BENSON IDAHOSA UNIVERSITY
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INAUGURAL LECTRE 4TH

SERIES

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FROM GROWTH BIOLOGY TO HIV-ASSOCIATED NEPHROPATHY TO THE DISCOVERY OF ANTI-CANCER AGENT: ECONOMIC IMPLICATION

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EARLY SIGNS OF MY INTEREST IN THE UNRAVELING OF SCIENTIFIC MYSTERIES

- As a child, I took interest in plants and animals. I took special interest in the mystery of a bicycle standing on two tyres while in motion. A bicycle, if moving fast enough tends to maintain an upright position … a phenomenon many believe today to be driven mainly by gyroscopic forces. This type of force is also crucial for an airplane functionality in the Sky. The last Century witnessed a lot of scientific breakthroughs including the Albert Einstein's $E=mc^2$ (the world's most famous equation).It has been postulated that Genes and Cancer topic represents one of the 10 scientific mysteries of the 21st century.

- While growing up (during my Elementary School days), I cultivated beans, maize, etc., behind my mother's kitchen; I watered the plants every morning and watched them grow.
- It was always amazing to me to see a new leaf sprout of these plants. Retrospectively, I guess these were early signs of my interest in scientific discovery which today has culminated into the presentation/lecture underway.

CHAPTER 1

- **GROWTH AND REGULATION OF PORCINE SATELLITE CELLS**
- **Objective**: To provide insights on lipogenesis, lipolysis, and mitogenesis.
- **Relevance**: Medical/Agricultural
- The use of growth promotants Ractopamine (RAC)
  Isoproterenol (ISO)
  Clenbuterol (CLE)

The chemical structure of commonly use BAA in meat Animals (Izevbigie Ph.D) dissertation, Michigan State University

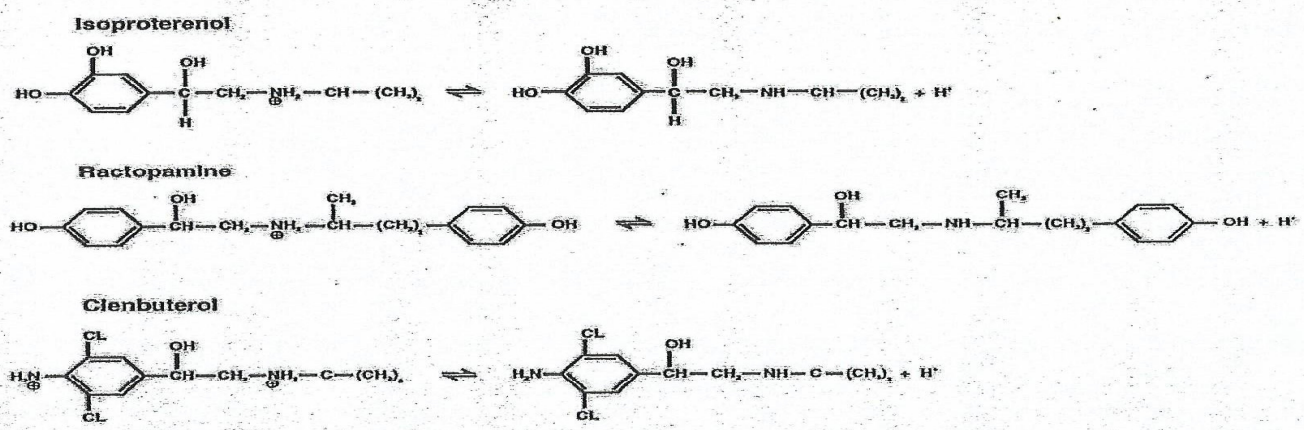
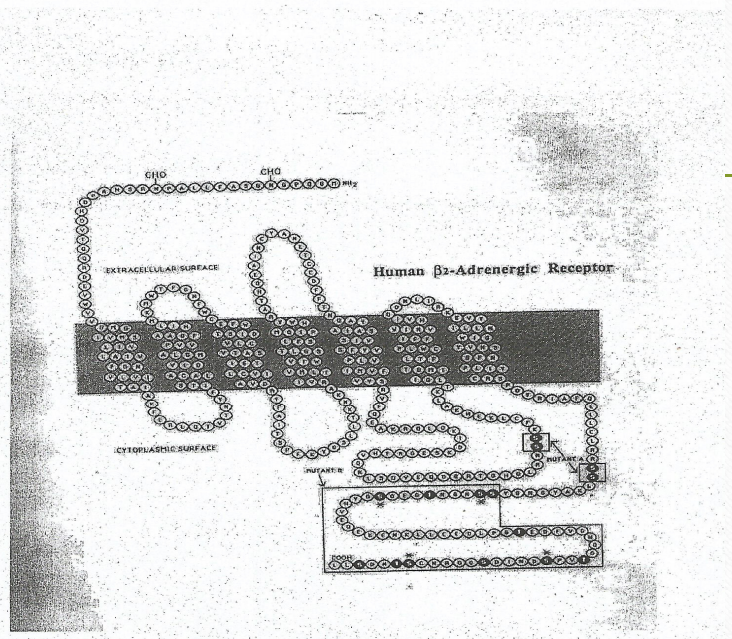


Figure 1. The chemical structure of commonly used βAA in meat animals.

Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

The Structure and membrane topology of human BAR (Housedoff et la, 1989) Reproduced with permission Biol.Chemistry



The structure and membrane topology of human β2-AR (Hausdoiff et al., 1989). Reproduced with Permission.

Figure 2

Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

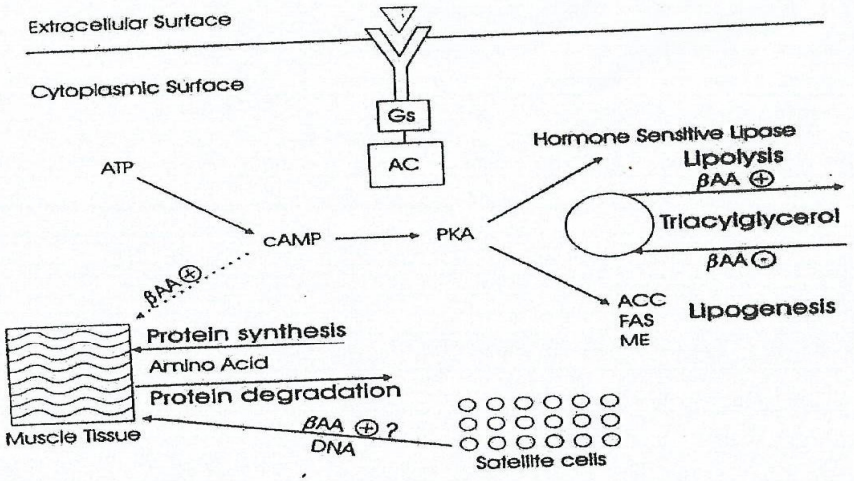


Figure 3

The signaline Cascade of βAA-mediated effects on protein synthesis, lipolysis, and lipogenesis.

\+ = stimulatory

\- = inhibitory

Source: Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

Results

Chamber Determination

Results of the cell quantitations studies are presented in Figure 5. Each data point represents the mean of six incubations.

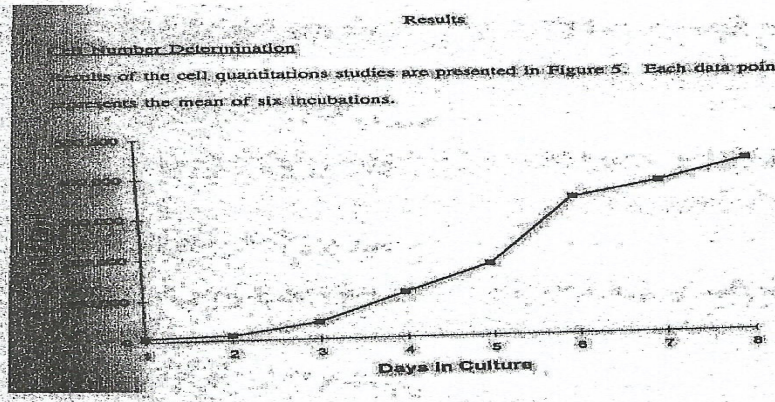


Figure 4A

Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

DNA Determination

Results of DNA quantitation are shown in Figure 6. Each data point represents the mean ± SD of six incubations.

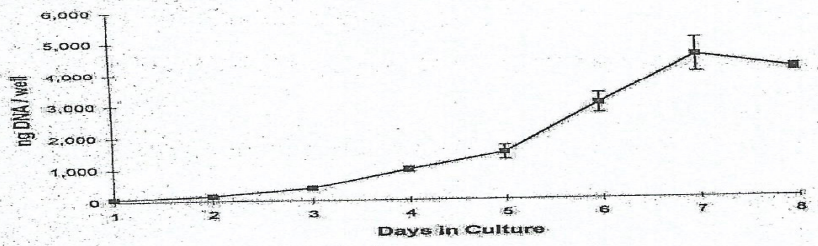


Figure 4B DNA quantitation studies.

Satellite Cell Differentiation

Results of the differentiation studies are shown in Table 2. Data are means ± SD of six incubations. Porcine satellite cells were grown as described in the Materials and Methods section. Cells were differentiated in serum-free medium supplemented with or without 10^{-3} cytosine β-D-arabinofuranoside (Ara-c). For the Ara-c-only treatment, serum-free medium was not supplemented with insulin.

Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

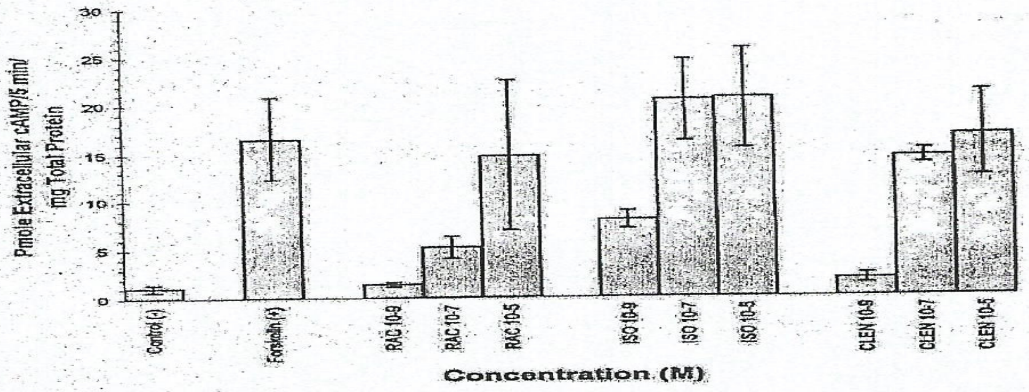


Figure 5 βAA-stimulated cAMP accumulation in differentiated C2C12 cells.

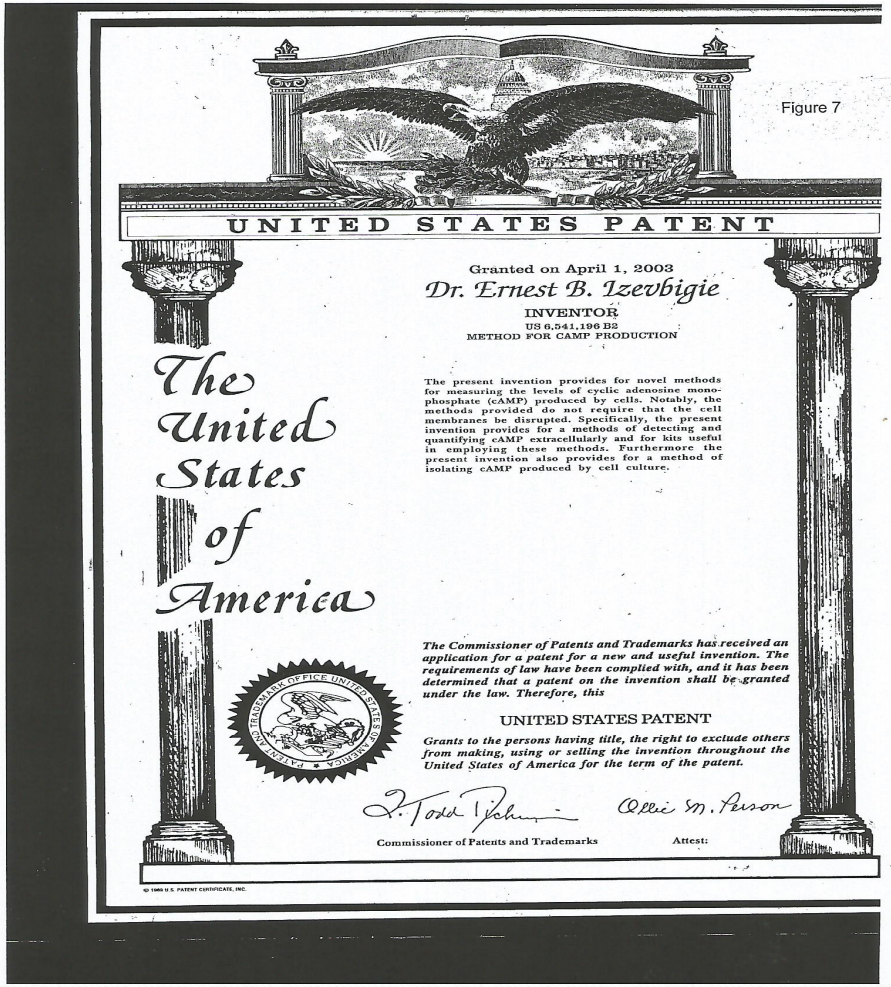
Note. C2C12 cells were grown and differentiated as described under the Materials and Methods section. Either ISO, RAC, or CLEN was added at a final concentration of 10^{-9}, 10^{-7}, or 10^{-5} M. Monolayers were then incubated at 37°C for 5 min and cAMP was quantified as stated under the Materials and Methods section. Data are means ± SD for three experiments done in duplicate.

Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

Figure 6 Cells grown in a 10^{-6} M insulin-only serum-free medium, after 72 h of incubation.

Izevbigie, Ph.D. Dissertation, Michigan State University, 1996.

Figure 7



UNITED STATES PATENT

Granted on April 1, 2003

Dr. Ernest B. Izevbigie

INVENTOR

US 6,541,196 B2

METHOD FOR CAMP PRODUCTION

The present invention provides for novel methods for measuring the levels of cyclic adenosine monophosphate (cAMP) produced by cells. Notably, the methods provided do not require that the cell membranes be disrupted. Specifically, the present invention provides for a methods of detecting and quantifying cAMP extracellularly and for kits useful in employing these methods. Furthermore the present invention also provides for a method of isolating cAMP produced by cell culture.

