24TH INAUGURAL LECTURE

TOPIC

ETHNOPHARMACOLOGY IN THE TRIANGLE OF MAN, ANIMAL, AND DISEASE

BY

PROFESSOR OLATUNDE PETER AJAGBONNA
DVM (IBADAN), M.SC (LAGOS) PH.D
(UDUS, SOKOTO)
Professor of Pharmacology and Toxicology

UNDER THE DISTINGUISHED CHAIRMANSHIP OF
PROFESSOR MICHAEL U. ADIKWU, FAS, FPSN FSTAN
VICE-CHANCELLOR, UNIVERSITY OF ABUJA

25TH MAY, 2017.

PROTOCOL

The Vice-Chancellor

Deputy Vice-Chancellors (Academic and Administration)

Registrar, Bursar, and University Librarian

Provost, Deans, and Directors present

Head of Departments/Units

Distinguished members of Senate

My Lord Spiritual and Temporal

Distinguished Professional Colleagues

Distinguished Guests and Friends of the University

My Students

Gentlemen of the Press

Ladies and Gentlemen.

Preamble

Mr. Vice-Chancellor Sir, an inaugural lecture is an occasion of significance in an academic staff member's career at the university. It provides newly appointed professors with the opportunity to inform colleagues, the campus community and the general public of their work to date, including current research and future plans. It is our own coronation in academy.

Professors are usually required to give their inaugural lecture within twelve months of their appointment but in Nigerian universities, the time after the appointment when the lecture is given varies according to other commitments in the university. This lecture was to have been delivered in 2006, but I had a clarion call to join late Prof. I. Ajogi to establish a Faculty of Veterinary Medicine at this University. The battle to establish the Faculty took us another six years of sleepless nights, prayers and fasting before we could secure both the NUC and VCN accreditation to train veterinary doctors in this University. It was first of the four new programs to get out of the wood, for this,

I am grateful to the Almighty God, having also been part of the foundation staff that established the elite Veterinary School of Usmanu Danfodio University in the city of the Caliphate (Sokoto). Although, this lecture is coming today, 14 years after I was appointed as the first Professor of Pharmacology and Toxicology in 2003 in the department and the first indigenous professor in the history of Faculty of Veterinary Medicine, Sokoto.

I feel particularly happy and honoured on this occasion to stand before such an august audience as gathered here today. This inaugural lecture is the 24th in the series of such inaugural lectures of this University and incidentally, the first from the Department of Veterinary Pharmacology and Toxicology and also the very first from the Faculty of Veterinary Medicine in this University.

My Early Life and Some Defining Moment in my Career

There is a saying that, you do not have to feel trapped by your circumstances, you can always live a fruitful and fulfilling life, believing that God will provide the means to accomplish the impossible. This statement summarizes my early life, struggles and the rough path i took to where I am today.

- Born at Nigerian independence to an illiterate mother and father in the popular Okun town of Egbe in Kogi State.
- Afflicted with a strange disease at the very early stage in life, when Medicare was almost non-existence. I was humanly condemned to die within two weeks, and was told the prayer of my mother who just lost a son before me was God; please just spare this one, even if he was not going to amount to anything. Mr. Vice chancellor sir, glory be to God I am not only alive today, I am 57 years old and stand before you as a Professor of Pharmacology and Toxicology to deliver the 24thinaugural lecture of this University.
- 1994 – 1995, after my selection for the commonwealth elite scholarship for a Ph.D. in the United Kingdom, the joy was also short-lived because

Nigeria was suspended from the commonwealth of Nations following the killing of Ken SaroWiwa. My dream of doing a Ph.D. in the U.K was instantly aborted. It took me almost another three years to overcome this great set back. I picked up the pieces left by this development and settled for a local Ph.D. and the Lord provided the divine speed that enabled me to finish in a record time of two years 6 months.

My Journey into Ethnopharmacology

Mr Vice Chancellor sir, with a letter of admission already in my hand to study for my Masters at the prestigious University of Ibadan, I met a friend and a brother now Prof. F.B.O Mojiminiyi, who encouraged me to go to University of Lagos, where I needed to take an entrance examination before being considered for admission. Little did I know he was God sent to direct my path to an administrator, a father and a mentor per excellence Prof. Olusoga Sofola (former Provost, DVC Academic and Research, University of Lagos, who not only taught me the rudiment of research but gave me unfettered access to his vascular smooth laboratory the first then in the country. I picked my first M.Sc. in physiology from this laboratory working on the toxicity of the two common trypanocidal drugs (Samorin and Berenil) on the cardiovascular system. (Ajagbonna et al., 1995, 1996)

I was almost rounding up my dissertation when a memorandum of understanding between World Health Organization (WHO) and the Faculty of Pharmacy, University of Lagos gave rise to a centre for medicinal plant research. Curiously, in our laboratory because of our on-going work on cardiovascular system/blood pressure, the popular plant Hibiscus Sabdariffa extract (Zobo/Ishapa) was tested on animal and found to lower blood pressure in a normotensive animal, the result from this plant incidentally set the tone for my journey into the field of ethnopharmacology.

The excitement thereafter was that if Zobo could lower blood pressure, a peep therefore into the forest of Nigeria may likely reveal another plant with beneficial effect against many other diseases of man and animal.

I, therefore, decided to enrol for another M.Sc and later a Ph.D. in Pharmacology, a complete shift from my first love (physiology) to pharmacology, where I had to carry stem, root, bark and leaves of plants for almost three decades now. Many have asked me on several occasions, are you a herbalist, a veterinarian or a pharmacologist, because for many years now, my close research associates, are those people that sell, prescribe or know something about herbs and how they are prepared.

Mr Vice chancellor Sir, the topic of my lecture today is therefore titled Ethnopharmacology in the Triangle of Man, Animal, and Disease.

God in the Origin of Medicine to Man and Animal

- And the Lord God planted a garden eastward in Eden, and there He put the man whom he had formed and out of the ground made the Lord God to grow every TREE that is pleasant to the sight and good for medicine (Gen. 2:8-9).
- Then God said, let the earth bring forth GRASS, the HERBS that yield seed and the fruit tree that yield fruit according to its kind and God saw that it was good and He approved it.
- In Ezekiel 47:12, God said again that fruit trees of all kinds will grow on both banks of the river, their LEAVES will not wither nor will their fruit fail. Their FRUIT will serve for food and their LEAVES for HEALING/MEDICINE (NIV).
- In Ps. 104:14, the Lord caused the grass to grow for the cattle and herbs for the service/healing of man.
- For man believes he may eat all things, but he who is weak/sick eats only VEGETABLES/HERB (Rom. 14:2).

- And Prophet Isaiah prescribes in Isaiah 38:21 let the people take a lump of fig and apply it as POULTICE/PLASTER on the boil and he shall recover.
- And in the middle of its street and on either side of the river was the tree of life, which bore twelve fruits, each yielding its fruits every month. The LEAVES of the trees are for HEALING of the nations. (Rom. 22:2).

Mr Vice Chancellor Sir, the above biblical injunctions from God, have given approval and holy seal on the use of the medicinal plant for the healing of man and animal diseases and perhaps lay a good ethnobotanical foundation for us today as pharmacologists and scientists who now research into developing a drug from these natural products/plants.

Doctrine of Signature

In 1652, the great English herbalist, Nicolas Culpeper wrote that "by icon or image of every herb, man first found out their virtues". He was expressing a widely held belief that dated at least to biblical times in the axiom.

Similar SimilibusCurantur

Or

"Like cure like"

This is the basis of what is called Doctrine of signature in medicinal therapy. The doctrine is several hundred years old and states that a clue to the medicinal use of a plant is given by one of its features (shape, colour, taste) (Court, 1999).

Many scientists have however queried the scientific basis of this doctrine, but today investigative science has shown that this doctrine was outstandingly correct.

- Thus aphrodisiac plants often display parts resembling sexually relevant organs e.g. various orchid spp (Orchidaceae) have two ovoid bulbs resembling a pair of testicles.

- Peanut**:** Resemble the testicle, arginine the main components of Viagra comes from peanuts.
- Walnut**:** Resembles the brain, today over 3 dozen of neurotransmitters have been investigated for brain function.

Traditional Medicine/Herbal Medicine and Its Importance

According to WHO, this is defined as the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different countries, whether explicable or not used in the maintenance of health as well in the prevention, diagnosis, improvement or treatment of physical and mental illness.

- According to WHO estimates, about 80% of the world population rely on traditional systems of medicines for primary health care.
- Herbal medicines are generally affordable and easily accessible.
- Effective in treating chronic conditions.
- Reduced risk of side effects.
- The present drug development is based on the paradigm of a single compound, single target. While diseases still waiting for cures or treatment are mostly multifactorial. The traditional medicine, however, represents a more holistic approach to multifactorial treatment for a multifactorial disease.
- The synergistic effect of the multiple compounds, present in the whole plants allows the herbal medicine to act more gently, safely and effectively than the single targeted/single active ingredients around which a potential pharmaceutical product is generally formulated.
- More than 50% of all novels drug that comes to the market in the past 30 years are a natural product or natural product derived.

  Quinine – antimalarial (cinchona tree)

  Pigenil --anti-cancer (prinus Africana)

Turmeric – anticancer agent (ginger family)

Artemisin – antimalarial (Artemisia annua)

- Renewed interest in the herbal product for the development of alternative health care products have among the government of different countries, UN agencies, (WHO), investors, industry, the public at large is today a major issue for traditional medicine development.
- Drug resistance by microbes to contemporary drugs, scarcity of the effective therapeutics for complex diseases like diabetes, heart disease, cancer where more than 70% of the modern drug remain ineffective.
- The most vital point for growing interest in plant-based drug discovery is the economy and the discovery of synthetic drugs that require a large amount of money and time. The plant based drug research promises cost-effective drug development and lowers the health care cost.

Transforming Plant into Medicine

The tropical forest is reported as one of the hot spots of biodiversity and has almost twice as many alkaloids producing species as do temperate plants. Only 2% of these plants have been thoroughly screened (Olfield, 1981, Eisner, 1990).

- The question will be how then can we advance in the search for medicine with a vast number of plants that are available in our forests? (There are three major approaches to address this ;)
- Massor-blind screening approach simply collects any plants, screen everything collected. It requires an enormous investment of money, time and a fair amount of luck.
- Chemotaxonomy – Oriented approach: This is a more guided approach that select for screening plant that belong to certain families or genuses that are likely to contain certain classes of compounds.

- Tapping the experience of local people on the use of flora (plant) for medicines (via ethnobotany) this last approach is known as ETHNOPHARMACOLOGY.
-

What then is Ethnopharmacology?

- The word of ethnopharmacology comes from the Greek word ethnos meaning (people), pharmacon, meaning (drug) and old French word -ology (the scientific discourse, study or theory).
- Ethnopharmacology is, therefore, the scientific study of people and their use of various drugs especially plants.
- The term was first used in 1967 and is nowadays much more broadly defined as the observation, identification description, and experimental investigation of the ingredients of indigenous drugs, mostly plants and their effects on man and animal. (Heinrich and Gibbson, 2011).
- This field involves documentation, quantitative evaluation of use, management and experimental assessment of natural products used by man in the treatment of human and animal disease, where plants form the dominant component over other natural resources (Pandey 2010).

The advantages of drug development based on ethnopharmacology;

- Enables plant selection
- A lead from traditional use allows narrowing the pharmacology study.
- A lead from traditional modes of preparation provides clues to active chemical components.
- Lower laboratory investment.
- Shorten the research – productivity cycle (cut in R & D cost).

Triangle of Life

- A triangle is defined as the structure or system composed of three interrelated objects or a polygon with three sides.
- There is in real life, a triangular relationship amongst man, animal, and disease.

