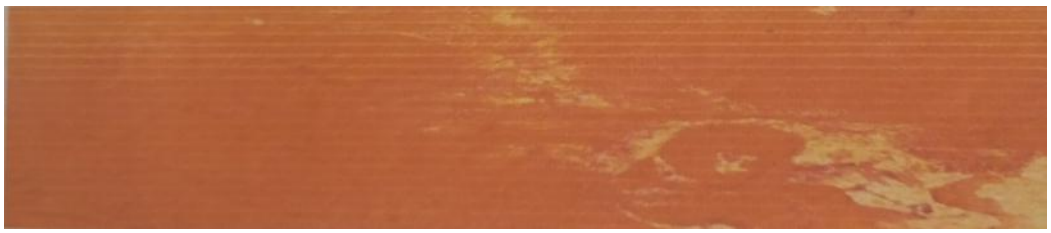

Figure 3.10b.
Section showing a normal and healthy tissue of the prostate. An indication that the prostate organ is healthy.
Staining uptake H& E
Magnification x200

GROUP ELEVEN, Sour-sop Control (SCc (2000mg/kg/)

Figure 3.11a.
Tissue section showing a normal cytoarchitecture of prostate.
Staining uptake H & E
Magnification x200

Figure 3.11b.
Section showing a normal cytoarchitecture of the prostate tissue. An indication that the prostate organ is a healthy one.
Staining uptake H & E
Magnification x200

The H & E in the staining uptake represent Haematoxanthine and Eosin respectively. The differences incolour appearance of the various slides are due to staining uptake of the different slides which mav be due to thickness of tissues on the different slides.

TABLE 1, The results for Prostate Specific Antigens (PSA)

GROUP AND DESCRIPTION	SAMPLES	PSA (ng/ml)
HC	A	0.080
	B	0.098
	C	0.025
	D	0.089
	E	0.080
TG a	A	0.064
	B	0.060
	C	0.056
	D	0.060
	E	0.058
TG b	A	0.033
	B	0.025
	C	0.070
	D	0.029

	E	0.048
VC	A	0.021
	B	0.018
	C	0.015
	D	0.019
	E	0.017
VS a	A	0.037
	B	0.021
	C	0.081
	D	0.051
	E	0.059
VS b	A	0.091
	B	0.008
	C	0.010
	D	0.051
	E	0.009
SC a	A	0.022
	B	0.034
	C	0.063
	D	0.023
	E	0.042
SC b	A	0.016
	B	0.021
	C	0.020
	D	0.019
	E	0.021

GROUPS	PSA (ng/mLx100)
HC	7.44±1.27°
TGa	5.96±0.13*
TGb	4.10±0.82*
VC	1.80±0.10*
VSa	4.98±1.01
VSb	3.38±1.64
SCa	3.79±0.71
SCB	1.96±0.10

Where
HC=Hormone Control
TG= Test Group^
VC=Vehicule ans Soursop

SC=Soursop Control
The small letters **a** and **b** represent the graded doses 500mg/kg and 1000mg/kg respectively.

DISCUSSION

Prostate Specific Antigen (PSA) is an androgen-regulated serine protease and member of the tissue kallidrein family of proteases (Youssed and Diamandis, 2001). However, its major cinical relvance is as a biomarker to detect prostate conditions such as neoplasm and to assess responses to treatment. High serum level PSA is indicative of a diseased of a diseased prostate.

In this study, it was observed that PSA level in the HC group is high, indicative of a diseased prostate. In group TG a, the graded dose of 500mg/kg, there was a reduction of PSA level when compared the PSA level from HC group, but still above normal level. The PSA level from animals in group TG b, the graded dose of 1000mg/kg was normal, an indication that at this dose, 1000mg/kg of *annomamuricata* extract BPH was prevented from being induced.

The results of the histopathology and that of the prostate specific antigen (PSA) are consistent as they correspond, high serum PSA level hormone control group correspond to hyperplesia in the tissue section as viewed histopathologically.

The prostate of an animal with BPH, when examined histologically is expected to exhibit some stromal proliferation and glandular hyperplesia. The prostate from the animals in the hormone control group show mild hyperplesia some stromal proliferationwhich led to an increased size and weight of the prostate organ. At the dose 500mg/kg, there was an improved appearance in tissues sectioning histologically, but tissue lacks the appearance of the normal healthy tissue. However, at the dose 1000mg/kg, tissue section has the appearance of a normal healthy tissue. This is to say that at the graded dose of 1000mg/kg there was a prevention of prostate hyperplesia.

The development of disease is also associated with enhancedproliferation and suppressed apoptosis of prostatic cell. Polupeptide growth factors plau an important role in disease development. Effect a Annonamuricata is supposed to be due to its reported anti-inflammatory and anti-proliferative action.

CONCLUSION

In conclusion, extracts of Annonamuricata (at the graded dose of 1000mg/kg) significantly inhibited the prostate growth in experimental animals induced with BPH. The possible mechanism of Annonamuricat extract might be its reported anti-inflammatory and anti-proliferative activity. Further on inhibition properties of extracts of Annonamuricata on the isolated preparations and measurement of DHT znd estradiol is warranted.

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