The United States of America

The Commissioner of Patents and Trademarks has received an application for a patent for a new and useful invention. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law. Therefore, this

UNITED STATES PATENT

Grants to the persons having title, the right to exclude others from making, using or selling the invention throughout the United States of America for the term of the patent.

D. Todd Dickinson — Commissioner of Patents and Trademarks

Attest:

Results

- Satellite cells grew well in culture (Fig. 4B)
- βAA's significantly stimulated 3^1-5^1 cyclic-Adonosine Monophosphate cAMP release (Fig. 5).
- βAA (Isoproterenol, Ractopanine, and Clenbuterol) significantly stimulated the growth of porcine satellite cells.
- Satellite cells grew well in culture (Fig. 4AB).
- Multi-nucleated muscle myofibers were produced in culture (Fig. 6).
- Novelty – Biological method for cAMP production different from chemical synthesis method.

Conclusion

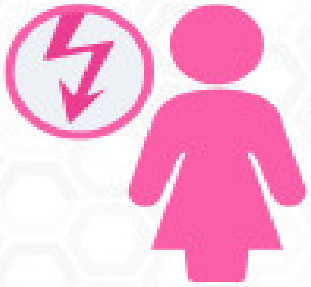
βAA's may contribute to muscular growth and thus depress the percentage fat and subsequently cardiovascular disease risk.

CHAPTER 2

- **Objective:** Investigate/assess anti-proliferative (anti-cancer) activities of some Nigerian botanicals on estrogen receptor-positive/negative human cancerous cells.
- **Relevance**: Medical

- **B**reast cancer is the most commonly diagnosed cancer in women, representing approximately 30% of all types of cancer in women (Cancer Statistics & figures, American Cancer Society). One out of every eight women will be diagnosed with breast cancer in her lifetime. It is estimated that in the United States in 2002, breast cancer will have accounted for more than 28% (232,680) of all new cancer (810,320) and 14.5% of cancer-related deaths (American Cancer Society, AMS).

BREAST CANCER



is the 2nd leading cause of cancer death among women in the U.S.

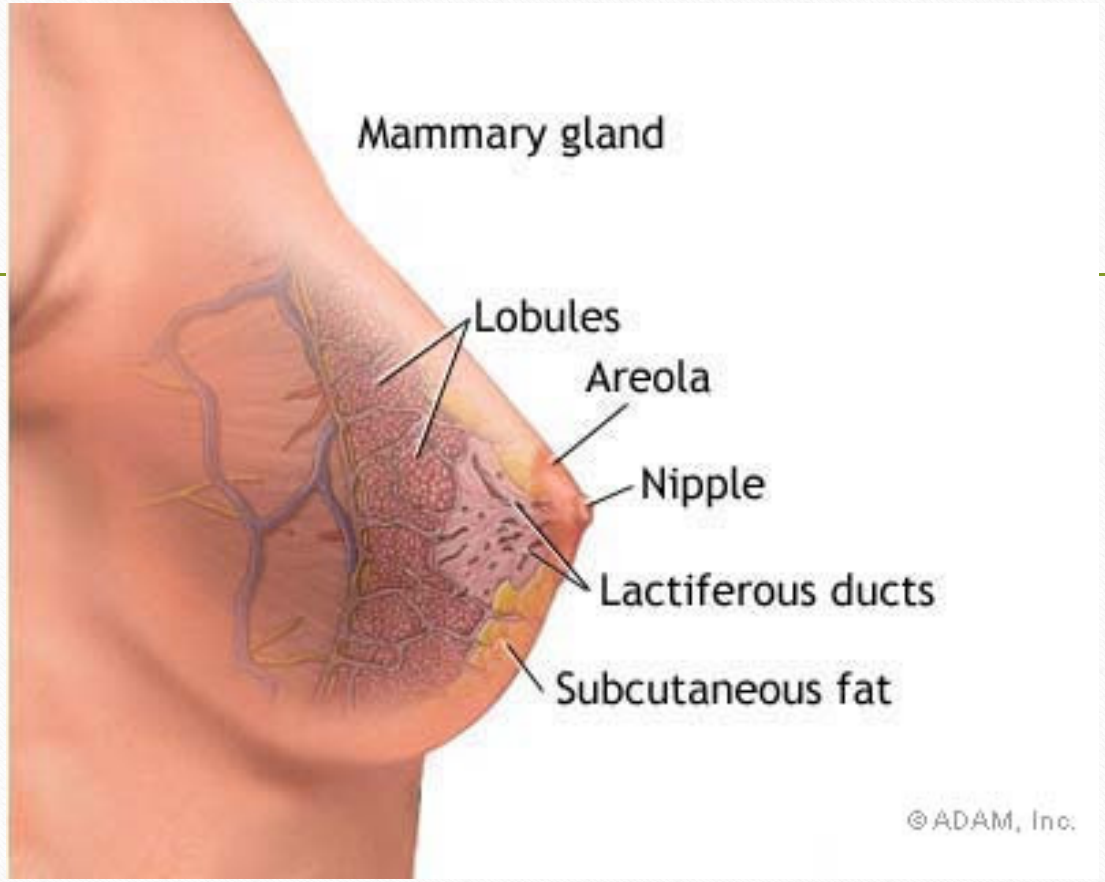
Source: Centers for Disease Control and Prevention

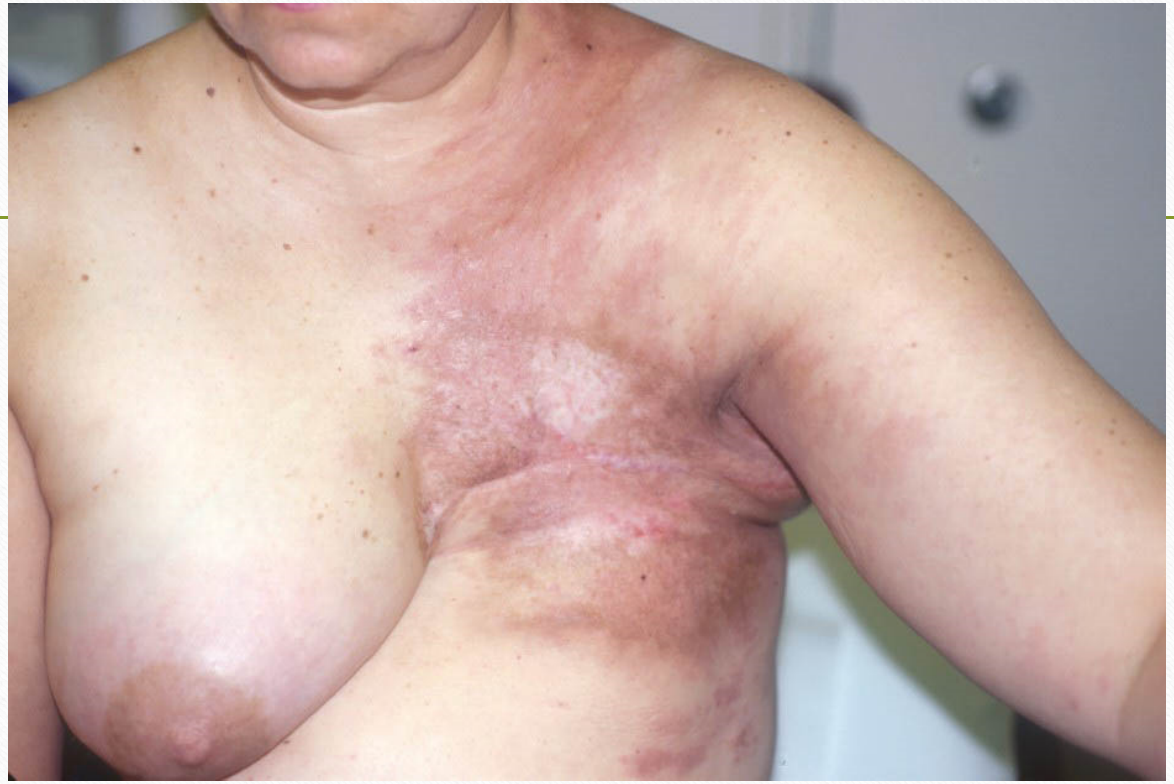
Estimated Number* of New Cancer Cases and Deaths by Sex, US, 2014

	Estimated New Cases			Estimated Deaths		
	Both Sexes	Male	Female	Both Sexes	Male	Female
All Sites	1,665,540	855,220	810,320	585,720	310,010	275,710
Oral cavity & pharynx	42,440	30,220	12,220	8,390	5,730	2,660
Tongue	13,590	9,720	3,870	2,150	1,450	700
Mouth	11,920	7,150	4,770	2,070	1,130	940
Pharynx	14,410	11,550	2,860	2,540	1,900	640
Other oral cavity	2,520	1,800	720	1,630	1,250	380
Digestive system	289,610	162,730	126,880	147,260	84,970	62,290
Esophagus	18,170	14,660	3,510	15,450	12,450	3,000
Stomach	22,220	13,730	8,490	10,990	6,720	4,270
Small intestine	9,160	4,880	4,280	1,210	640	570
Colon†	96,830	48,450	48,380	50,310	26,270	24,040
Rectum	40,000	23,380	16,620			
Anus, anal canal, & anorectum	7,210	2,660	4,550	950	370	580
Liver & intrahepatic bile duct	33,190	24,600	8,590	23,000	15,870	7,130
Gallbladder & other biliary	10,650	4,960	5,690	3,630	1,610	2,020
Pancreas	46,420	23,530	22,890	39,590	20,170	19,420
Other digestive organs	5,760	1,880	3,880	2,130	870	1,260
Respiratory system	242,550	130,000	112,550	163,660	90,280	73,380
Larynx	12,630	10,000	2,630	3,610	2,870	740
Lung & bronchus	224,210	116,000	108,210	159,260	86,930	72,330
Other respiratory organs	5,710	4,000	1,710	790	480	310
Bones & joints	3,020	1,680	1,340	1,460	830	630
Soft tissue (including heart)	12,020	6,550	5,470	4,740	2,550	2,190
Skin (excluding basal & squamous)	81,220	46,630	34,590	12,980	8,840	4,140
Melanoma-skin	76,100	43,890	32,210	9,710	6,470	3,240
Other nonepithelial skin	5,120	2,740	2,380	3,270	2,370	900
Breast	235,030	2,360	232,670	40,430	430	40,000
Genital system	338,450	243,460	94,990	58,970	30,180	28,790
Uterine cervix	12,360		12,360	4,020		4,020
Uterine corpus	52,630		52,630	8,590		8,590
Ovary	21,980		21,980	14,270		14,270
Vulva	4,850		4,850	1,030		1,030
Vagina & other genital, female	3,170		3,170	880		880
Prostate	233,000	233,000		29,480	29,480	
Testis	8,820	8,820		380	380	
Penis & other genital, male	1,640	1,640		320	320	
Urinary system	141,610	97,420	44,190	30,350	20,610	9,740
Urinary bladder	74,690	56,390	18,300	15,580	11,170	4,410
Kidney & renal pelvis	63,920	39,140	24,780	13,860	8,900	4,960
Ureter & other urinary organs	3,000	1,890	1,110	910	540	370
Eye & orbit	2,730	1,440	1,290	310	130	180
Brain & other nervous system	23,380	12,820	10,560	14,320	8,090	6,230
Endocrine system	65,630	16,600	49,030	2,820	1,300	1,520
Thyroid	62,980	15,190	47,790	1,890	830	1,060
Other endocrine	2,650	1,410	1,240	930	470	460
Lymphoma	79,990	43,340	36,650	20,170	11,140	9,030
Hodgkin lymphoma	9,190	5,070	4,120	1,180	670	510
Non-Hodgkin lymphoma	70,800	38,270	32,530	18,990	10,470	8,520
Myeloma	24,050	13,500	10,550	11,090	6,110	4,980
Leukemia	52,380	30,100	22,280	24,090	14,040	10,050
Acute lymphocytic leukemia	6,020	3,140	2,880	1,440	810	630
Chronic lymphocytic leukemia	15,720	9,100	6,620	4,600	2,800	1,800
Acute myeloid leukemia	18,860	11,530	7,330	10,460	6,010	4,450
Chronic myeloid leukemia	5,980	3,130	2,850	810	550	260
Other leukemia‡	5,800	3,200	2,600	6,780	3,870	2,910
Other & unspecified primary sites‡	31,430	16,370	15,060	44,680	24,780	19,900

*Rounded to the nearest 10; estimated new cases exclude basal cell and squamous cell skin cancers and in situ carcinomas except urinary bladder. About 62,570 carcinoma in situ of the female breast and 63,770 melanoma in situ will be newly diagnosed in 2014. †Estimated deaths for colon and rectal cancers are combined. ‡More deaths than cases may reflect lack of specificity in recording underlying cause of death on death certificates and/or an undercount in the case estimate.

Source: Estimated new cases are based on 1995-2010 incidence rates reported by the North American Association of Central Cancer Registries, representing 89% of the US population. Estimated deaths are based on 1995-2010 US mortality data, National Center for Health Statistics, Centers for Disease Control and Prevention.


Mammary gland
Lobules
Areola
Nipple
Lactiferous ducts
Subcutaneous fat
©ADAM, Inc.



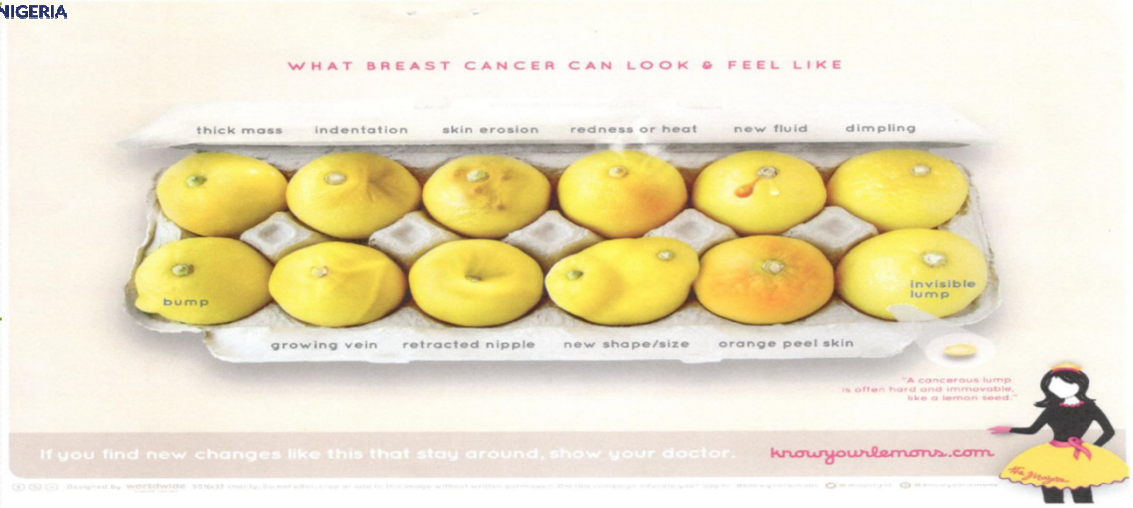


Figure 11. Some Symptoms of Breast Cancer.