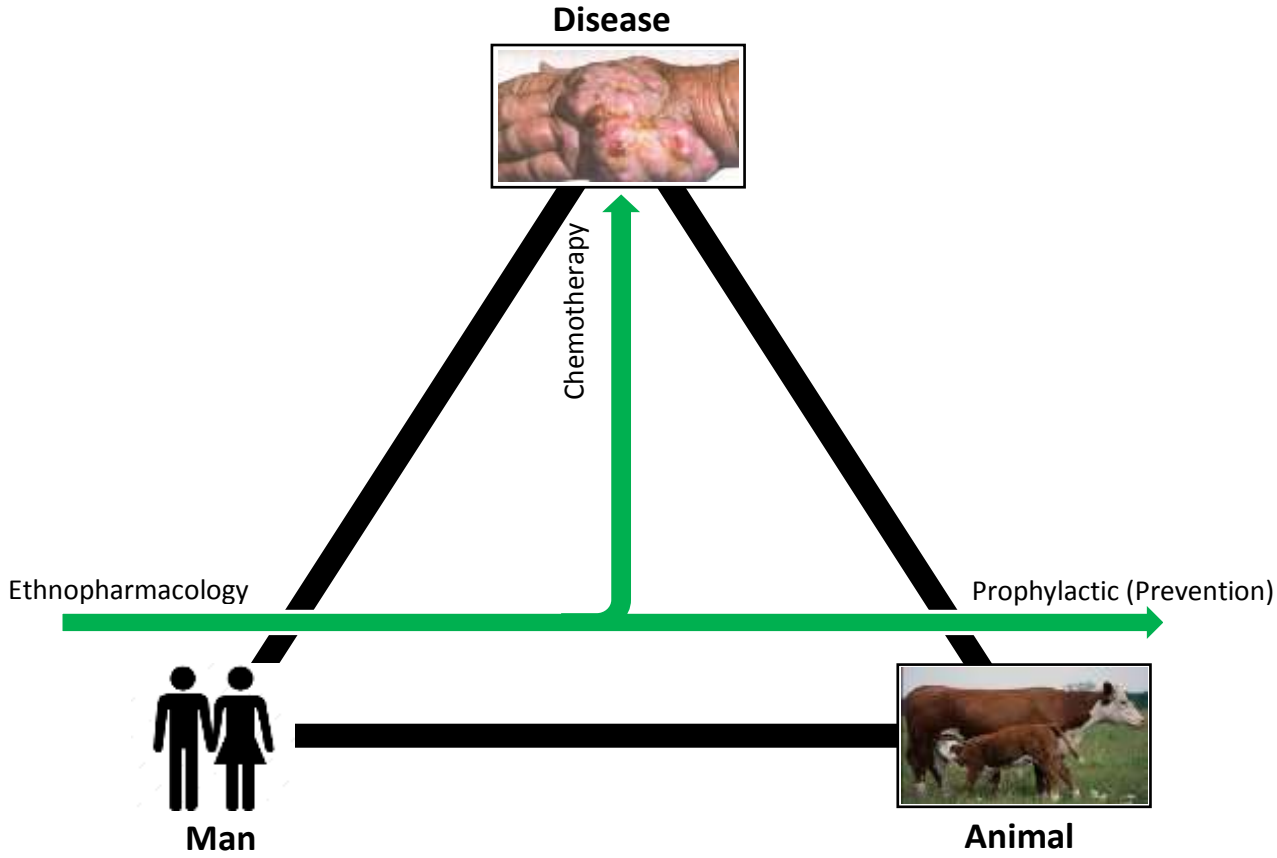

Fig. 1. Navigation of ethnopharmacology horizontally and vertically through the triangle

- In this triangle, man and animal are both at the base carrying a big burden called disease.
- Navigation of ethnopharmacology through this triangle could either be horizontally or vertically.
- It is the navigation of the major components of ethnopharmacology (medicinal plant) that have been my main focus of research as it affects man, animal, and disease.
- The topic of my discussion today "Ethnopharmacology in the triangle of man, animal, and disease, reflects the results of my teaching, research and service to academic development in Nigeria and globally.

My Research with Colleagues

MrVice-Chancellor Sir, my research with colleagues over the last twenty-seven years has centred on the following:

- The ethnopharmacological survey, collection, documentation, and identification of bioresources (medicinal plant) used in traditional/folklore medicines for the management of diseases of man and animals.
- Scientific validation of the claimed therapeutic efficacy of the medicinal plant using several in vitro and in vivo models.
- Elucidation of the several mechanisms of action of these plants in comparison to the existing synthetic drugs.
- Toxicological consequences (if any) at short, medium and long term.
- Developing a value-added product for commercial exploitation.

How then have we gone about achieving this?

- Experimental Animal model

  Research animals are valuable tools for understanding the pathophysiology and in developing therapeutic interventions for any disease. These animals are therefore used in basic medical and veterinary research (Samardeep 2013; XIA-Fang leong 2015).
- Over the last two decades we have therefore used several of these models to study the aetiology, development, progression and therapeutic intervention for diseases like hypertension (Adegunloye, Ajagbonna et al., 1996; Ajagbonna, 2000;Ajagbonna and Mojiminiyi, 2002) diabetes (Ajagbonna and Adebayo, 1999; Adeneye, Ajagbonna et al., 2007; Onakpa, Ajagbonna et al., 2012)Diarrhea (Etuk, Ajagbonna et al., 2007; Ode, Ajagbonna et al., 2012) and trypanosomosis (Ajagbonna et al.,

2003, 2005, 2006; Biobaku and Ajagbonna, 2008; Peni, Ajagbonna et al.,2009; Shamaki, Agaie, Ajagbonna et al., 2012, Peni,Agaie, Ajagbonna et al., 2012). Malaria (Ajagbonna et al., 2002).

Advantages of these models:

- Relative ease to manage and shorten life span
- Similarities to human (on the anatomical basis and physiological functions) e.g. chimpanzee and mice share about 99% and 98% of DNA with human respectively.
- Very useful in drug safety testing.
- The 3RS principle of replacement, reduction and refinement threw up some pertinent questions.
- Can we replace animal with in vitro model?
- Can we reduce the number of animals to be used in our research?
- Can we minimize pain and suffering of test animals?

Diseases in the triangle

Hypertension

Hypertension has always been regarded as a disease of affluence but this has changed drastically in the last two decades with average blood pressure now higher in Africa than in Europe and the United State of America and the prevalence increasing among the poor section of the society. In sub-Saharan Africa, in 2000 80 million adults were reported to be hypertensive and this figure is projected to rise to 150 million by 2025 (Vijver et al., 2013).

There is also the issue of related complication like shock, heart failure and diabetes becoming increasingly more common in Africa (Vijver et al., 2013).

The disease in Africa has been partly linked to high intake of sodium, mostly from salt used to preserve food or to make it tastier.

Salt and Hypertension

Reports in the literature have shown that hypertension can be induced in human and experimental animals by excess salt consumption (Luft, 1989; Forte et al 1989; Adegunloye and Sofola 1996; Ajagbonna and Mojiminiyi, 2001; Ajagbonna et al 2002).

The response of the blood pressure to an acute change in salt intake has been used to determine what is referred to as salt sensitivity. An animal model for salt-sensitive hypertension may be obtained through the feeding of rats with 8% salt diet (Sofola et al., 2002; Mojiminiyi, Ajagbonna et al., 2017).

Hypertension, especially salt-sensitive hypertension is more common in the black race than in Caucasians (Luff and Winberger, 1997; Mojiminiyi, Ajagbonna et al 2007)

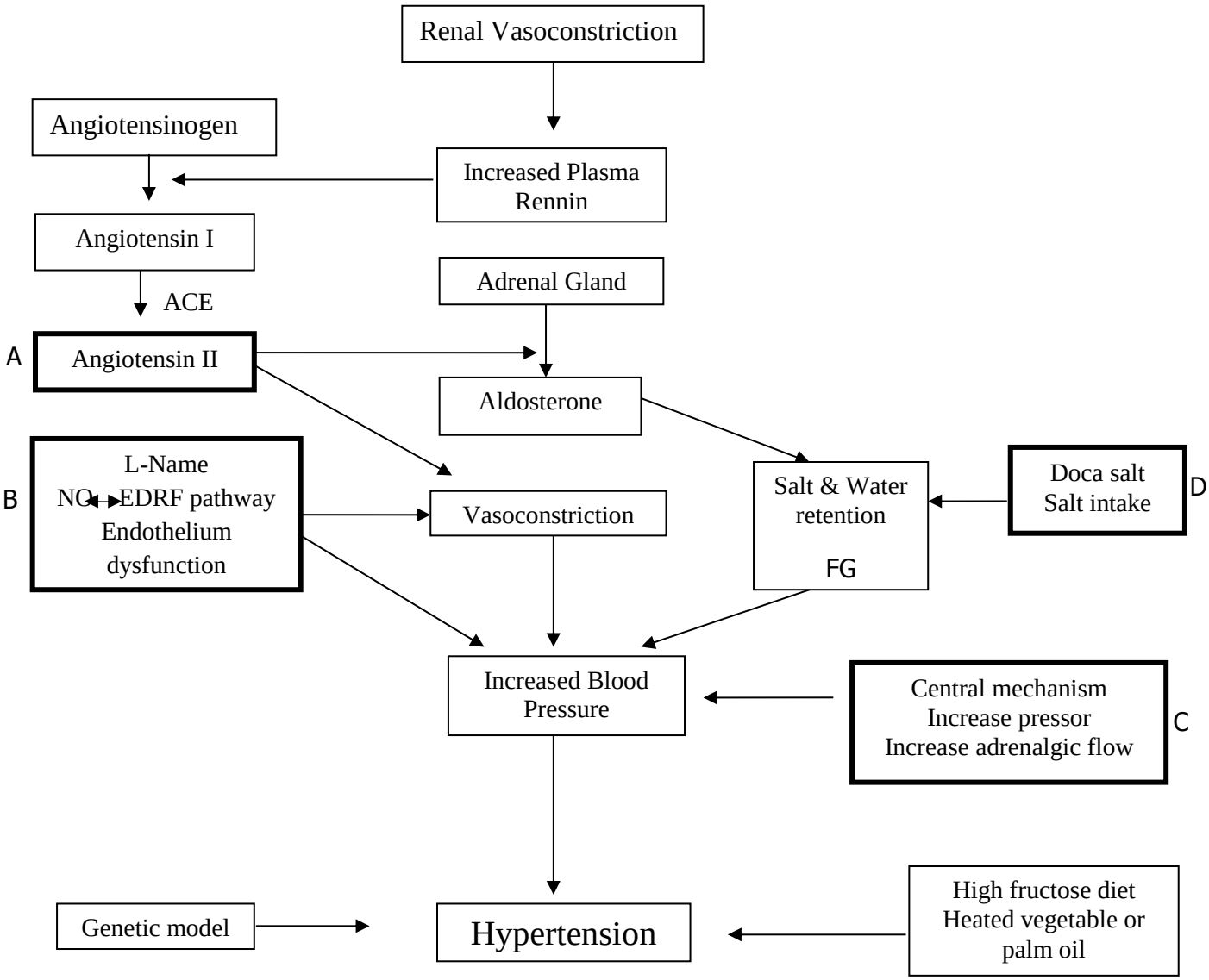

Fig. 2. Multifactorial mechanisms involved in the pathophysiology of hypertension

Some features of salt hypertension

- Dysfunction of the central adrenergic mechanism.
- Overstimulation of sympathetic nervous system.
- Inhibition of Na-K-ATPase activity (Hypokalaemia).
- Attenuation of K^+-induced vascular relaxation.
- Endothelium dysfunction.
- Inability to excrete excess salt.
- Increase vascular reactivity or sensitivity.

Management of Hypertension

Several antihypertensive drugs are available today all over the world and include:

- Diuretic e.g. furosemide
- Sympatholytic e.g. guanethidine
- Vasodilators e.g. minoxidil
- Calcium channel blockers e.g. nifedipine
- Angiotensin converting enzyme inhibitors e.g. captopril (Falase and Salako 1979)

What are the limitations of the current drug?