Risk Factors.

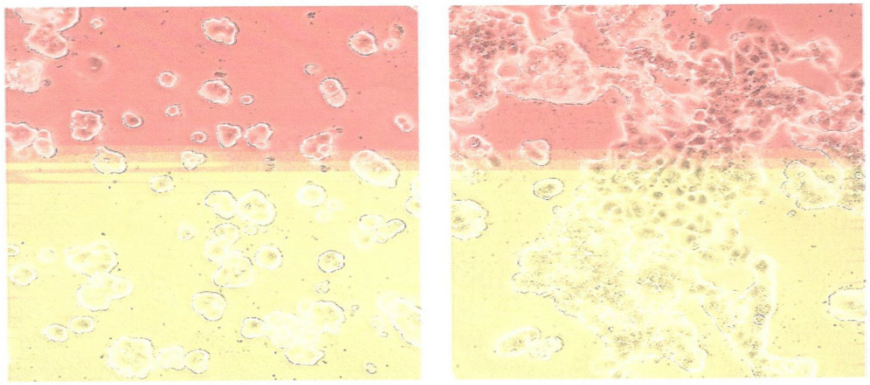
- Uncontrollable risks.
  - Early menarche
  - Late menopause
  - Age
  - Genes
  - Gender
- Controllable risks.
  - Alcohol
  - Use of birth control pills and hormone replacement therapy.

Risk Factors Cont’

- Exposure to high levels of radiation.
- Sedentary lifestyle
- Poor diet.
- Obesity.

11/6/2015 1980.ashx (800×600)

ATCC Number: **HTB-22**
Designation: **MCF-7**



11/6/2015 cells-hi.jpg (500×396)

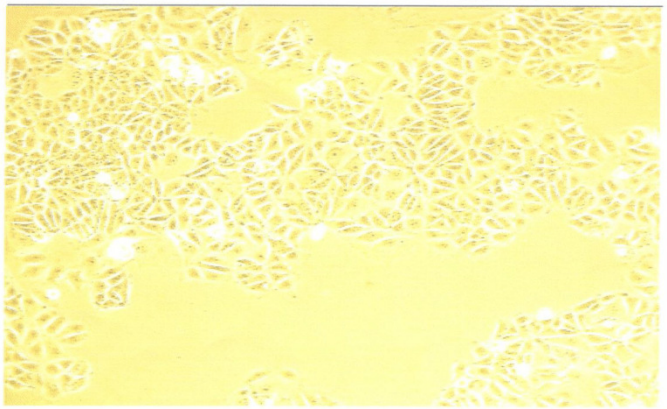


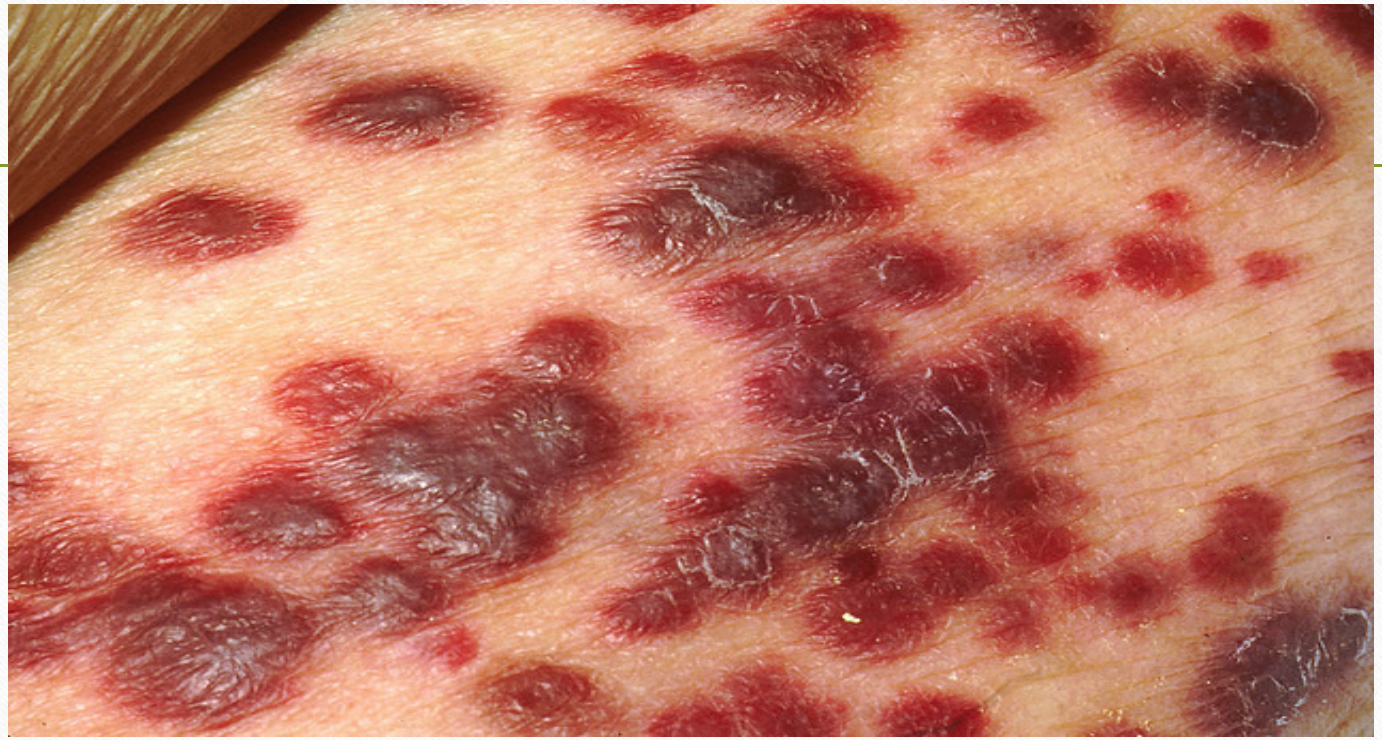
Figure 8.

MCF-7/HTB-22 Growing in Culture ATCC, 2015

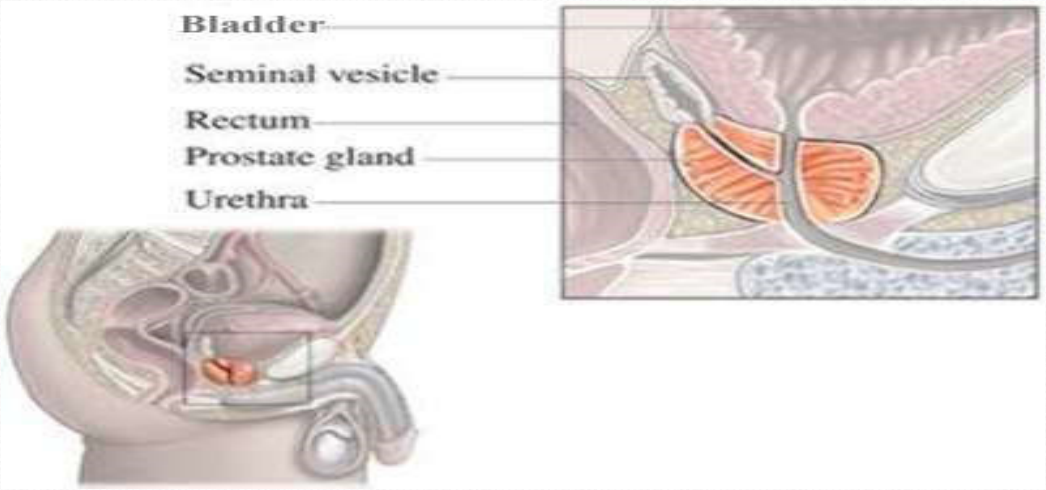
WORLD HEALTH ORGANIZATION POSTULATES THREE LEADING TYPES OF CANCER IN AFRICA

- BREAST
- PROSTRATE
- CERVICAL CANCER

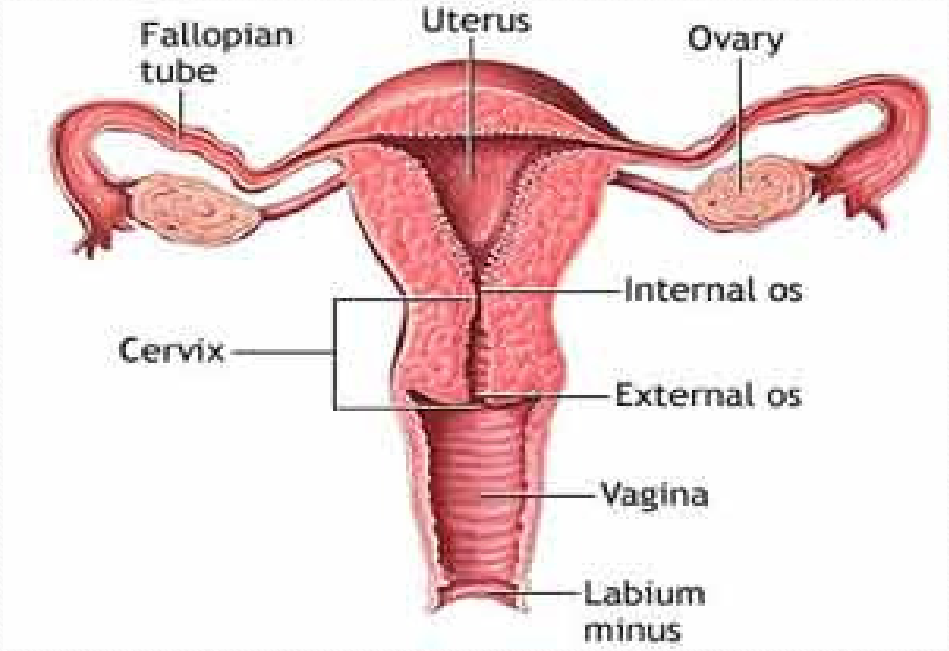
HIV-Associated Kaposis Sarcoma



Anatomy of the Human Prostate Gland



Anatomy of the Female Reproductive Organ



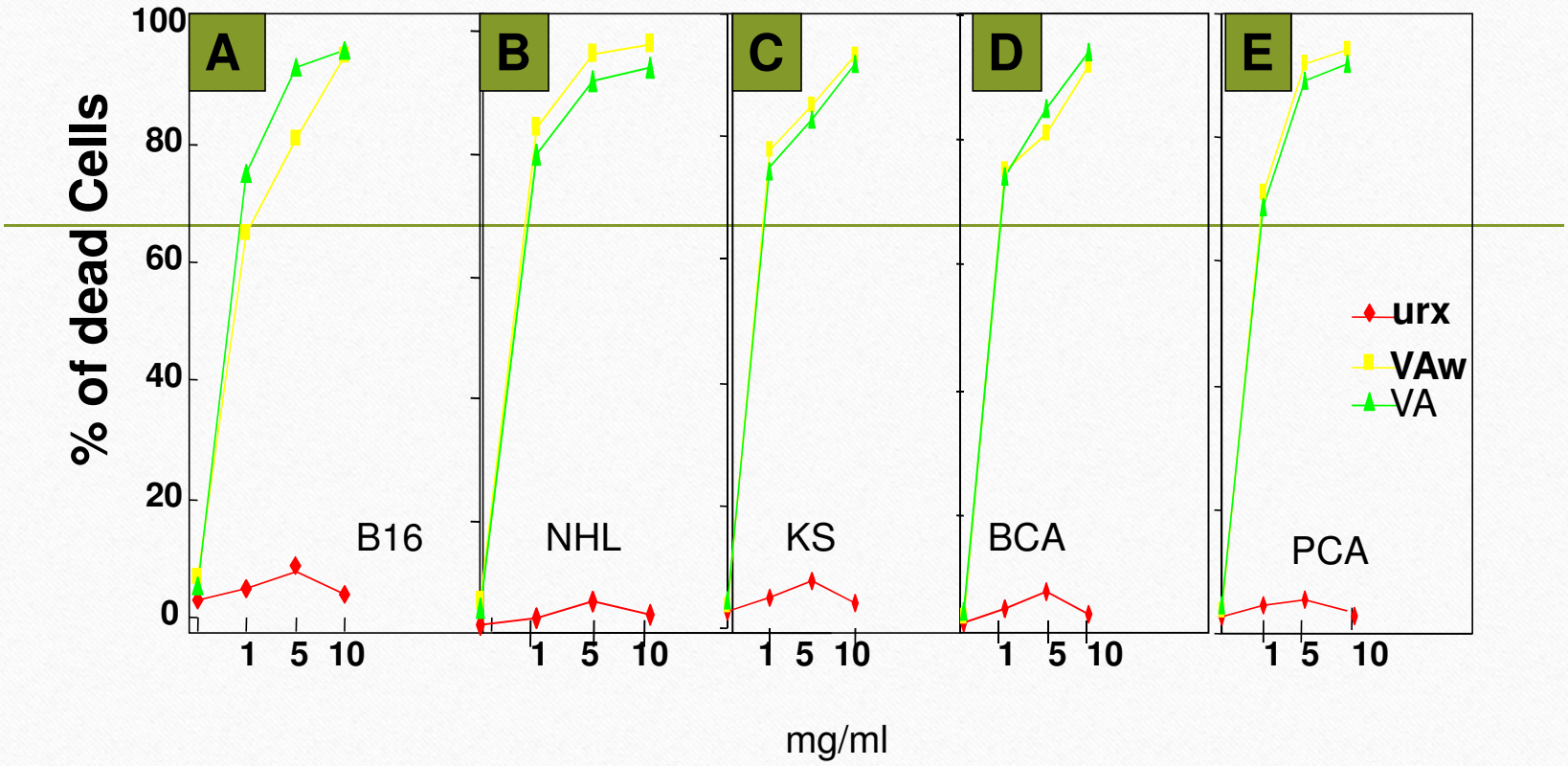
Evidence for VA Health Benefits

- Anti-cancer (Kupchan et al., 1969;Izevbigie, 2003)
- Anti-diabetes (Akah and Okafor 1992; Atangwho et al.,2009)
- Anti-malarial
- Anti-hypertension
- Anti-microbial or parasitic (Akinpelu, 1999)
- Hepatoprotective (Iwalokun 2006)
- Lipid-lowering activity (Akah and Okafor, 1992)

OBJECTIVE ONE

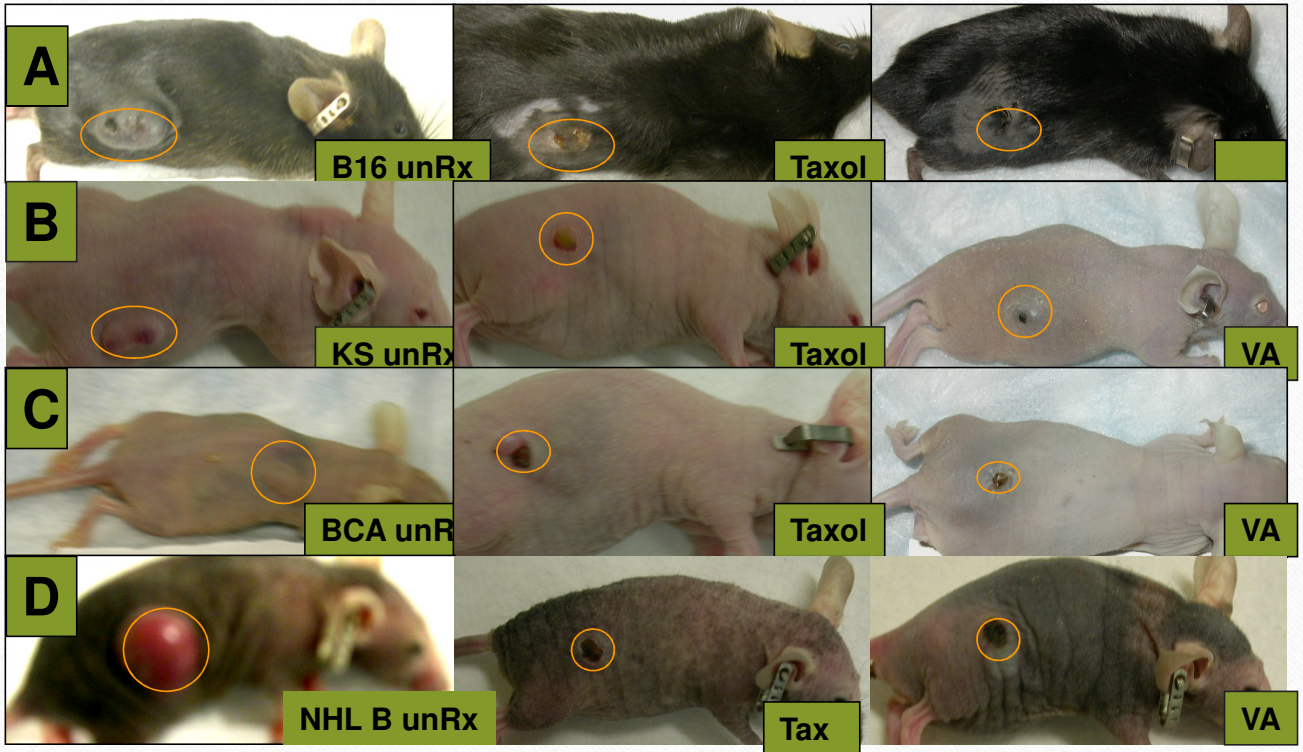
- To evaluate the effect(s) of a novel water-soluble leaf extract of *Vernonia amygdalina* (VA) on human breast cancer cell proliferative activities.
- MCF-7 cell line, considered a suitable model, was used in this study.

Fig. 1 . VA Extracts Abrogate the Growth of Five Types of Tumoral Cells In Vitro



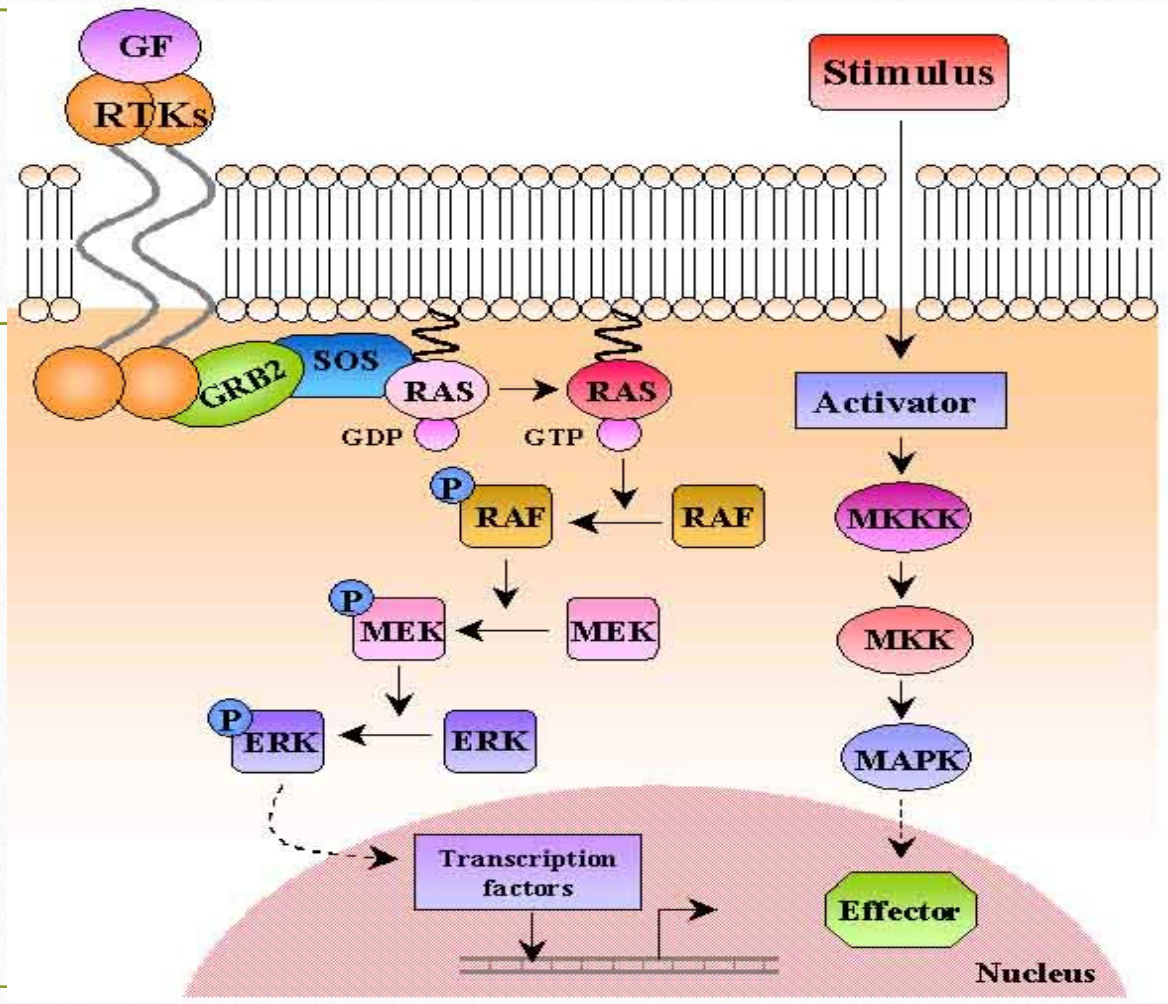
Objective Three: To Assess the Therapeutic Index of VA in Nude Mice

Fig. 2. Photographs of Xenografted Mice Treated with VA Extracts

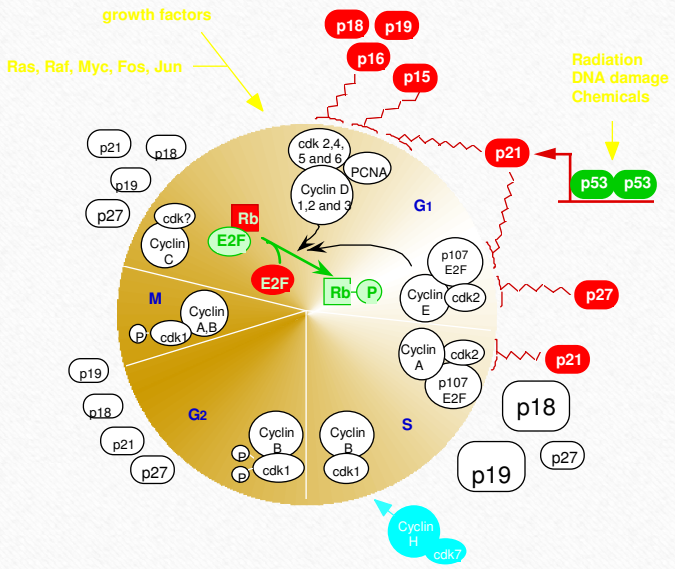


Mechanisms of Actions of VA

- MAPK activity inhibition
- Microtubule Destabilization
- Pro-Apoptosis
- Anti-tumorigenesis, metastasis, invasion by c-Myc and NF-Kb inhibition
- Anti-Multi-drug resistance


GF
RTKs
Stimulus
GRB2
SOS
RAS
RAS
GDP
GTP
Activator
P
RAF
RAF
MKKK
P
MEK
MEK
MKK
P
ERK
ERK
MAPK
Transcription factors
Effector
Nucleus

Cell Cycle Regulators/Signal Transuction



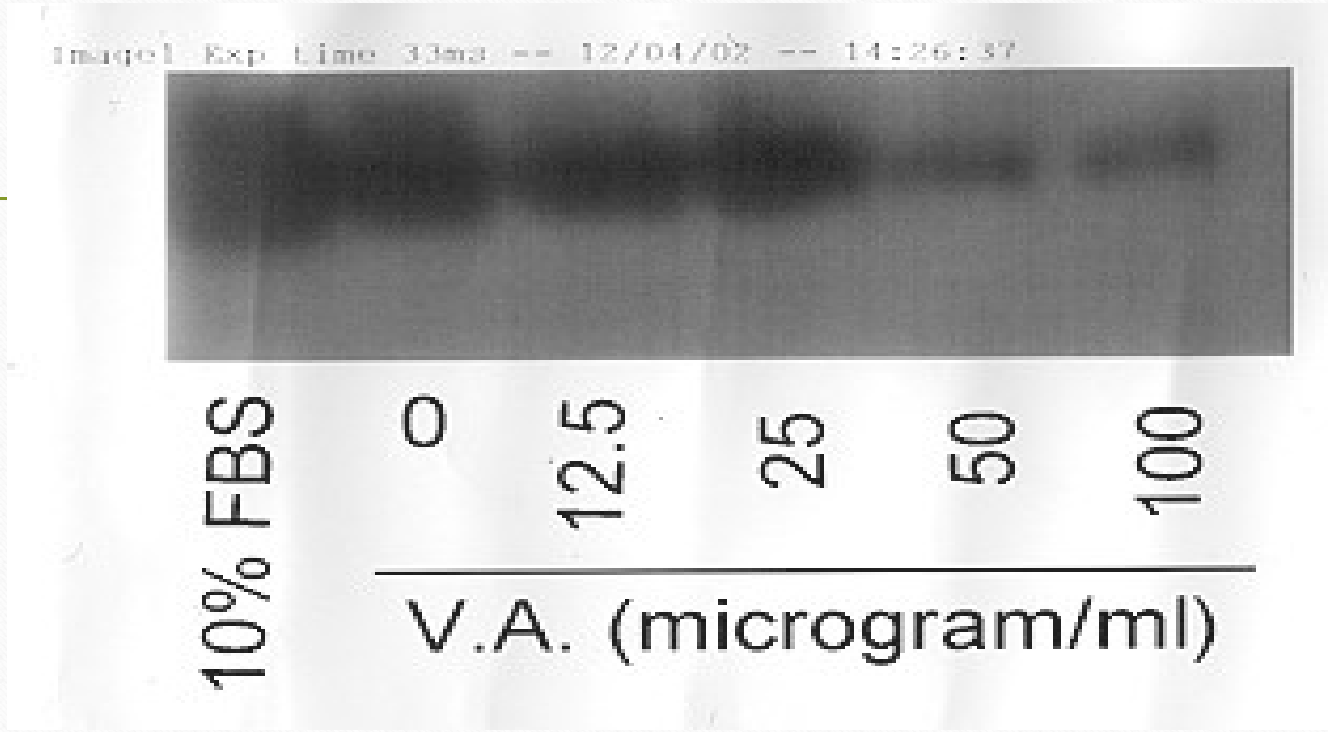
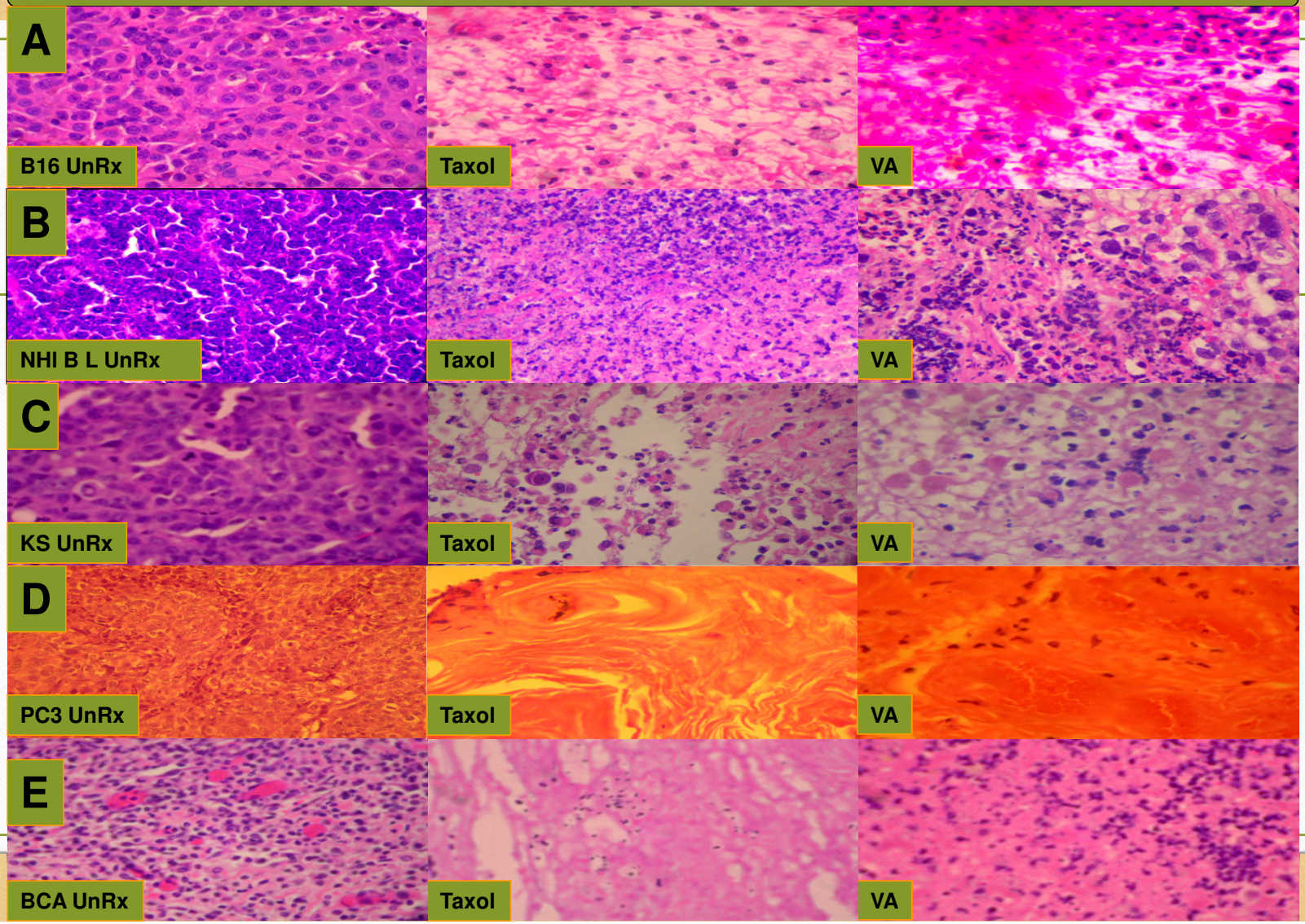
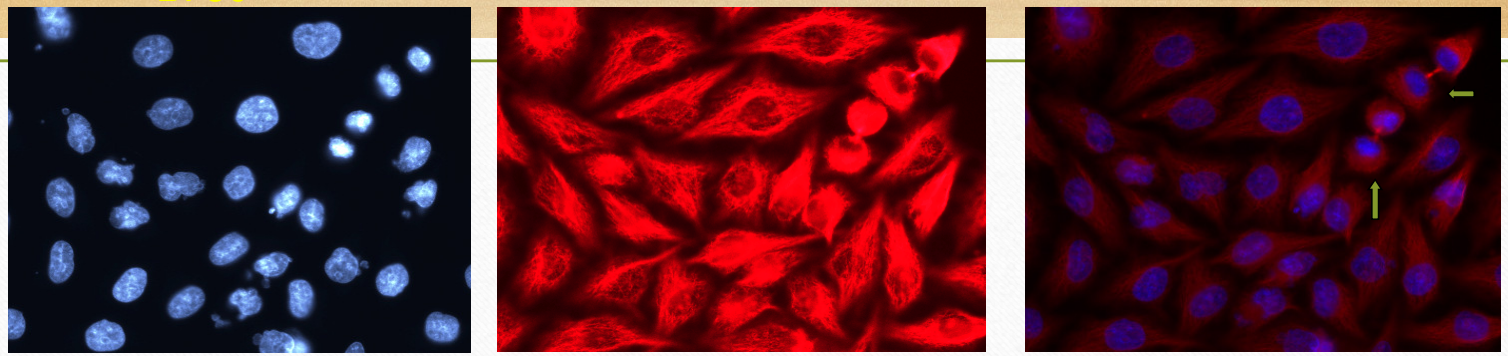