- Intolerable side effects.
- The high cost of the drugs.
- Drug resistance.
- The frequency of administration.
- Number of drugs in one prescription.
- The downturn in the economy which has diminished the importation of these drugs.

As a result of the foregoing and the present poor health care system in most African countries, one rational way out is to explore the possibility of reviving and modernizing the ethnomedical activities that have long been forgotten.

Contribution of ethnopharmacology

- The last two decades have witnessed a concerted effort in our laboratory to investigate local plants with hypotensive and anti-hypertensive therapeutic values.
- We have validated and documented the following plants as potential hypotensive and antihypertensive agent.
- Hibiscus sabdariffa (HS).

- Rhaptopetalum coriaceum olio (RCO)
- Musanga cecropioides(MCW)
- Cassia occidentalis.
- Viscum album.

Pharmacological effects of RCO, MCW and HS



RCO **MCW** **HS**

- Invitro (Isolated rat aorta)
- In vivo (whole animal)

(a) In vivo

Animal (Anaesthetized with urethane and chloralose)

- Femoral vein → Injection of drugs
- Femoral artery (Both annulated) → Statham pressure transducer

↓

Grass polygraph for displaying Blood pressure

- CVS manoeuvres used for mechanistic studies
  - BCO
  - Vagotomy
  - Cholinergic blockade (atropine)
  - Effect of pressor agents (Bay K 8644 & noradrenaline)

    Histaminergic blockade (H1&H2)

(b) In vitro

- Precontraction with 10^{-7} MNA (ROC) and 60MmKcl (PSC) Contractile responses were carried out + or – graded conc of the Extract.

Fig.3. Schematic representation of general methodology in hypertensive studies

RESULTS OBTAINED

Results from many studies in our laboratory have demonstrated that intravenous injection of an aqueous extract of MCW, RCO, and HS all produced a dose-dependent fall in systolic, diastolic pressure and mean arterial pressure.

Table 1a: Effect of MCW extract on systolic blood pressure (SBP) mmHg, diastolic blood pressure DBP, mean arterial pressure (MAP) and heart rate (Beats/min) in rats.

Parameter	Control	0.0005 mg/kg	0.001 m/kg	0.005 mg/kg	0.05 mg/kg
SBP	105.13 ± 10.27	83.74* ± 10.25	49.93* ± 18.44	47.98* ± 11.37	12.08* ± 08.27
DBP	80.48 ± 09.65	59.37* ± 13.76	27.65* ± 18.04	27.35* ± 09.66	07.51* ± 06.01
MAP	88.70 ± 09.75	67.49* ± 12.55	35.08* ± 18.17	34.23* ± 10.17	09.02* ± 06.76
HR	414 ± 24	390* ± 18	389* ± 15	302* ± 35	290* ± 20

Values are mean ± SEM

*represent P< 0.005

Table 1b: Effect of graded doses of RCO (mg/kg) on mean arterial blood pressure (MAP) and heart rate (HR)

Parameter	Control	01	0.25	0.5	1	2
MAP	99.4 ± 0.74	93.70* ± 0.42	81.20* ± 0.41	80* ± 0.5	68* ± 0.3	60* ± 1.0
Heart Rate	399 ± 20	397 ± 15	398 ± 17	388* ± 15	392* ± 14	387* ± 17

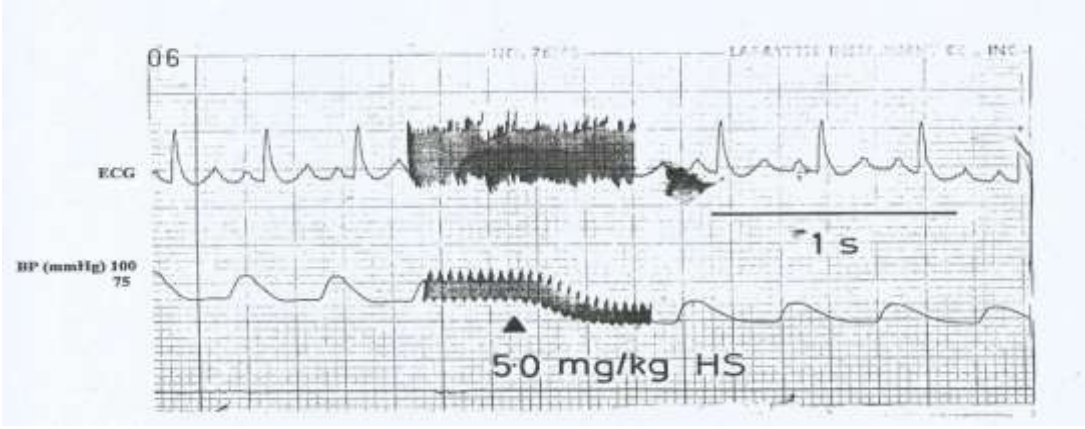

Fig 4A: Effect of graded doses of intravenous injection of HS extract on blood pressure and ECG in dogs

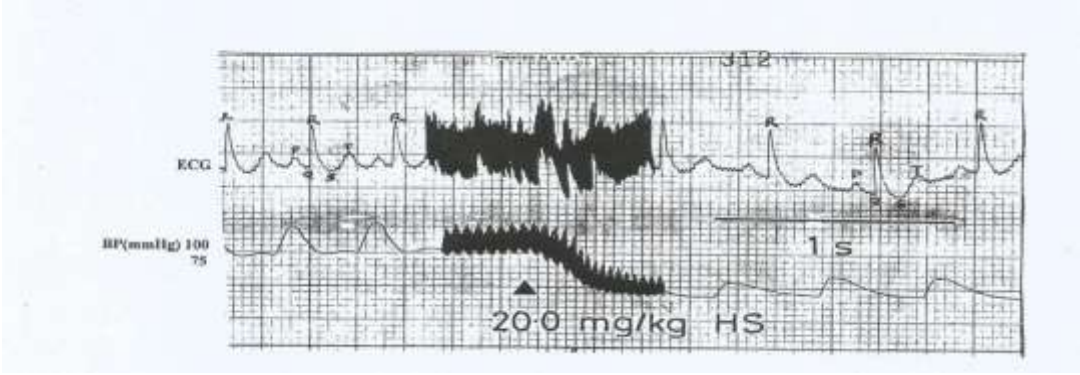

Fig.4B Effect of intravenous injection of HS extract (20.0mg/kg) on blood pressure and electrocardiogram in dogs.

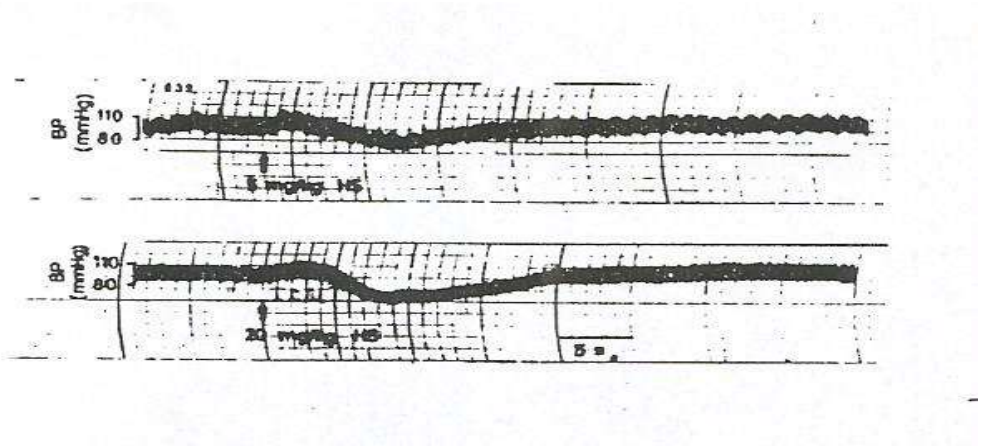

Fig. 5 Effect of graded doses of calyx extract of HS on Blood pressure in rats

Table 1c: Effect of graded doses of calyx extract of HS on heart rate

Doses	Pre-injection heart rate	Post injection heart rate
1	138 ±12	136 ± 14*
2.5	140 ± 13	136 ± 12*
5.0	140 ± 10	120 ± 12*
10.0	145 ± 12	116 ± 10*
20.0	142 ± 10	105 ± 13*

Values are meant ± SEM

* $P < 0.05$

Mechanistic Studies

Agents that lower blood pressure can do so by interfering with any of the blood pressure regulatory mechanism.

Sympatholytic mechanism

Occlusion of the common carotid arteries stimulates the sympathetic nervous system thereby increasing the blood pressure. Fig 6 shows that the increased MAP induced by bilateral carotid occlusion (BCO) was attenuated by the injection of MCW extract, its hypotensive effect may, therefore, be via a sympatholytic mechanism. Similar results were also obtained for HS and RCO extract (Adegunloye, Ajagbonna et al., 1996; Ajagbonna, 2000).

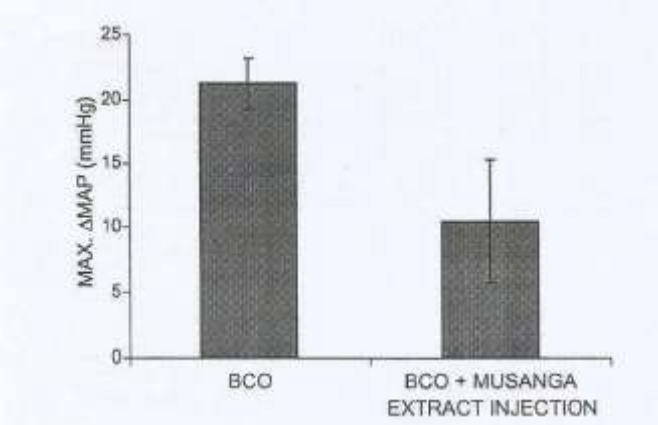

Fig. 6. Effect of intravenous injection of 0.05mg/kg of Musanga stems bark aqueous extract on the maximal increase in MAP∆(max MAP) induced by BCO.

Effect on ACE inhibitor (captopril)

In the treatment of hypertension, inhibition of the angiotensin converting enzyme is established as one of the modern therapeutic approaches. The hypothesis was tested for MCW, where administration of captopril 5mg/kg significantly attenuated the magnitude of MCW – induced fall in MAP (fig 7).

ACE blockade results in the inhibition of angiotensin II (a potent vasoconstrictor and activator of aldosterone secretion) with subsequent vasodilator effect.

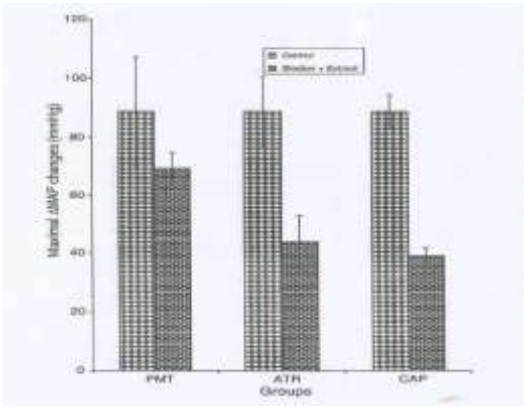

Fig. 7. Effect of I.V injection of 0.05mg/kg of MCW on max. MAP using different blockers PMT: promethazine+ cimetidine, ATR: Atropine, and CAP: captopril

Effect of vagotomy, atropinisation and histaminergic blockade on blood pressure lowering effect of the extract.(HS& MCW)

PARASYMPATHETIC STIMULATION: Pharmacological agents that activate central parasympathetic output or augments ACH action at neuroeffector site will lower the blood pressure.

- The attenuation of the response to MCW by atropine suggests that extract may have an acetylcholine – like effect (negative inotropic and chronotropic effects). This hypothesis was also tested for RCO and similar result and conclusion reached (Adegunloye, Ajagbonna et al., 1996; Ajagbonna 2000).

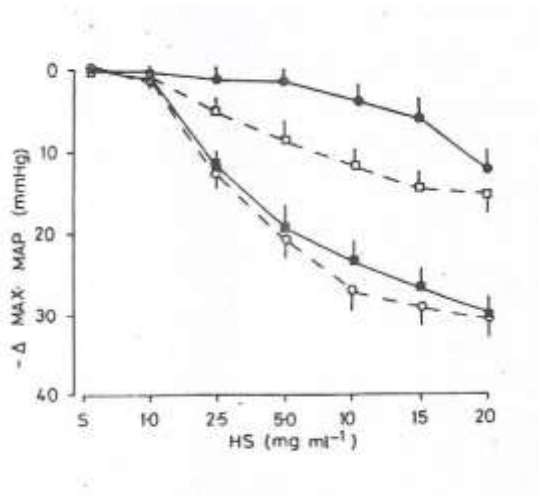

Fig. 8. Graph showing intravenous dose-response to the extract of calyx of HS (○ - - - ○, control) and the effect of vagotomy (■----■) ,atropinisation (●----●) and histaminergic blockade (□ ----□)on the responses.

- Fig. 8. Shows that sectioning of the vagal nerves did not alter the hypotensive effect of HS. The attenuation of the response to HS by atropine suggests that the extract may have an acetylcholine- like effect as seen in MCW. Histamine is released locally in the body and it possesses a vasodilatory effect. The histamine effect is mediated through H1 and H2 Receptors which can be blocked with promethazine and cimetidine respectively. The observation that histaminergic blockade

reduced the hypotensive effect of HS indicate that the effect may be dependent on a histamine related mechanism.

- **Effect of the extract on Bay K 8644 and noradrenaline:** Bay K 8644 is a calcium channel agonist, while noradrenaline (NA) stimulate ca^{2+} influx and release from the intracellular store of vascular smooth muscle. RCO from our study significantly reduces the pressor responses to NA and Bay K 8644.

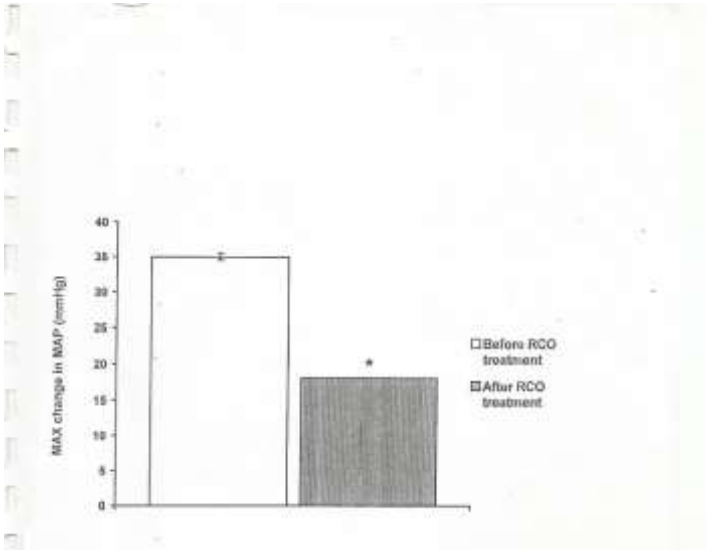

Fig. 9. Maximal changes in MAP (mmHg) induced by NA before and after RCO (2.0mg/kg) treatment. * $P<0.05$ compared with control before RCO

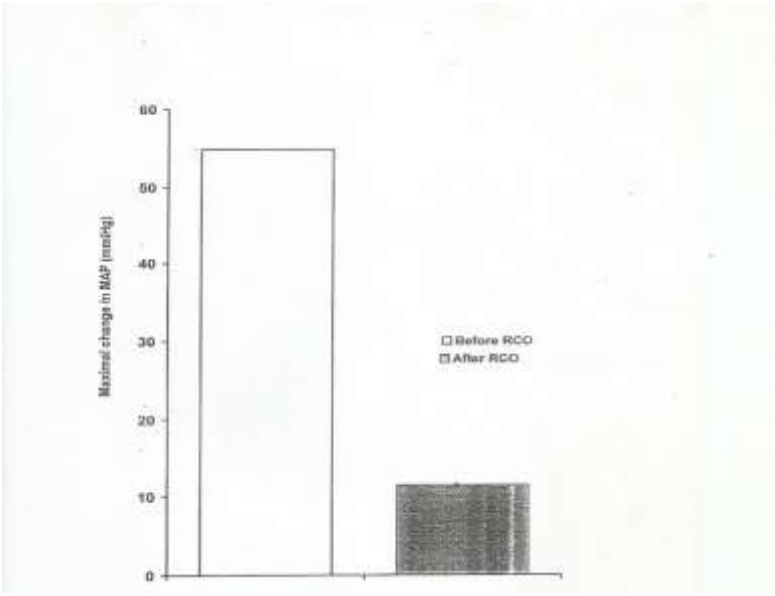

Fig. 10. Maximal changes in MAP (mmHg) induced by Bay K8644 before and after RCO (2.0mg/kg) treatment. *P<0.05 compared with control before RCO.

It is, therefore, possible that the extract may inhibit calcium influx into vascular smooth muscle and/or have some effects on the intracellular release of ca^{2+}, an effect reported in this study.

Effect of the extract on vascular smooth muscle

An agent that relaxes vascular smooth muscle could either be direct or indirect.

The mechanism by which these extracts HS, RCO, MCW relaxes vascular smooth muscle and thus lower blood pressure was investigated. We used 2mm ring segment of the isolated aorta of rats.

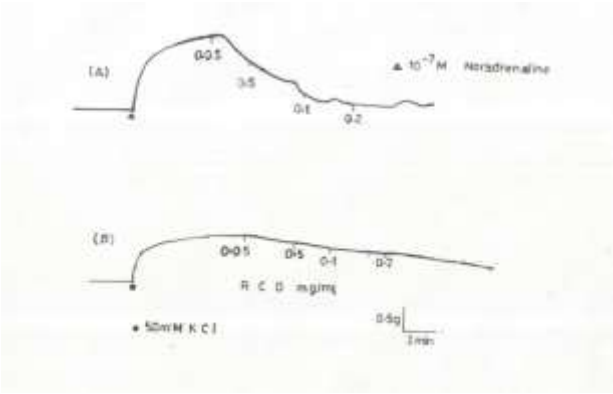

Fig. 11.Typical tracing of the Relaxation Response to RCO Extract following Precontraction of Aortic ring with 10^{-7} M NA and 50mM KCl

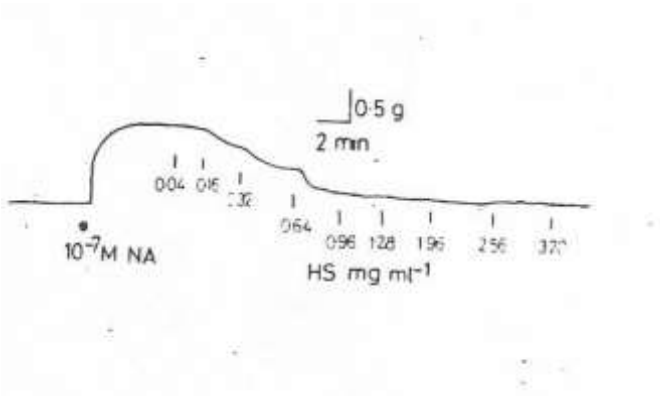

Fig. 12.Typical tracing of the Relaxation Response to HS Extract following Precontraction of Aortic ring with 10^{-7} M NA.

DIRECT VASORELAXANT EFFECT

- RCO, HS, and MCW all have a direct relaxant effect on vascular smooth muscle (fig11&12).
- Cumulative addition of the extract (RCO) relaxes aortic ring precontracted with NA and KCl (fig13&14).

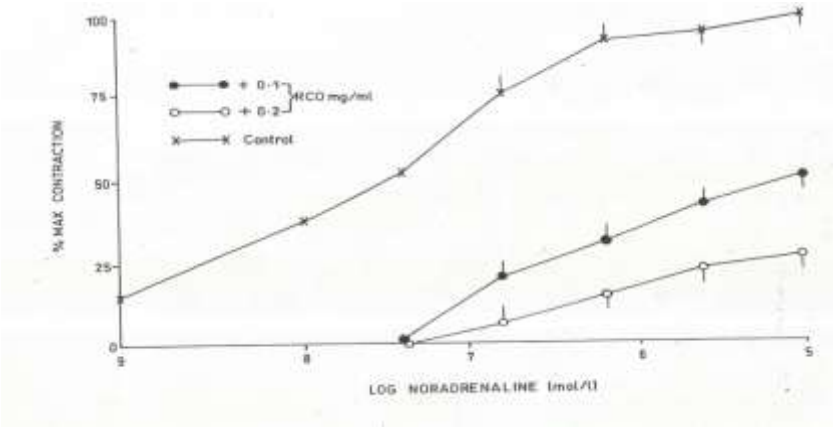

Fig. 13. Contraction Responses to NA in the absence (control) and the presence of a graded concentration of RCO extract.

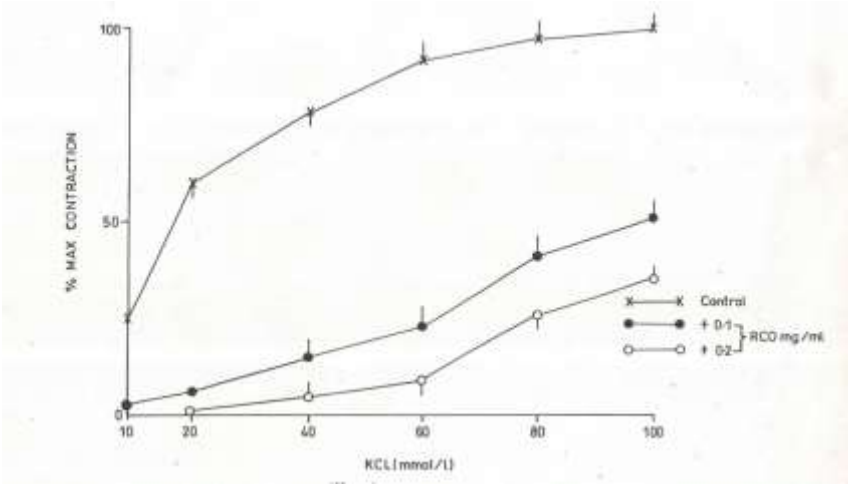

Fig. 14. Contraction Responses to KCl in absence (control) and presence of graded concentration of RCO extract

- Similarly, RCO/HS significantly attenuated the contractile response to $Cacl_2$ in NA and KCl stimulated rings (fig 15&16).

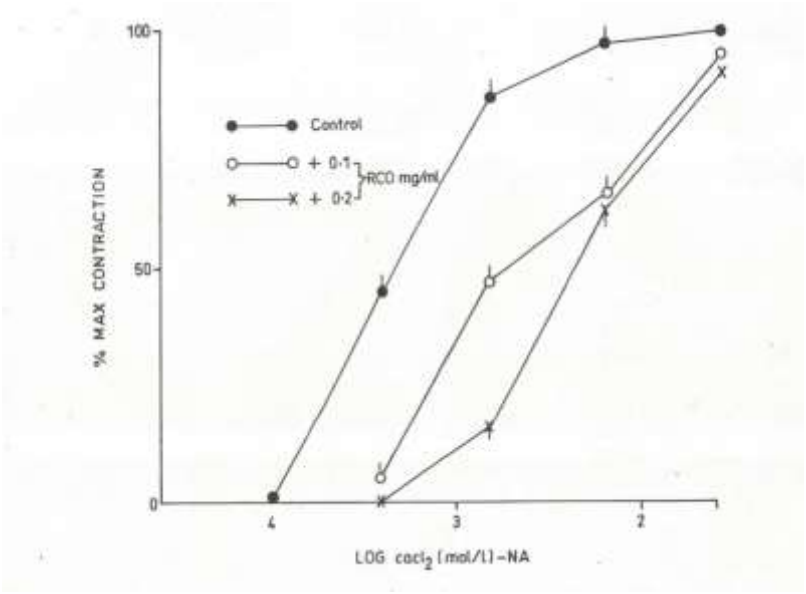

Fig. 15. Contraction responses to Aortic Rings to $CaCl_2$ in the presence of NA (10^{-5} M) and following Pre-treatment with various concentrations of RCO extract.