Image1 Exp Time 33ms -- 12/04/02 -- 14:26:37
10% FBS
0
12.5
25
50
100
V.A. (microgram/ml)

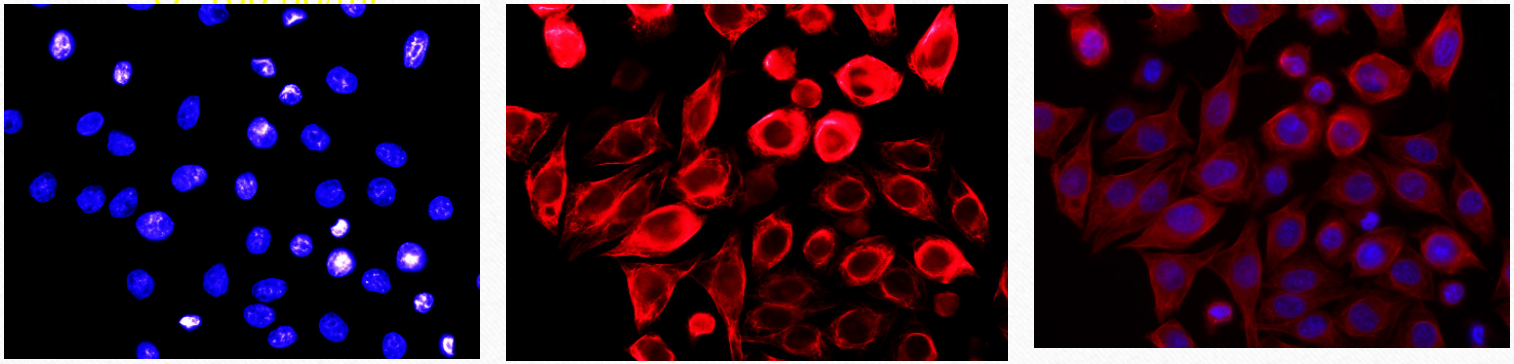
Fig. 3. VA induced Cellular Morphological Changes by H & E stains



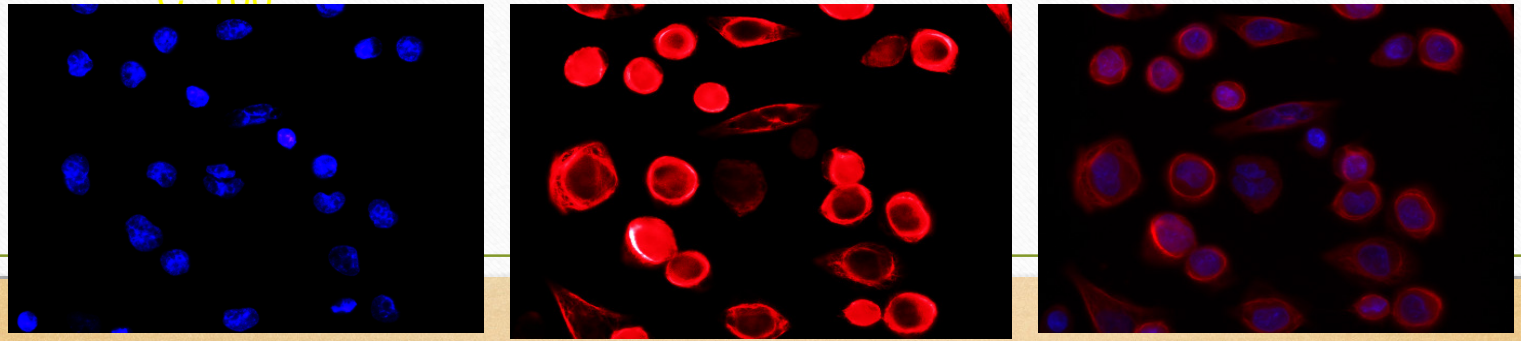
B. 50



C. 100 ug/ml



C. 100

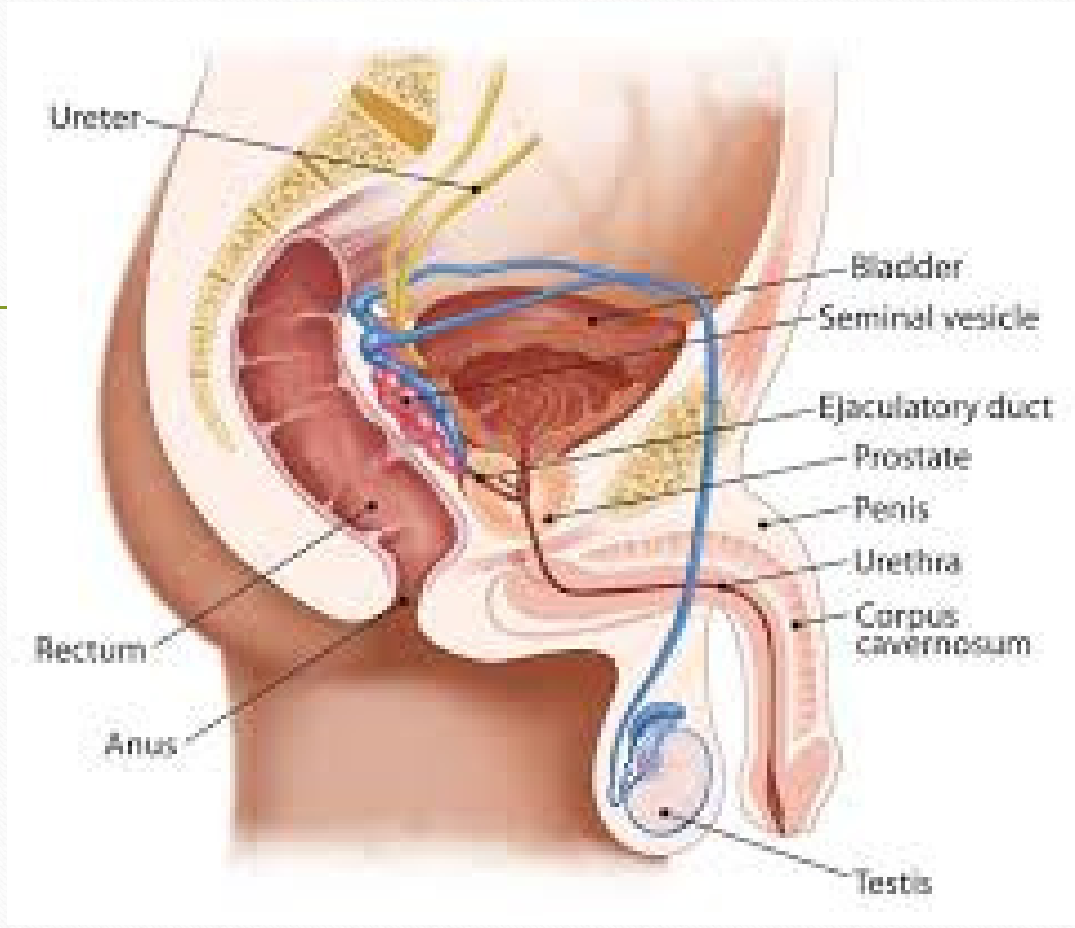


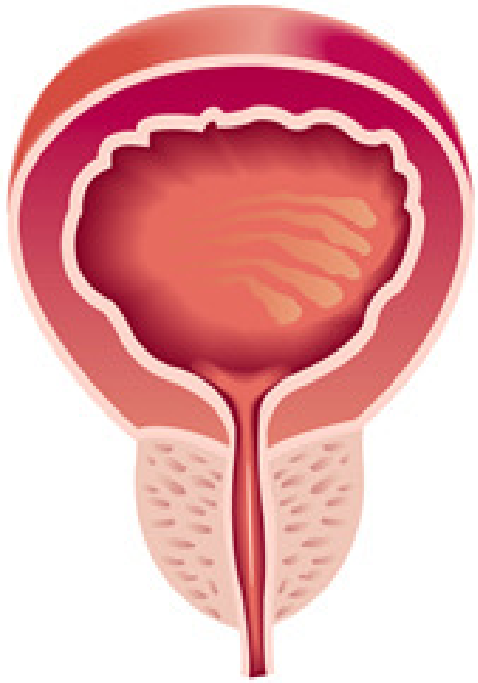
CHAPTER 3

- **PROSTATE ADENOCARCINOMA**
- Prostate cancer (PC), the most common type of cancer found in American men, accounts for 10% of all cancer-related deaths in men (Cancer Statistics & Figure, 2014). Risk factors associated with PC include age, familial history, ethnicity and hormonal status with seventy-five percent of all cases of PC found in men 65 years or older (Ramon and Denis, 2007).