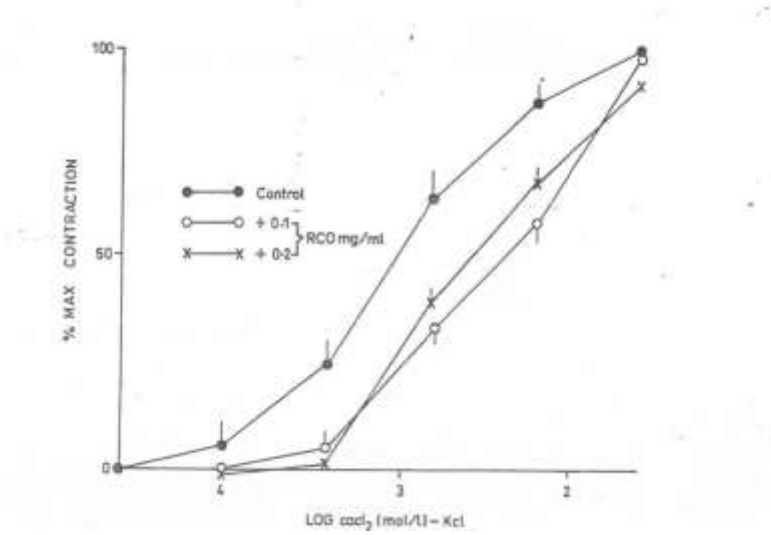

Fig. 16. Contraction responses to Aortic Rings to $CaCl_2$ in the presence of KCl (50mM) and following Pre-treatment with various concentrations of RCO extract.

- RCO/HS both significantly reduced the magnitude of NA-induced phasic contraction.

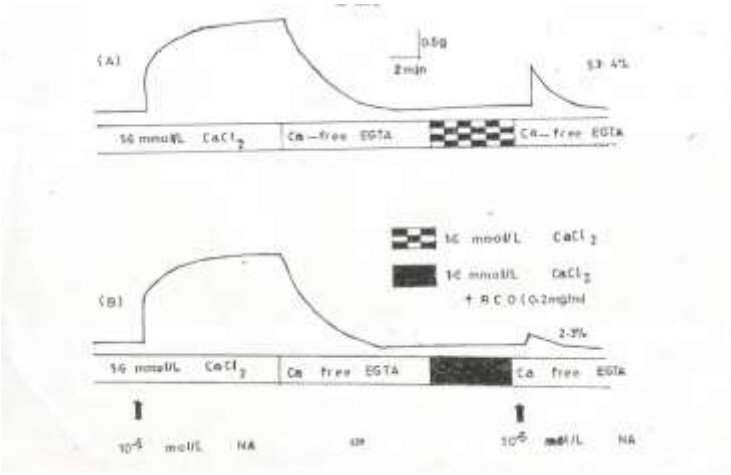

Fig. 17. Typical tracing illustrating the protocol used to study, the effect of RCO extract on phasic response to NA (intracellular Ca^{2+} store).

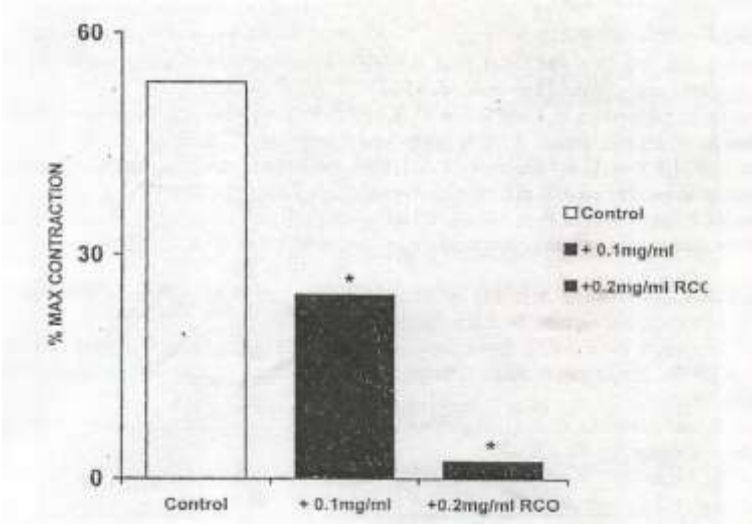

Fig. 18. Maximal phasic contraction achieved in the control and following RCO treatments.

- The result put together suggest that RCO relaxes VSM via a mechanism that inhibits Ca^{2+} influx through the receptor-operated channel (ROC) and potential sensitive channel (PSC) and also an inhibition of Ca^{2+} release from the intracellular store.
- Ca^{2+} channel blocker e.g. nifedipine have in the last decade become widely accepted as the effective treatment of hypertension. (Aloamaka and Osunkwo, 1989). It, however, mediates a selective inhibition of Ca^{2+} entry through the PSC. Similarly, at a therapeutic concentration,

nifedipine has no effect on the intracellular mobilization of Ca^{2+} (Nayler and Procewilson 1981).

- The result from our studies, however,suggest that RCO for example, affected ROC, PSC Ca^{2+} channel as well as inhibiting the release of an intracellular store of Ca^{2+}, suggesting therefore that the extract may be more effective as a Ca^{2+} channel blocker than nifedipine.

Effect on membrane stabilization and stimulation of Na-K-ATPase system

- Membrane stabilization reduces both electrical and mechanical activity of VSM (Webb and Bohr, 1978).
- It is also known that activation of Na-K-ATPase activity produces membrane hyperpolarization and hence reduces both the excitability and contractility of VSM.
- Agents that stabilize the membrane or activate Na-K-ATPase will, therefore, depress the contractile responses of VSM and thus lower blood pressure.
- We, therefore, decided to examine the effect of HS extract on this hypothesis.

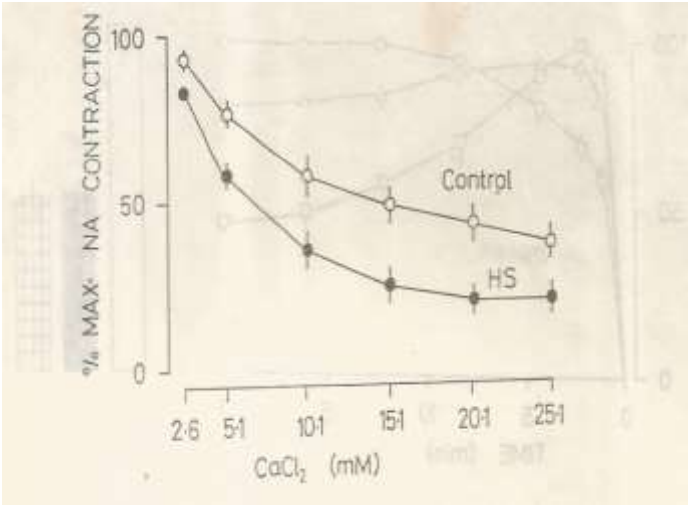

Fig. 19. Relaxation responses to $CaCl_2$ following precontraction with 10^{-7} M NA in the absence (control) and in the aortic rings treated with 0.6mg/ml HS.

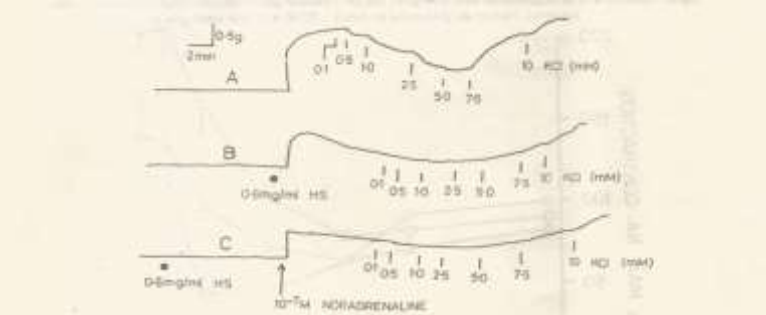

Figure 2: Typical tracings showing contractile responses to noradrenaline and relaxation responses to KCl (A) The aortic ring was precontracted with 10^{-5}M NA in KCl-free PSS and when the contraction had reached a steady level KCl was added cumulatively. (B) 0.6mg/ml HS was added to KCl-free PSS containing the aortic rings and a minute after the addition of HS the tissue was stimulated with 10^{-5}M NA. As soon as the contraction reached a peak it started relaxing and after it had relaxed to a steady level KCl was added cumulatively. (C) 0.6mg/ml HS was added to the KCl-free PSS containing the aortic rings for 10 minutes prior to stimulation with 10^{-5}M NA. The NA-induced contraction was highly attenuated with little relaxation. Cummulative addition of KCl was carried out after the aortic rings had relaxed to a steady level.

The results in (Fig. 19 and 20) where $Cacl_2$ induced dose-dependent relaxation was enhanced by the incubation of the aortic ring with 0.6mg/ml HS shows that HS attenuated contractile response to NA (1441.67± 49mg) vs. (941.67 ± 5.01mg) occurred by stabilizing the membrane and stimulating Na-K ATPase activity. (Adegunloye, Ajagbonna et al, 1993).

Antihypertensive effect of the extract on salt loading hypertension

- Six to eight weeks of daily administration of salt to rats resulted in a significant elevation of systolic, diastolic and MAP (i.e. hypertension) when compared to control rats without salt in their diets. This suggests that the rats were salt sensitive and confirm the earlier findings from our laboratory (Sofola et al., 1993; Ajagbonna, 2000, Ajagbonna and Mojiminiyi 2007, Mojiminiyi, Ajagbonna et al., 2012).

Table 2: The mean arterial pressure (MAP) of control rats and those given salt, HS salt + HS and furosemide

Group	Mean Arterial Pressure mmHg
Control	113.15 ± 2.96*
Salt	184.60 ± 29.76
HS	89.98 ± 7.43*
Salt HS	119.35 ± 8.94*
Furosemide	94.90 ± 11.45 *

* $P < 0.05$vs salt group

- These results showed for the first time that concurrent administration of HS/RCO extract and salt diet did not result in the induction of hypertension, thus suggesting that both extracts may likely be a potential prophylactic agent in salt-sensitive hypertension.
- Most essential hypertensive patients in Africa and in diaspora are salt sensitive.
- The systolic blood pressure was significantly attenuated by the two extracts, the systolic blood pressure is a function of the left ventricular contraction and this suggests that HS/RCO reduces left ventricular contraction and possibly left ventricular hypertrophy.
- The cardiac weight and cardiac weight index of salt loaded rats are significantly higher than the group treated with HS calyx extract, this further suggest that.
- HS extract has the potential to reduce hypertrophy associated with salt-induced hypertension which is similar to the result observed by Odigie et al., 2003 in Renovascular hypertensive rats.

Table 3: The absolute cardiac weight and the cardiac weight index (cardiac wt/body weight) of control rats, those given salt, HS, salt + HS and furosemide group

Group	Absolute weight (g)	Cardiac weight index
Control	0.69 ± 0.02*	2.82 ± .05**
Salt	0.82 ± 0.04	3.56 ± 0.15
HS	0.56 ± 0.003**	2.86 ± .04**
Salt + HS	0.672 ± 0.03**	3.17 ± .05*
Furosemide	0.69 ± 0.01*	3.35 ± 0.04

* $P < 0.05$vs salt

** $P < 0.01$ vs. salt

- Similarly, diastolic pressure was attenuated by RCO/HS extract, implying that the extract might have reduced or attenuated peripheral resistance, results similar to the one reported in normotensive rats.