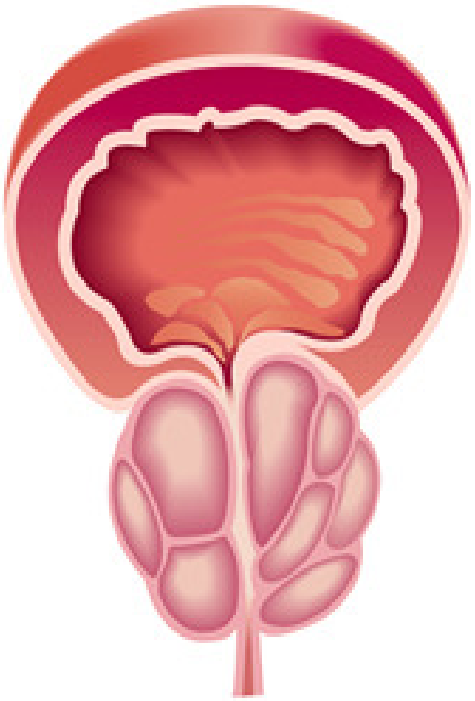
OBJECTIVE

- ASSESSMENT OF THE ANTIPOLIFERATION ACTIVITY OF VERNONIA AMYGDALINA IN PROSTRATE ADENOCARCINOMA CELLS

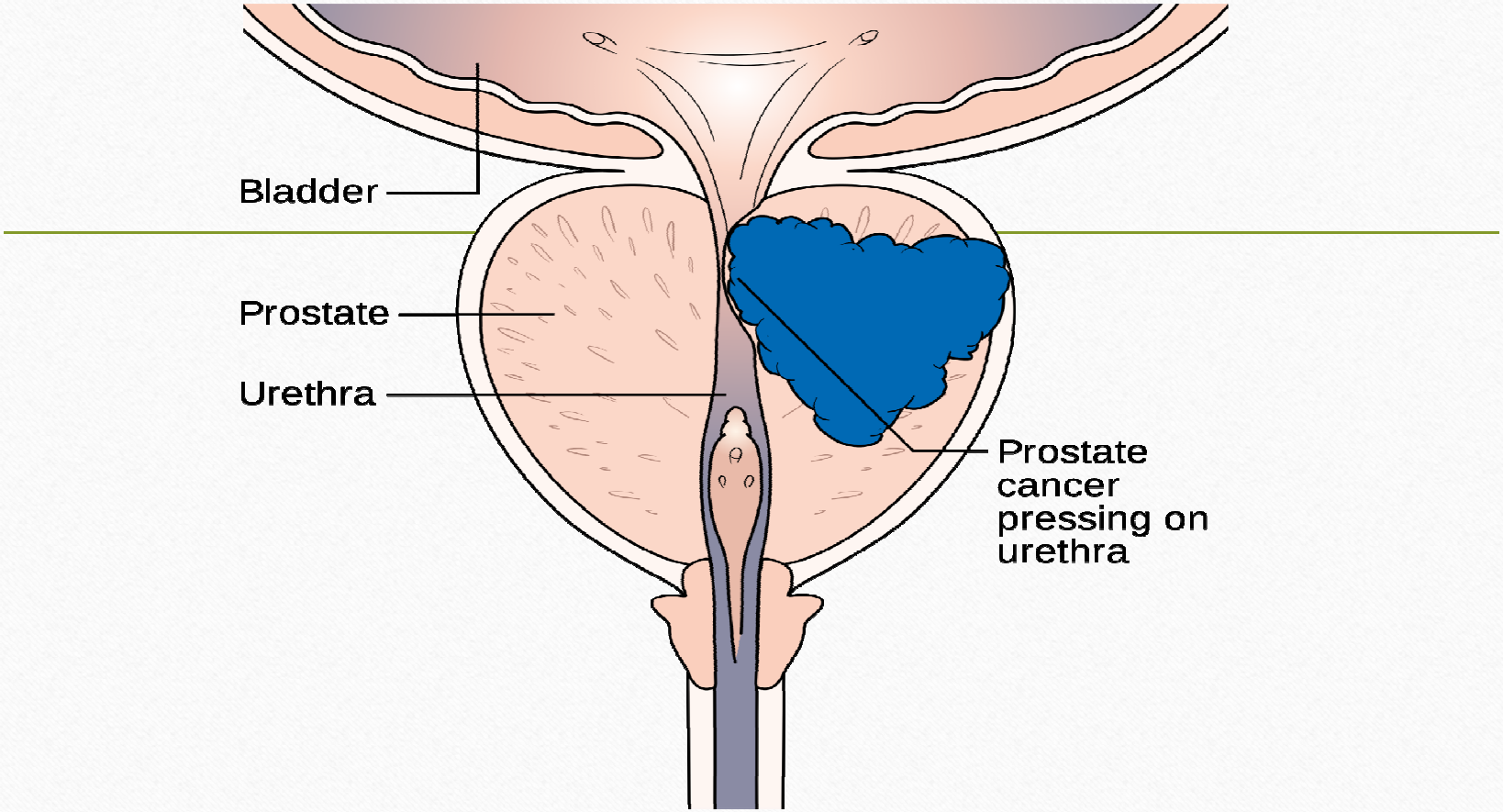

Ureter
Bladder
Seminal vesicle
Ejaculatory duct
Prostate
Penis
Urethra
Corpus cavernosum
Rectum
Anus
Testis



Normal prostate



Prostatic hypertrophy


Bladder
Prostate
Urethra
Prostate cancer pressing on urethra

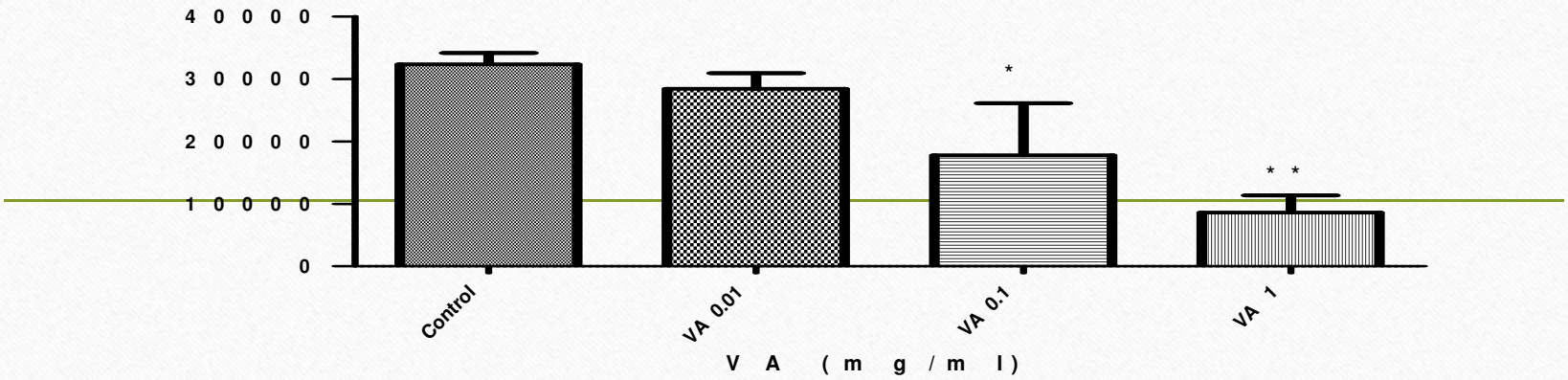


Figure 15: Concentration-dependent Inhibition of DNA Synthesis by VA extracts. Cells at the logarithmic growth phase were treated with 0.01, 0.1, and 1 mg/ml VA for 18h before the addition of 1 µCi/ml [3H] thymidine. Each data point represents the mean of three independent experiments done in triplicates (n=9). Exposure of cells to 0.01, 0.1, and 1mg/ml inhibited DNA sythesis by 12, 45, and 73% respectively. DNA synthesis was determined as described under Materials and Methods sectiopn. * represents $p < 0.05$; ** $p <$ 0.01.

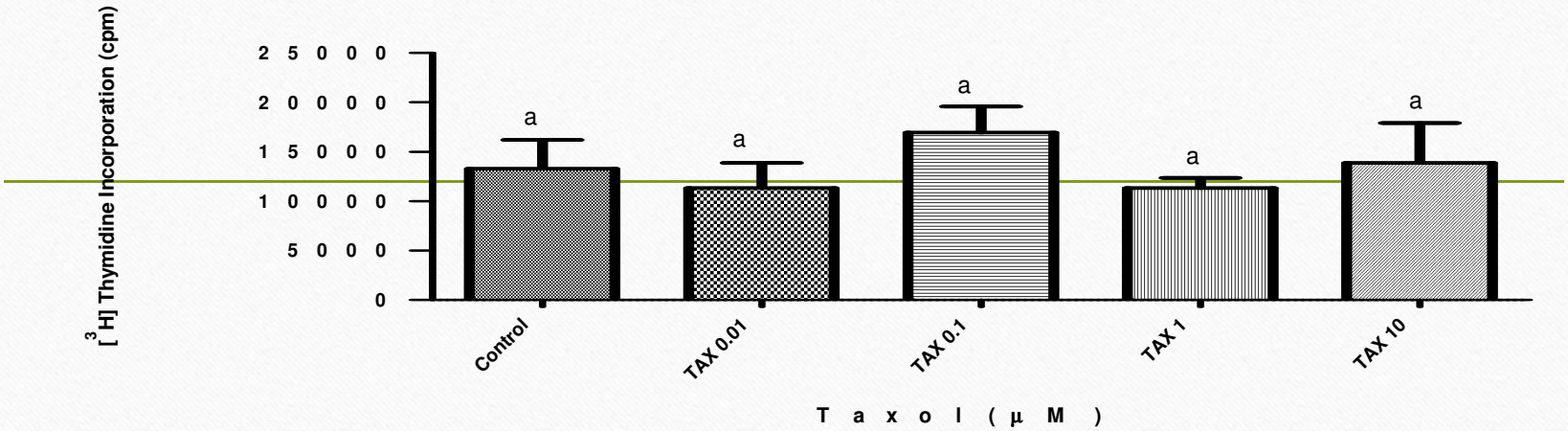
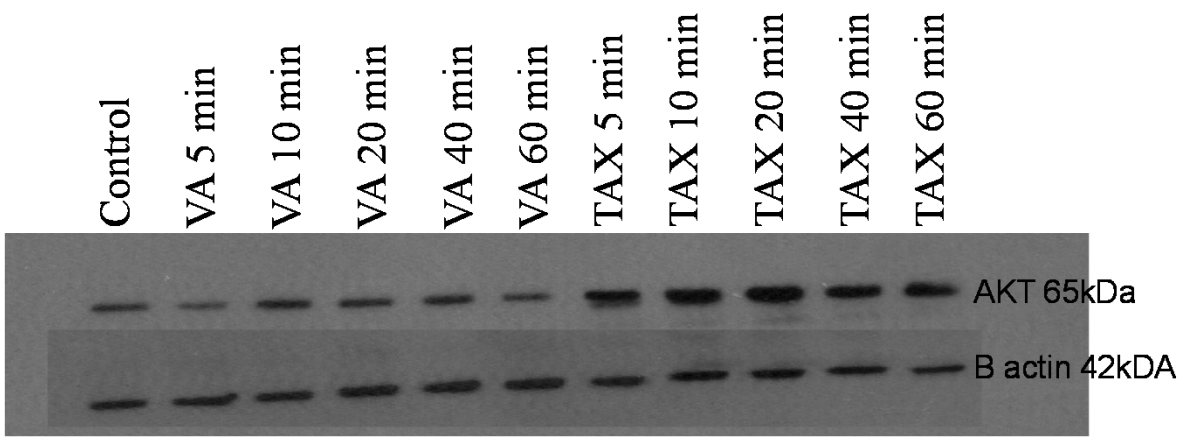
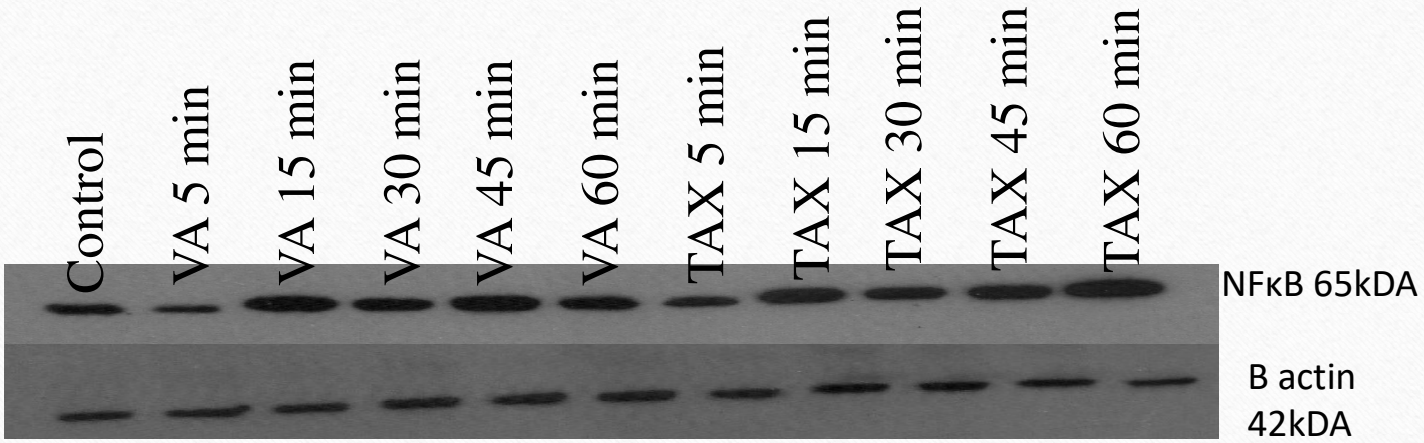


Figure 16: Insensitivity of PC-3 Cells treated with various concentrations of Taxol. The cells at the logarithmic growth phase were treated with 0.01, 0.1, and 1 mg/ml Taxol before the addition of 1 μCi/ml [3H] thymidine. Each data point represents the mean of three independent experiments done in triplicates (n=9). Data means represented by the same letter are not statistically different from each other.

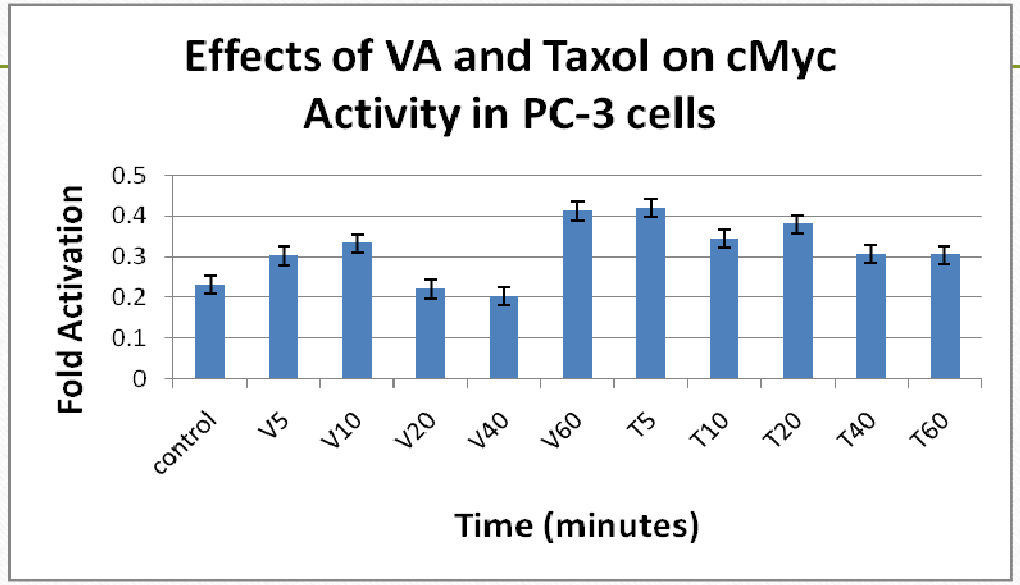
Pro-cancer Molecule, AKT



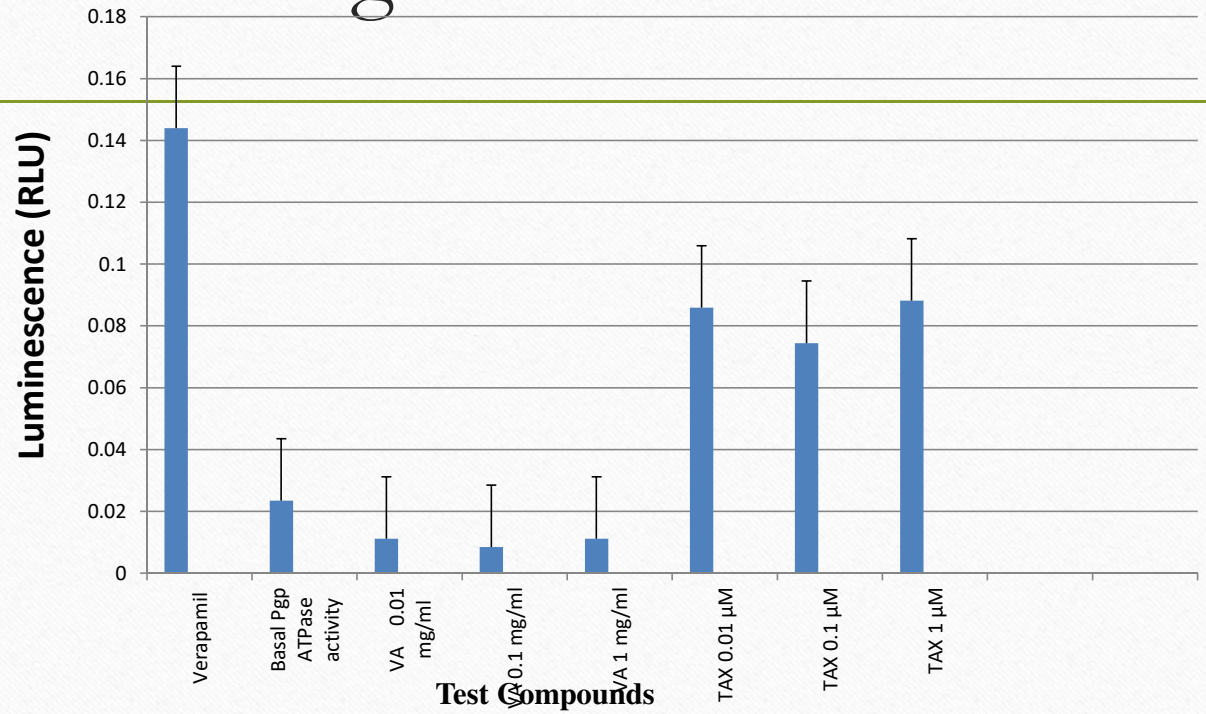
Pro-cancer Molecule, NF-kB



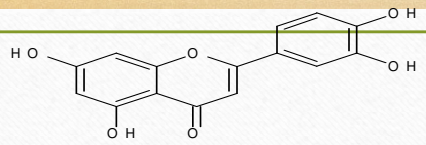
Proto-oncogene



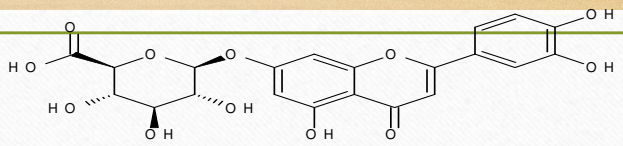
Multi-Drug Resistance Molecule



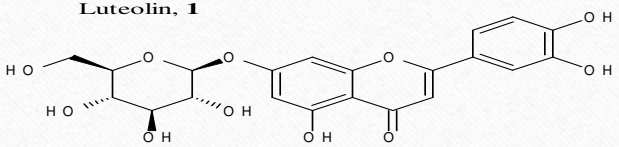
ACTIVE COMPONENT OF THE VERNONIA AMYDGLALINA



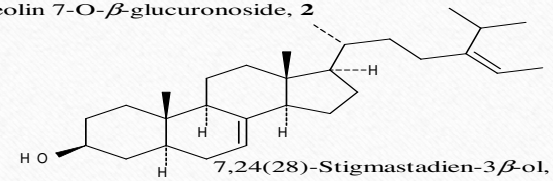
Luteolin, **1**



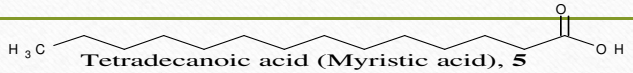
Luteolin 7-O-β-glucuronoside, **2**



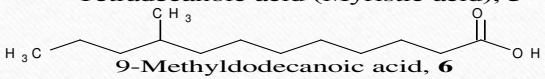
Luteolin 7-O-β-glucoside, **3**



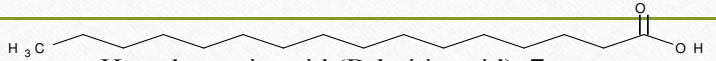
7,24(28)-Stigmastadien-3β-ol, **4**



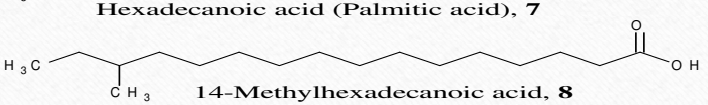
Tetradecanoic acid (Myristic acid), **5**



9-Methyldodecanoic acid, **6**



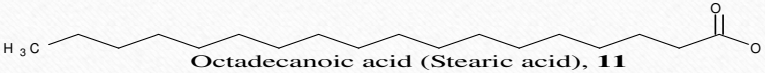
Hexadecanoic acid (Palmitic acid), **7**



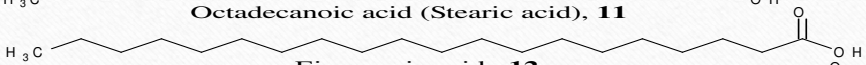
14-Methylhexadecanoic acid, **8**

(Z,Z)-9,12-octadecadienoic acid (Linoleic acid), **9**

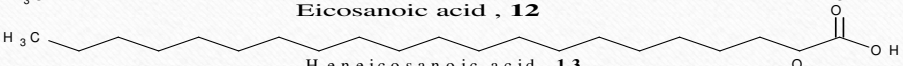
(Z,Z,Z)-9,12,15-octadecatrienoic acid (α-Linolenic acid), **10**



Octadecanoic acid (Stearic acid), **11**

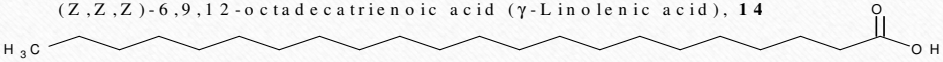


Eicosanoic acid , **12**

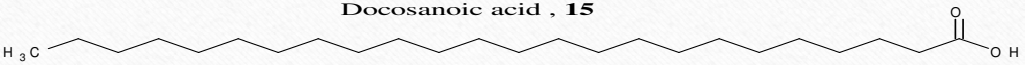


Heneicosanoic acid, **13**

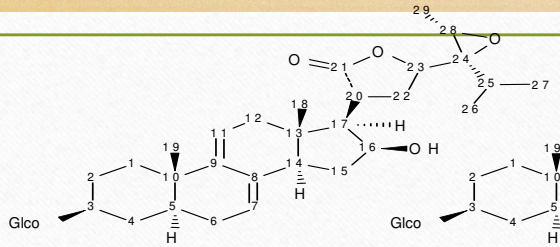
(Z,Z,Z)-6,9,12-octadecatrienoic acid (γ-Linolenic acid), **14**



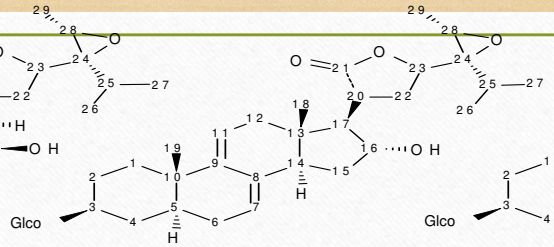
Docosanoic acid , **15**



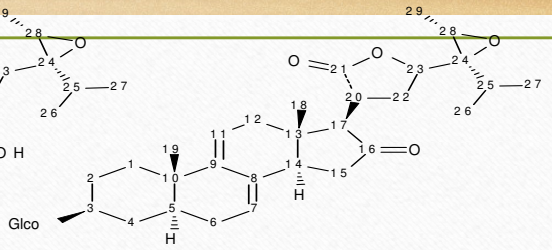
Tetradocosanoic acid , **16**



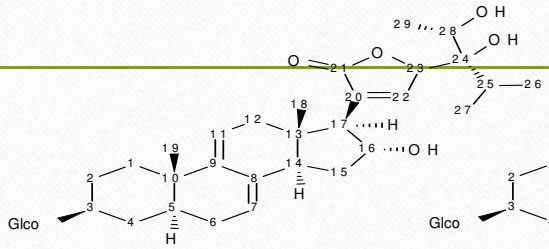
Vernonioside A1, **17**



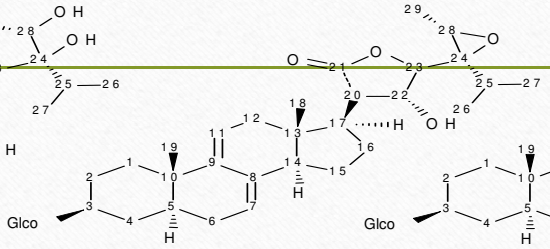
Vernonioside A2, **18**



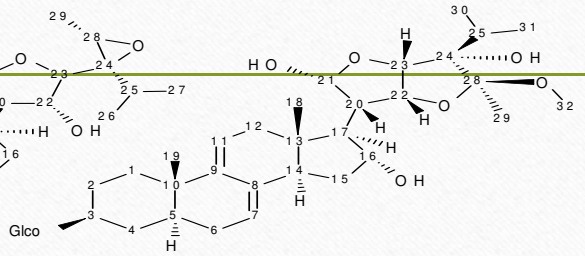
Vernonioside A3, **19**



Vernonioside A4, **20**



Vernonioside B1, **21**



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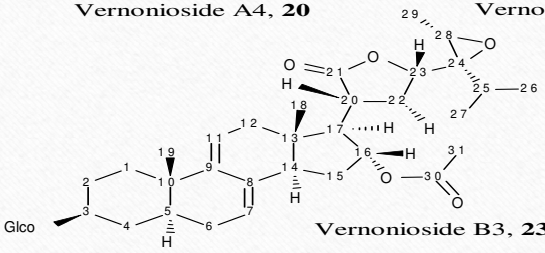
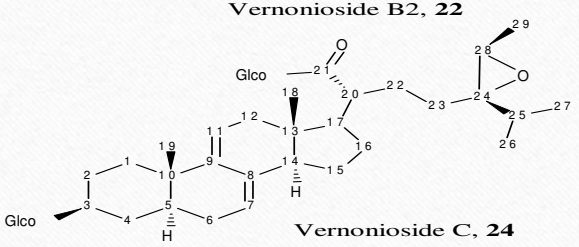
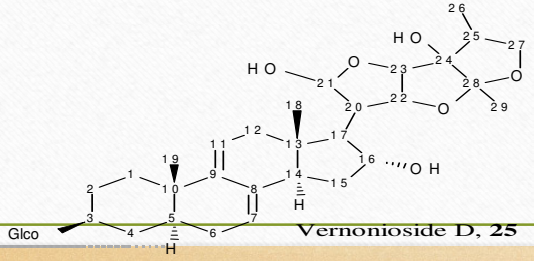


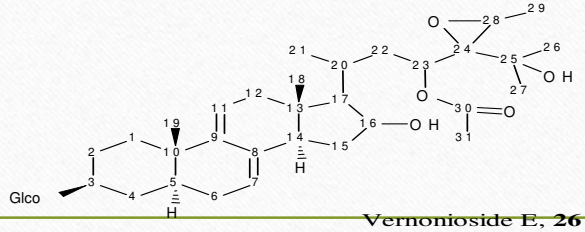
Vernonioside B3, **23**



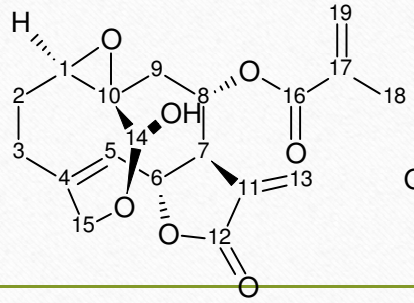
Vernonioside C, **24**



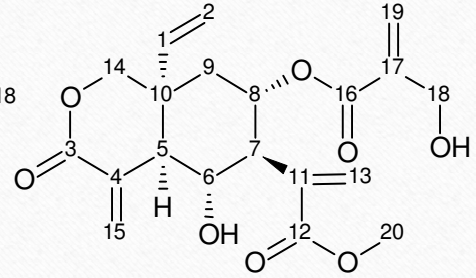
Vernonioside D, **25**



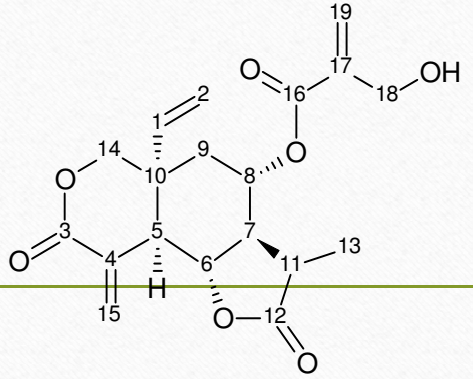
Vernonioside E, **26**



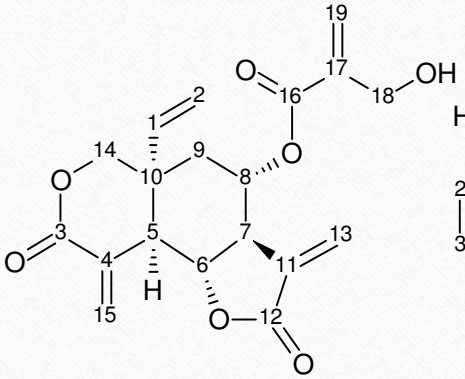
Vernolide, **27**



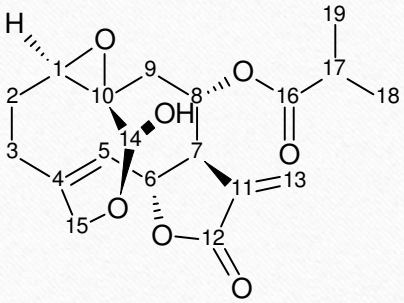
Vernodalol, **28**



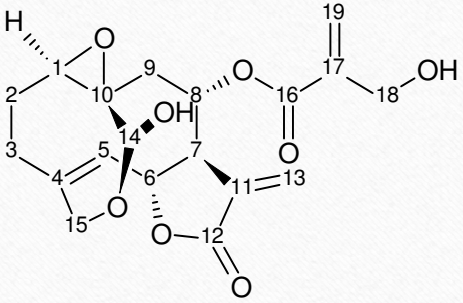
11,13-Dihydrovernodalin, **29**



Vernodalin, **30**



Vernomygdin, **31**



Hydroxyvernolide, **32**

Sesquiterpene Lactones Activities

1. SLs induce Apoptosis
2. SLs inhibit MAPK/ERKs Activities
3. SLs down-regulate pro-metastasis molecule factor (NF kappa B) expression
4. SLs inhibit IMP dehydrogenase activity
5. SLs inhibits Aromatase activity
6. SLs and Immuno-modulatory activities
7. NCI (Dictionary of cancer terms) defines SLs “a substance found in some plants. SLs may have anti-inflammatory & anti-cancer effects. Plants containing SLs have been used in some cultures to treat certain medical problems”.