- The tachycardia (increase HR) that usually characterizes salt-induced hypertension was also attenuated by concurrent treatment of HS/RCO and salt diet.
- Guyton has postulated that kidney remains the most important factor in the genesis of salt-induced hypertension and that inability to excrete excess salt leads to the development of hypertension. The anti-hypertensive effect of therapeutic agents like furosemide has been linked to natriuresis and diuresis.
- RCO/HS from our results reduces the increase blood pressure from salt loading by an increase in water and electrolyte excretion.

Table 4: Effect of RCO extract on the mean urine volume of rats treated daily for a period of six weeks with sodium chloride

Treatment Group	Mean urine volume (ml) in various weeks						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Control	10 ± 1.0	15 ± 2.0	15 ± 1.5	15 ± 2.0	10 ± 1.7	12 ± 2.0	13 ± 3.0
Salt alone	10 ± 2.0	20 ± 1.5	30 ± 2.5	35 ± 1.7*	50 ± 1.7*	60 ± 3.0*	65 ± 18*
Salt + RCO	11 ± 1.5	30 ± 2.0	45 ± 2.6	55 ± 1.8*	80 ± 3.0*	80 ± 2.5*	78 ± 1.5*

Values are means ± SEM of six observations; *Values are significantly (P < 0.05) different from the control

Table 5: Effect of RCO extract on the mean Na^+ excretion in the urine of rats treated daily for a period of six months with sodium chloride

Treatment Group	Mean urine volume (ml) in various weeks						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Control	108 ± 0.74	107 ± 0.94	106.2 ± 0.87	105 ± 0.8	106± 0.47	105 ± 1.0	108 ± 0.70
Salt alone	107 ± 0.5	130 ± 0.40	137 ± 0.6*	145 ± 1.6*	146.2 ± 1.2*	148.5 ± 1.1*	150 ± 1.9*
Salt + RCO	107 ± 0.5	139 ± 0.74*	155.7 ± 1.08*	163.5 ± 1.1*	157.2 ± 0.5*	155 ± 1.2*	158 ± 1.35*

Values are means ± SEM of six observations; *Values are significantly (P < 0.05) different from the control

- Administration of salt to rats in this study resulted into hypokalaemia which was prevented by treatment with RCO/HS extracts. Previous studies have shown that salt-induced hypertension was reduced by addition of potassium in the drinking water of rats; nifedipine in the same

study could not reverse the salt-induced hypokalaemia (Nwaigwe and Sofola 1989).

- In our own study with RCO/HS, correction of hypokalaemia may have contributed to antihypertensive effect by making adequate K^+ available for the membrane, via the activation of the Na-K-ATPase pump which was inhibited during salt loading
- Elemental analysis of RCO/HS extract shows potassium to be the major element.
- The extract also contains a large amount of ascorbic acid which is an antioxidant; this may also be responsible for the antihypertensive effect of this extract.
- An antioxidant like ascorbic acid is well known to stimulate the synthesis of nitric oxide (No), a potent vasodilator. (Zakari 2005; Mojiminiyi, Ajagbonna et al., 2012).
- There was also salt-induced enhancement of vascular sensitivity to agonist and to depolarization. These results agree with observation in human (Aoki et al 1982; Ebeigbe and Ezimokhae, 1998).
- Treatment with either HS/RCO decreased the enhanced contractile responses to isolated rat aorta in NA and Kcl precontraction, confirming our earlier report in normotensive rats.(fig 21&22)

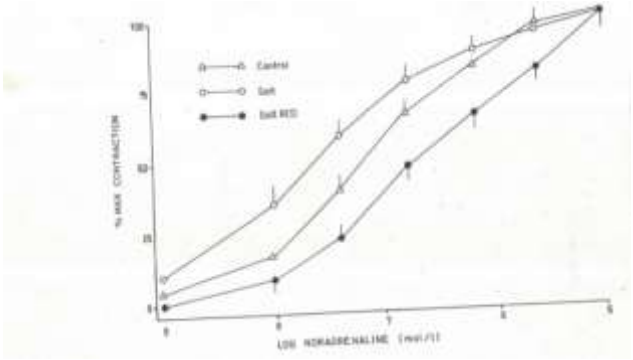

Fig. 21. Contraction responses of Aortic Rings from control, Salt loaded and Salt plus RCO treated rats to NA.

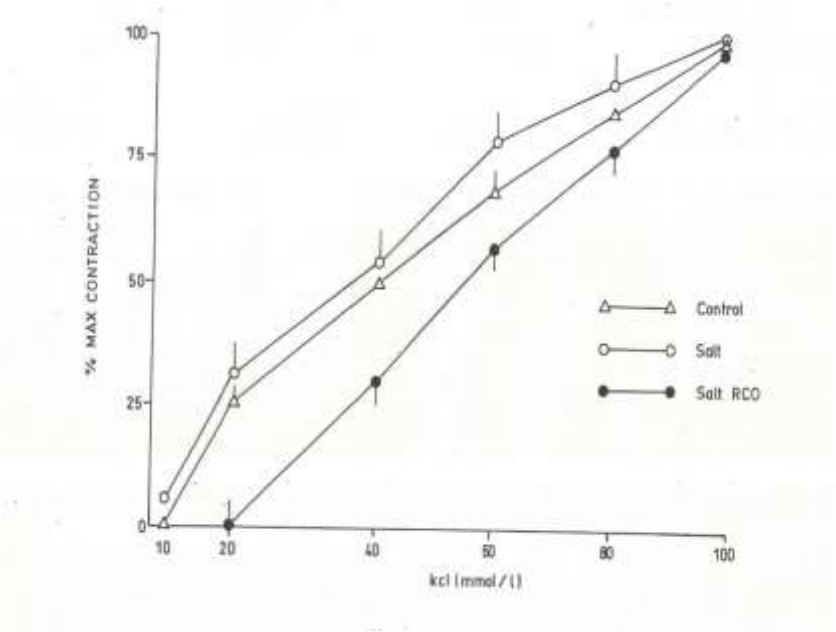

Fig. 22. Contraction responses of Aortic Rings from control, Salt loaded and Salt plus RCO treated rats to KCl.

Endothelium dysfunction and hypertension

- Nitric oxide (NO) produced by endothelium nitric oxide synthase (eNOS) represents an antithrombotic and anti-atherosclerotic principle in the vasculature.
- NO is a potent vasodilator and contribute to blood pressure control. Mice with a disrupted eNos gene are hypertensive and endothelium-dependent. No mediated vasodilatation is inhibited.
- Based on these antihypertensive and anti-atherosclerotic effects, the enhancement of endothelia NO production could be of prophylactic or therapeutic interests.
- This hypothesis was tested with some of the plant extracts that we have reported over the years to have vasodilatory effects.

Effect of RCO on EDRF (NO)

Table 6: Relaxation response in aortic rings control, indomethacin, methylene blue and endothelium-denuded group.

Treatment	% Maximal Relaxation
Control	73 ± 3.0
Indomethacin	64 ± 3.5
Methylene blue	24 ± 3.2*
Endothelium-denuded	15 ± 3.0*

* P < 0.05 compare to control

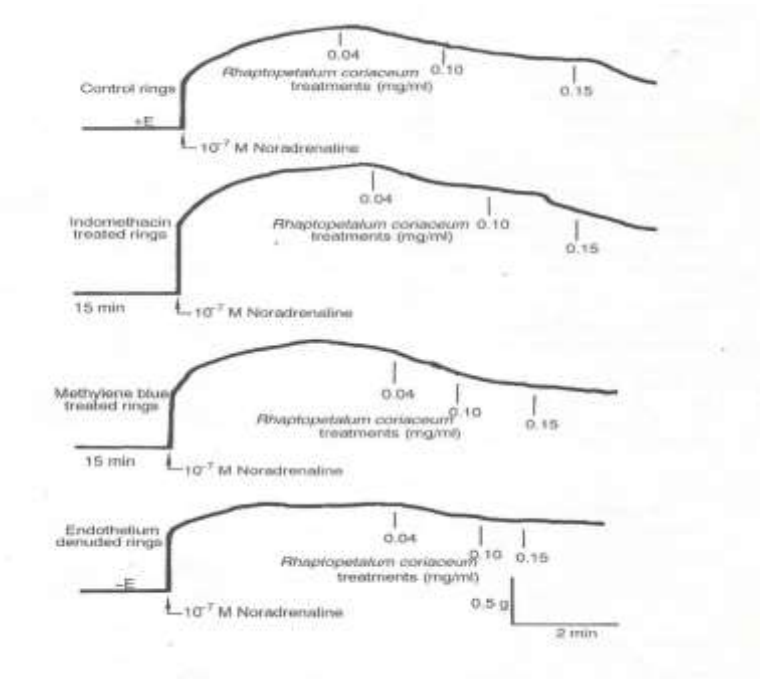

Fig. 23. Typical Tracings of the Effect of RCO on Aortic Rings precontracted with 10^{-7}M NA with Endothelium (+E) or without Endothelium (-E), Rings treated with Methylene Blue (MB) and Indomethacin.

- Attenuation of the relaxation in endothelium-denuded rings shows that the relaxation is endothelium dependent.
- Agents that elicit endothelium-dependent relaxation cause elevation of cyclic guanosine monophosphate (CGMP) levels within VSM and methylene blue prevents the formation of CGMP by inhibiting guanylatecyclase. The presence of methylene blue in this study

significantly attenuated RCO - induced relaxation, suggesting that the endothelium-dependent relaxation observed was linked to intracellular elevation of CGMP.(fig 23)

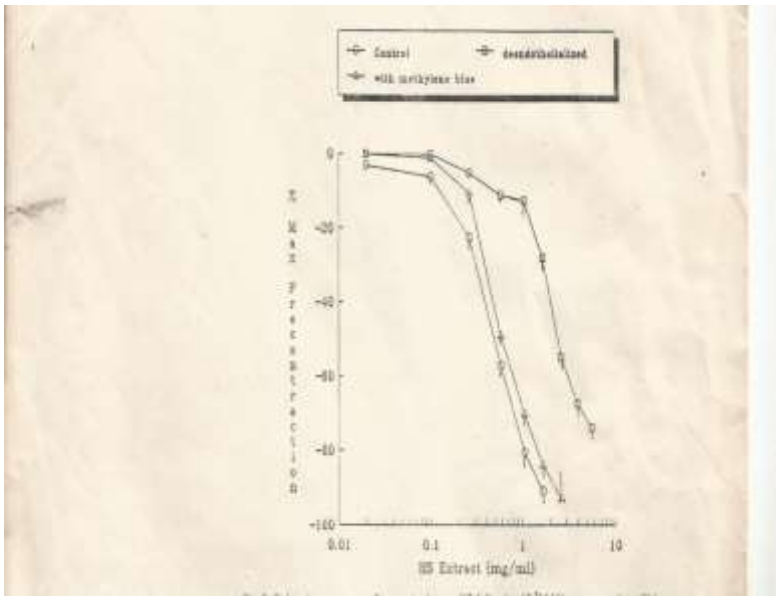

Fig. 24.Relaxation responses of rat aortic rings to HS following 10^{-7}M NA contractions.

Treatment of RCO on vascular endothelium-dependent relaxation in salt – induced hypertension

- In various types of hypertension e.g. renal, salt hypertension and genetic hypertension, endothelium-dependent relaxation is depressed and it is known that relaxation response to Acetylcholine (ACh) determines the magnitude of EDRF release by any agent, therefore its antihypertensive effect (Palmer et al 1987, Ajagbonna and Mojiminiyi, 2002).

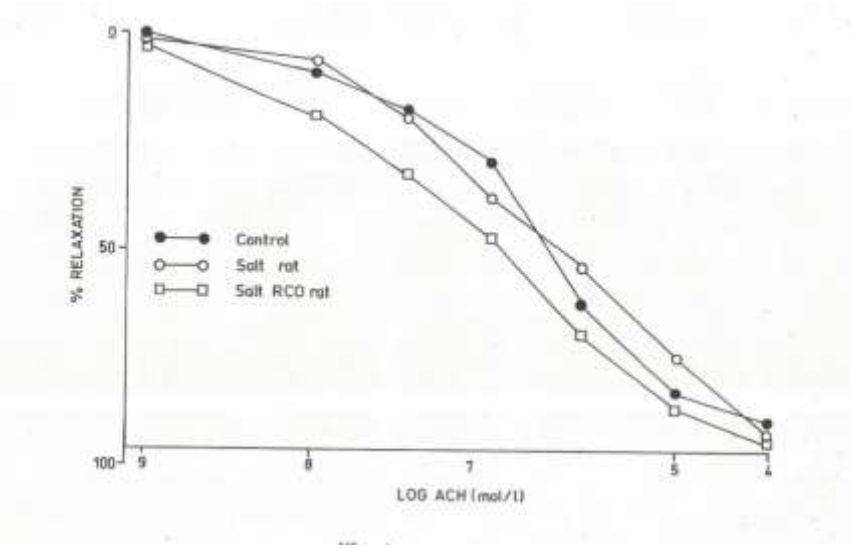

Fig. 25. Relaxation Response of Aortic Rings of control, Salt loaded and Salt loaded plus RCO to acetylcholine

- Fig (25) shows that the relaxation response of the aortic ring to Ach was highest with salt-RCO treated rings. This may suggest more liberation of EDRF (NO) following RCO treatment which may be responsible for the vasodilator effect with a consequent decrease in blood pressure.
- The IC_{50} values which are a reflection of the sensitivity of the aortic ring to Ach further show that salt + RCO treated rings were more sensitive to Ach.

Table 7: Values of IC_{50} (M) and tension (mg) in response to Ach in precontracted aortic rings from control, salt loaded and salt + RCO rings.

Parameter	Control	Salt alone	Salt + RCO
IC_{50}(M)	$3.1 \pm 8 \times 10^{-7}$	$3.6 \pm 0.5 \times 10^{-7}$	$1.4 \pm 0.9 \times 10^{-7}$
Tension	1075 ± 20	1090 ± 15	1045 ± 30

Result here is very significant

- A reduced vasodilatory response to Ach has been frequently reported in vascular tissue from both human and animal with diabetes.
- Endothelium dysfunction in human penile corpora cavernosa was also reported for type I and type II diabetic patients this has been linked to

erectile dysfunction. Consumptions of these extracts may, therefore, be beneficial, in ameliorating this dysfunction.

Effect of Hibiscus Sabdariffa in human studies

- Human studies from our laboratory have also shown that the calyx extract of hibiscus sabdariffa has a hypotensive effect and antihypertensive effect (Odigie, 2002).

For example:

- In a clinical trial involving 54 patients with moderate essential hypertension, daily consumption of the extract (two spoonfuls of blended tea of the extract boiled in one glass of water for 20 – 30 min) resulted in about 11% decrease in systolic and diastolic blood pressure 12 days after the treatment. Three days after cessation of the treatment, the blood pressure rose again by about 6 – 8%.
- A double-blind placebo – controlled clinical trial has also provided gratifying evidence of the antihypertensive effect of the calyx extract. These results were not significantly different from those obtained for patients on ACE (captopril) treatment (Herrera – Arellano et al., 2004).
- No adverse effects were found with either treatment, confirming the effectiveness and safety of the extract as reported in many of our animal studies. Ajagbonna and Adebayo, 2001, Ajagbonna and Onyeyili 2002; Adeneye, Ajagbonna et al., 2006; Pofi,Mojiminiyi, and Ajagbonna (2006).

Diarrhea Disease

- In developing countries, particularly in Africa, low level of hygiene and sanitation has exposed the people to a wider array of microbial pathogens, which increases their susceptibility to microbial infections (Fennell et al., 2004).

- Worldwide, the prevalence of diarrhoea is high and accounts for more than 5-8 million deaths each year in infants and children less than 5 years (Venkatesan et al., 2005). The prevalence in North Eastern Nigeria is estimated to be as high as 22%.
- Diarrhoea (scours) is also common in newborn calves, lambs and kids. The clinical presentation can range from mild diarrhoea to profuse acute diarrheal which may result to death in a few as 12 hours. It has been named as one of the limiting factors to sustainable livestock production in Africa.

Medicinal Plant and diarrhoea

- Presently several plants are available and used in the control of diarrhoea in man and animal.
- We have conducted an ethnobotanical survey of medicinal plants use for the control of diarrhoea in Sokoto State Nigeria.
- The plants documented were identified and ranked based on information frequency of citation. The ten top rank plants were selected (Table 10) and screened for acute toxicity or (safety) and antidiarrheal potential.

Table 8: Ethnobotanical profile of selected Nigerian medicinal plants used in this study.

S/N	Family	Species	Local Name (Hausa)	Plant Part Used
1	Mimosaceae	Acacia nilotica	Bagaruwa	Bark
2	Combrelacea	Terminalia macroptera	Bayankada	Bark
3	Rubiaceae	Mytragne Africana	Dafa	Bark
4	Anacardiaceae	Lannea acida	Faru	Bark
5	Cambrelaceae	Ampelocissos grantii	Gogododo	Root
6	Leguminosae	Pilostigma reticulatum	Kalgo	Bark
7	Campylacantha	Acacia polyancartha	Karo	Bark
8	Cambrelaceae	Angiossus leiocarpus	Marke	Bark
9	Euphorbia pilulifera	Euphorbia hirta	Nonkuchiya	Arieal part
10	Polygulaceae	Sercuridaca longipendunculata	Sanya	Bark

Table 9: Effect of limit dose test (Acute toxicity) of selected plants in rats.

S/N	Plant Species	No. of Animal	Dose (mg/kg) body wt	Short term result (48hr)	Long term result (14 days)
1	Acacia nilotica	5	3000	Survival	Survival
2	Terminalia macroptera	5	3000	Survival	Survival
3	Mytragne Africana	5	3000	Survival	Survival
4	Lannea acida	5	3000	Survival	Survival
5	Ampelocissos grantii	5	3000	Survival	Survival
6	Pilostigma reticulatum	5	3000	Survival	Survival
7	Acacia polyancartha	5	3000	Survival	Survival
8	Angiossus leiocarpus	5	3000	Survival	Survival
9	Euphorbia hirta	5	3000	Survival	Survival
10	Sercuridaca longipendunculata	5	3000	Survival	Survival

Antidiarrheal Potential of the Plants

MrVice-Chancellor sir, for any agent to be accepted as an antidiarrheal, it must meet what is now known as GEIGER criteria;

- Inhibition of the production of wet or unformed faeces in animals.
- Inhibition of the production of watery stool and or fluid evacuation in the animal.
- Inhibition of gastrointestinal propulsive action.
- These ten top ranked plants were therefore tested against the hypothesis of Geiger to determine their pharmacological potential as an antidiarrheal agent.

Results

Table 10: Effect of selected Nigerian medicinal plants on castor oil induced diarrhoea

S/N	Plant Species	Onset of wet stool (hr)	Average number of wet stool after 8hrs	Standard deviation
1	Acacia nilotica	3	0.625 ± 0.183*	0.518
2	Terminalia Macroptera	1	1.250 ± 0.164	0.463
3	Mytragne Africana	1	1.500 ± 0.189	0.535
4	Lannea acida	5	0.375 ± 0.183*	0.518
5	Ampelocissos grantii	3	0.625 ± 0.183*	0.518
6	Pilostigma reticulatum	2	0.875 ± 0.350*	0.991
7	Acacia polyancartha	3	1.000 ± 0.267*	0.756
8	Angiossus leiocarpus	1	1.000 ± 0.267*	0.756
9	Euphorbia hirta	5	0.375 ± 0.183*	0.518
10	Sercuridacalongipendunculata	1	1.125 ± 0.350	0.991
11	Standard with diphenoxylate + Atropine	7	0.250 ± 0.144	0.463
12	Castro oil plus normal saline (control)	1	2.250 ± 0.164	0.463

*P<0.05 compared with control.

- All the ten plants extract like the standard antidiarrheal agent (diphenoxylate) inhibited the frequency of defecation, reduced the liquid content of the faecal droppings. The onset of diarrhoea was also significantly delayed in most of the plants.

Table 11: Effect of selected Nigerian medicinal plants on gastrointestinal motility in rats.

S/N	Plant Species	Mean % total length of small intestine travelled by activated charcoal	Standard deviation
1	Acacia nilotica	60.532 ± 1.800*	4.040
2	Terminalia Macroptera	62.440 ± 1.810*	4.050
3	Mytragne Africana	73.270 ± 1.530*	3.420
4	Lannea acida	55.866 ± 1.700*	3.900
5	Ampelocissos grantii	65.372 ± 1.380*	3.600
6	Pilostigma reticulatum	63.780 ± 2.420*	5.400
7	Acacia polyancartha	70.540 ± 2.420*	5.000
8	Angiossus leiocarpus	60.200 ± 1.100*	2.460
9	Euphorbia hirta	57.620 ± 1.950*	4.3600
10	Sercuridacalongipendunculata	65.540 ± 0.400*	0.905
11	Standard with diphenoxylate + Atropine	54.552 ± 1.290	2.870
12	Castro oil plus normal saline (control)	99.600 ± 0.290	0.650

*P<0.05 compared with control.

- All the ten plants significantly decreased the propulsion of activated charcoal meal through the gastrointestinal tract.

Table 12: Effect of selected Nigerian medicinal plants on intestinal fluid accumulation.

S/N	Plant Species	Mean body wt./20 x weight of intestine	Standard deviation
1	Acacia nilotica	41.334 ± 2.190*	4.911
2	Terminalia Macroptera	44.676 + 3.370*	8.350
3	Mytragne Africana	63.690 ± 2.870*	6.410
4	Lannea acida	35.250 ± 2.310*	5.169
5	Ampelocissos grantii	37.748 ± 0.590*	1.314
6	Pilostigma reticulatum	44.114 ± 2.510*	5.609
7	Acacia polyancartha	36.824 ± 1.200*	2.690
8	Angiossus leiocarpus	37.390 ± 1.240*	2.780
9	Euphorbia hirta	34.470 ± 1.660*	3.713
10	Sercuridacalongipendunculata	44.362 ± 0.150*	2.561
11	Standard with chlorpromazine	30.625± 0.610	1.353
12	Castro oil plus normal saline	79.144 ± 1.990	4.450

*P<0.05 compared with control.

Table 13: Antibacterial activity of selected Nigerian medicinal plants using disc diffusion method.

S/N	Plant species	Organism used, Conc. of plant extract & zone of inhibition (mm)								
		Salmonella			E. coli			Staph. aureus		
		40m g/ml	60m g/ml	80m g/ml	40m g/ml	60m g/ml	80m g/ml	40m g/ml	60m g/ml	80m g/ml
1	Acacia nilotica	-	-	-	-	-	-	-	-	-
2	TerminaliaMacroptera	2mm	3mm	4mm	-	-	-	-	-	-
3	Mytragne Africana	-	-	-	-	-	-	-	-	-
4	Lanneaacida	2mm	3mm	4mm	-	-	-	-	-	-
5	Ampelocissosgrantii	2mm	2mm	3mm	-	-	-	-	-	-
6	Pilostigmareticulatum	-	-	-	-	-	-	-	-	-
7	Acacia polyancartha	1mm	3mm	3mm	-	-	-	-	-	-
8	Angiossus leiocarpus	3mm	5mm	5mm	-	-	-	-	2mm	-
9	Euphorbia hirta	2mm	2mm	3mm	-	-	-	-	-	-
10	Sercuridacalongipendunculata	-	-	-	-	-	-	-	-	-
11	Chloramphenicol Hcl	6mm	8mm	9mm	4mm	5mm	5mm	2mm	3mm	3mm
12	Tetracycline Hcl	5mm	7mm	9mm	5mm	5mm	6mm	-	-	3mm
13	Control with normal saline	-	-	-	-	-	-	-	-	-

- = No zone of inhibition observed

The summary of these results showed that:

- Most of the extracts resulted in a marked reduction in the number of diarrhoea stools.
- Reduced volume of intestinal contents.
- Reduction in intestinal transit.