Conclusion

- VA inhibits the growth of cancerous cells under in vitro and in vivo conditions
- VA is well tolerated by animals
- Several mechanisms (oncogenic molecules; c-myc, NF-Kb, AKT activities are attenuated by VA
- VA promotes apoptosis and microtubules destabilization in cancerous cells.

Clinical Relevance

- These data further suggest that *VA* extracts represent very promising anti-caner agents… that warrant well-controlled clinical investigation.

Translation Continued

"The doctor of the future will give NO medicine, but will interest her or his patients in the care of human frame, in a proper diet, and in the cause and prevention of diseases"

Thomas A. Edison

US Inventor (1847-1931)

- **THE PLANT CALLED VERNONIA AMYGDALINA**
- Bitter leaves are derived from the Vernonia amygdalina plant. Vernonia amygdalina, a member of the asteraceae family, is a small shrub that grows in the tropical Africa. V. amygdalina is commonly called bitter leaf because of its bitter taste. The VA leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses.

- In the wild, Chimpanzees have been observed to instinctively ingest the VA plant leaves when suffering from parasitic infections. Many herbalists and naturopathic doctors recommend aqueous VA for their patients as treatment for emesis, nausea, diabetes, loss of appetite-induced ambrosia, dysentery, and other gastrointestinal (GI) Tract problems.

- Until the last decade or so, there were only anecdotal reports and claims to support the health benefits of VA. The anecdotal reports are now being supported by compelling scientific evidence that VA regimen or consumption (as dietary supplements) may provide multiple health benefits in the areas of:

Translation into Economic Value

Grand Summary

Mr. Vice Chancellor / Chairman Sir,

- The essence of Inaugural Lecture, perhaps amongst other things, is to provide rationale (s) why one is deserving of the award of the title "Full Professor" and further to demonstrate or show individual's intellectual contribution to knowledge and societal growth. It is in this premise, I state the following:

- Three (3) U.S patents (One Nigeria issued Patent) – Covering novel scientific concepts now used as reference points for other scientists.
- The phenomena/ biotechnologies described in the patents have been licensed to a biotechnology company in the U.S that commercialized the products called edoTIDE™
- University spin-off biotechnology company was established, edoBotanic LLC, with a current staff strength of 5 individuals, and counting. There are 3 distributors of edoTIDE™ in Nigeria
- edoTIDE™ is commercially available in the U.S., Nigeria and other parts of the Globe.
- edoTIDE™ products are registered by NAFDAC in Nigeria.
- Protocol to conduct Phase III clinical trial has gone through the Ethics Committee of the University of Benin, under revision for re-submission

- Many people, may be some in the audience including me, are beneficiaries of edoTIDE^{TM}
- The Edo State Government has graciously allocated a parcel of land measuring 50 hectares around the Obayanto Community for the Propagation, Processing into finished products, packaging of the bitter leaf plants.
- Healthier Edo State and Nigeria → results in contribution to GNP.
- Employment created by edoTIDE^{TM} products generate payment of taxation at local, state and Federal Government levels, thus, contributing to National Economy.
- Editorial Board of Technology and Innovation, U.S.A
- Positive publicity for Nigeria


edoBotanics™
edoTIDE plus™
Dietary Supplement
Promotes Healthy
Glucose Level*
60 capsules
750 mg
edoBotanics™
edoTIDE plus™
Dietary Supplement
Promotes Prostate &
Immune System Health*
60 capsules
600 mg

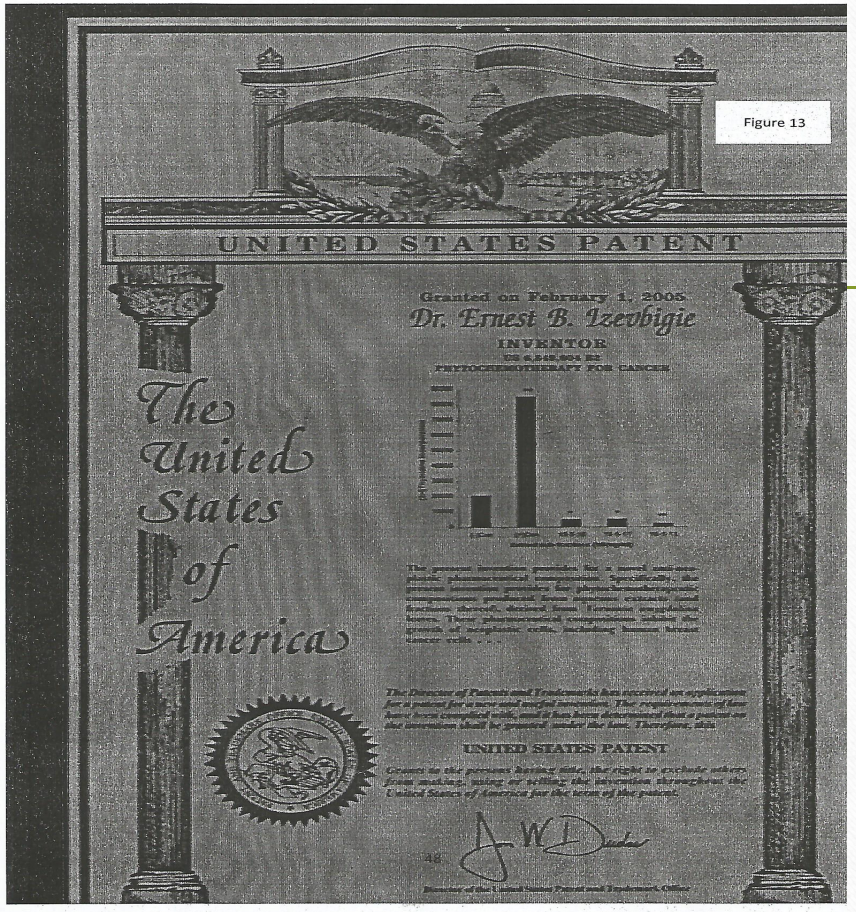


Figure 13
UNITED STATES PATENT
Granted on February 1, 2005
Dr. Ernest B. Izevbigie
INVENTOR
The United States of America
UNITED STATES PATENT

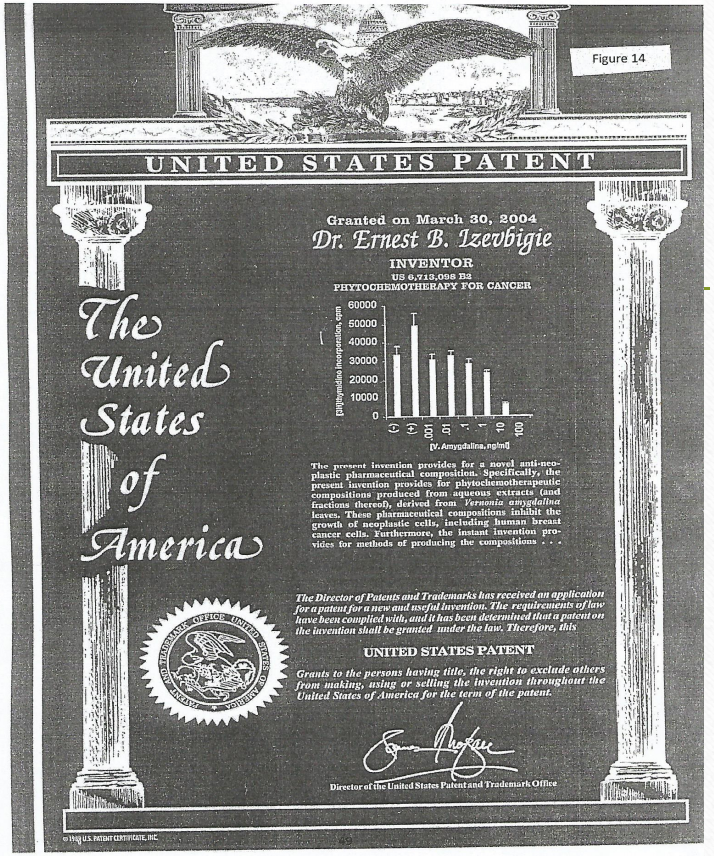
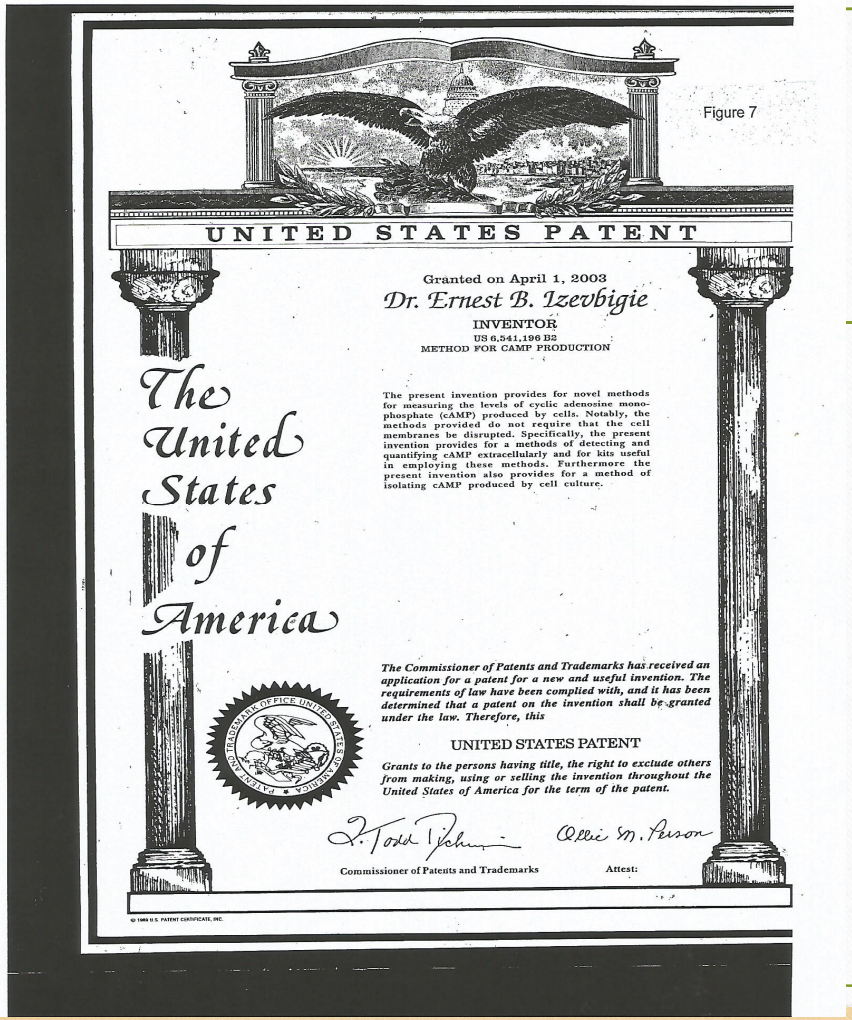


Figure 14
UNITED STATES PATENT
Granted on March 30, 2004
Dr. Ernest B. Izevbigie
INVENTOR
US 6,713,098 B2
PHYTOCHEMOTHERAPY FOR CANCER
The United States of America
[V.Amygdalina, ng/ml]
The present invention provides for a novel anti-neoplastic pharmaceutical composition. Specifically, the present invention provides for phytochemotherapeutic compositions produced from aqueous extracts (and fractions thereof), derived from Vernonia amygdalina leaves. These pharmaceutical compositions inhibit the growth of neoplastic cells, including human breast cancer cells. Furthermore, the instant invention provides for methods of producing the compositions . . .
The Director of Patents and Trademarks has received an application for a patent for a new and useful invention. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law. Therefore, this
UNITED STATES PATENT
Grants to the persons having title, the right to exclude others from making, using or selling the invention throughout the United States of America for the term of the patent.
Director of the United States Patent and Trademark Office

Figure 7



UNITED STATES PATENT

Granted on April 1, 2003

Dr. Ernest B. Izevbigie

INVENTOR

US 6,541,196 B2

METHOD FOR CAMP PRODUCTION

The present invention provides for novel methods for measuring the levels of cyclic adenosine monophosphate (cAMP) produced by cells. Notably, the methods provided do not require that the cell membranes be disrupted. Specifically, the present invention provides for a methods of detecting and quantifying cAMP extracellularly and for kits useful in employing these methods. Furthermore the present invention also provides for a method of isolating cAMP produced by cell culture.

The United States of America

The Commissioner of Patents and Trademarks has received an application for a patent for a new and useful invention. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law. Therefore, this

UNITED STATES PATENT

Grants to the persons having title, the right to exclude others from making, using or selling the invention throughout the United States of America for the term of the patent.

Commissioner of Patents and Trademarks

Attest:

ACKNOWLEDGEMENT

- Thank God for his blessings (Jeremiah 33:3; 1 Corinth 2:9-14)
- Dr. William Nelson, Johns Hopkins University School of Medicine, Baltimore, MD
- RCM1/NIH grant #s:G122RR1359-07S1; P20MDOOO53401

- **God** First and foremost, I would like to thank God for being greater than he who is in the world (1 John 4:4).
- **Family** To my loving wife, and 3 children for their support and sacrifice.

~~To my loving parent, Mr. Benjamin I.E. Izevbigie and Mrs. Esther E. Izevbigie~~ whose genetic materials I am, for their love guidance and support.

- **Clerical Support**

My appreciation is also extended to my clerical staff for their assistance in the preparation of this document.

- **Graduate Students /Research Scientists** who conducted some of these experiments under my direct supervision.
- Dr. Xuan Luo, China University.
- Dr. Keyana Cameron, University of Tennessee College of Medicine.
- Dr. Michael Opata, University of Texas at Garveston, TX.
- Brandon Hill, University of Alabana @ Birmingham, AL.
- Dr. Lecia Grasham – Robbinson, Jackson State University.

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