These signify the usefulness of these models used in this study and the efficacy of most of the extract as an antidiarrheal agent in accordance with the Geiger criteria.

- The result from the antibacterial studies where only a slight inhibitory activity was seen against salmonella suggest that the anti-diarrhea effects of most of the plants may be due to anti-secretory and anti-motility but not due to antimicrobial activity. (Etuk, Ajagbonna et al., 2008)

Disease burden in animal

A popular Arab proverb says; "A country rich in livestock is never poor and a country poor in livestock is never rich".

- Currently, almost two billion people over the world depend at least partly on domestic animals for their livelihood and 12% of these people completely depend upon them.
- Livestock sector generates 1.4% of world GDP. The growth rate is 2.2% consistently for the last decade. The current livestock sector contribution to GDP is 40% of agriculture and it is likely to rise to 50 to 60% of agriculture very soon (FAO 2006).
- Poor animal productivity is widely attributed to the occurrence and endemicity of certain animal diseases e.g. trypanosomosis.

Trypanosomosis

Introduction

- One of the most important diseases of livestock and human in sub-Saharan Africa, caused by several spps of trypanosomes.
- 20% of Africa's 173 million cattle are at risk of infection. In addition, 36 out of 52 African countries are endemic for sleeping sickness with 55 million people at risk of contracting the infection (Adeniyi, 1993; Cattand, 1995; Mikail and Ajagbonna, 2007).
- A lot of revenue is lost yearly due to mortality and low productivity of trypanosome infected livestock in many parts of Africa (Ajagbonna et al., 2005).
- The search for a vaccine against this disease remain elusive and present drug is beset with problems:
  - Drug resistance
  - Toxicity
  - High cost of treatment
  - Few available drugs
- We, therefore, proposed remedies from the medicinal plant can be an answer to this problem.
- Our first approach was to first appraise and investigated the toxicity of the two most important trypanocidal drugs (Samorin and berenil) current in use. Results from our laboratory are published in many journals showing the toxic effects of these drugs in the cardiovascular system (Ajagbonna 1991; Ajagbonna et al., 1993; Ajagbonna et al., 1995a and 1995b).
- The question from the observed toxicity result then include:
  - Can we reduce the observed toxicity?
  - Can we have a cheap, easily accessible natural product?

- Can the new remedy potentiate the existing drug to improve its efficacy without a relapse?

Medicinal Plants and Trypanosomosis

- We have investigated over the years the following plants as a potential trypanocidal agent to address the present challenges of the existing drug, these include:
  - Khaya Senegalensis
  - Allium sativum
  - Terminalia avicenoides
  - Securidaca longepedunculata
  - Jatropha gosspiifolia
- The criteria used in the assessment of trypanocidal efficacy
  (a) Invitro – absence of motility and mortality of the parasite.
  (b) In vivo – examination of blood daily for the parasite, the effect on haematology, death or complete protection without relapse. Effect of pre-treatment on infectivity.
  Behavioural changes such as movements, feeding, drinking, and alertness were equally monitored.

Results

Table 14: In vitro anti-trypanosomal activity of the aqueous stem extract of *gossypiifolia var. gossypiifolia*

Incubation Time (min)	PBS Parasitized blood	Concentration (mg/ml)					
		Extract					Samorin
	(control)	100	50	25	12.5	6.25	0.1
0	+++	++	++	+++	+++	+++	--
10	+++	--	+	+	+++	+++	---
15	+++	--	--	+	+++	+++	---
30	+++	NM	NM	--	+++	+++	NM
60	+++	NM	NM	-	++	+++	NM
90	+++	NM	NM	NM	++	++	NM
105	+++	NM	NM	NM	++	+	NM

+++ = highly motile; ++ = very motile; + = motile; -= sluggish; -- = very sluggish; --. = highly sluggish; NM = not motile;
PBS = phosphate buffered saline

Table 15: Results of infectivity test with the extracts of *Jatropha gosspiifolia var. gossypiifolia* in rats.

Groups (3 rats/group)	Concentration (mg/ml)	Time of infection (days)					
		10	**20**	**30**	**40**	**50**	**60**
A	6.25	-	++	+++	All rats died	-	-
B	12.5	-	++	+++	√	-	-
C	25.0	-	+	++	√	-	-
D	50.0	-	-	+	++	All rats died	-
E	100.0	-	-	+	+	++	++
F	0.1 (Samorin)	-	-	-	-	-	(Survived) Survived
G	PBS–Parasitized	+++	All rat died				

+ = 2 parasites/field; ++=6-8 parasites/field; +++=>60 parasites/field (massive infection); -=either no parasites or no rat

Table 16: Parasitemia per day of observation in different groups of rats with T. brucei infection

Treatment Group (rats)	0	1	3	5	7	9	11	13	15	17	19	21	25	30	35	40	45	50	55	60
A	0/5	0/5	3/5	5/5	5/5	4/4	2/2	1/1	%	%	%	%	%	%	%	%	%	%	%	%
B	0/5	0/5	2/5	5/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
C	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
D	0/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
E	0	0	0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F	0/5	0/5	3/5	0/5	0/5	3/5	2/5	2/5	2/5	2/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key:

A = Infected not treated

B = Diminazene aceturate (3.5mg/kg/bw) single standard dose i/p.

C = Diminazene aceturate (1.75mg/kg/bw) + Allium sativum bulb extract (20mg/kg) treated sub therapeutic dose.

D = Diminazene aceturate (0.875mg/kg/bw) + Allium sativum bulb extract (10mg/kg) treated sub therapeutic dose.

E = Pre-treated Diminazene aceturate (1.75mg/kg/bw) + Allium sativum bulb extract (20mg/kg) treated sub therapeutic dose.

F = Allium sativum bulb extract (40mg/kg) standard dose orally.

G = Uninfected not treated control

Table 17: Summary of a total number of death, cured animals and average survival period in the treatment and untreated groups.

Indices Determination	Infected + Diminazineaceturate alone	Infected + Khaya (40mg/kg) along	Infected + treated Khayasenegalensis Diminazineaceturate	Infected not treated	Uninfected untreated control
Total number of death	1	4	0	4	0
Number of animal cured	3	0	4	0	4
Average survival day	24	13	24	9	24

- **Observations from our studies**
  - The extract showed concentration and dose-dependent antitrypanosomal activity in both in vitro and infectivity studies.(Table 14&15)
  - Pre-treatment with the extract resulted in delayed onset/establishment of infection for 10 – 15 days. Pre-treatment with the combination, however, prevented the establishment of infection for 60 days. (prophylactic significant of the combination therapy.(Table 16)
  - Treatment with the extract alone resulted in a reduction of parasitaemia but not curative.
  - Berenil (Therapeutic dose) alone and its combination with extract resulted in complete clearance of the parasite, restoration of haematological indices toward control values.(Tables 16&17)
  - We observed that combination therapy was not only the best in terms of efficacy but was less toxic and less costly than monotherapy with either berenil or samorin.
  - All the plant extract at the dose used in this study appear to potentiate the chemotherapeutic activity of berenil and thus present a great potential for the treatment of African trypanosomosis.

- Drug combination is one of the few options suggested by many workers (Atougaand Costa 1999; Ajagbonna and Olaniyi, 1999; Anene et al 2001) to minimize current therapy failure due to drug resistance/toxicity in the face of a dearth of new trypanocide for the last 30 years.
- The animals were observed to be feeding and drinking normally, they were mentally alert and respond to external stimuli for the period of the infectivity test, showing safety in the use of the plant extract.

Toxicity studies/safety of the plant extract

- The lethal dose (Dose of the agent that will kill 50% of the animal population) or the acute toxicity was conducted using limit dose test of up and down procedure according to the OECD/OCDE test guidelines on acute oral toxicity at a limit dose of 3000mg/kg body weight.
- This was done for most of the extracts we have investigated in this presentation. The LD_{50} estimate was calculated to be greater than 3000mg/kg. (Adeneye, Ajagbonna et al 2006).
- Repeated dose toxicity studies were conducted by daily oral dosing of the animals for short, medium and long-term effect of these extracts.
- No fatality was recorded, no significant effect on both haematological, biochemical parameter measured and no significant damage to vital organs like liver, kidney, and heart was observed.
- These data suggest a safe therapeutic benefit for most of the extracts(Ajagbonna&Adebayo2001: Ajagbonna&Onyeyili,2002)

Herbal Contamination and Toxicity

- A common misconception about herbalism and use of natural products is that “natural” equal safe. Contaminants and adulterants of medicinal plants can be pharmacologically active and responsible for unexpected toxicity (Etuk, Egua and Ajagbonna2009).

- Similarly, contamination of herbal with a microorganism, fungal toxin e.g. aflatoxin, pesticide, heavy metals have all been reported (Felix et al., 2005).
- We have therefore examined the Nasara Pile Syrup (NPS), a commercial herbal medicine that enjoys wide patronage among the populace in Sokoto State Nigeria.

Toxicity Studies on NPS

- Graded doses (0.5, 1.0, 1.5 and 1.75ml/100g) of the herbal medicine were administered to some albino rats and their responses observed for 72hr to study the acute toxic effect of the herbal medicine.
- In the subchronic toxicity study, some rats were also treated orally with repeated doses of the medicine for 28 days after which the animals were sacrificed and vital organs like liver, kidney, and heart obtained for histopathological examination.

Result

- The results showed that administration of a single dose of the medicine did not produce any harmful effect or death in the animals within the 72hr period.
- In the repeated dose study, the herbal medicine produced death and some damage to the kidney, liver, and heart of the rats; this was evidenced by histopathological lesions in a dose-dependent manner. (Etuk, Igbokwe, Ajagbonna et al., 2008).

Heavy Metal and Microbial Contaminants in NPS

- The heavy metal and microbial contaminants levels were evaluated in NPS against the backdrop of reports of high levels of such contaminants in similar herbal products elsewhere in Nigeria, India, and China.

- Atomic absorption spectrophotometric technique was used for the analysis of the herbal product for the level of heavy metal contents, while the bacteria count was by pour plate culture method and the subsequent specific identification was achieved by subculturing and application of cowan and steel procedures (Egua, Etuk, and Ajagbonna, 2008).

Result

- The total microbial content in the NPS was found to be 2.86 x 10^7 CFU/ml.
- The bacterial identified included Bacillus cerus, Bacillus spharicus, Bacillus mycoides, and Bacillus lentus. The bacteria load was high enough to initiate gastrointestinal infection.

Table18: Heavy metal contents in Nasara Pile Syrup (NPS)

Heavy Metal	Extract Content (mg/l)	Reference Value (mg/l)
Zn	0.14	397.5
Cu	0.55*	0.11
Cd	0.24*	0.031
Cr	0.25*	0.105
Mn	1.60	14
Ni	1.48*	1.345
Pb	1.65*	0.005
Fe	1.80	320

* values exceeding reference value (Anon 2003).

- The results (Table18) showed that NPS contained some heavy metals in excess of the WHO permissible maximum limit for heavy metals in consumable items. Thus prolong consumption of this herbal product may result in heavy metal accumulation in the body and chronic poisoning. Therefore, the distribution and consumption of this herbal product should be strictly regulated by NAFDAC.

Phytochemical Analysis

Qualitative and quantitative phytochemical analysis was carried out on most of the plants presented here today according to the standard method. The major constituents common to most of the plants include alkaloid, tannin, and flavonoids.

ISSUES AND CHALLENGES TO THE DEVELOPMENT OF HERBAL MEDICINE IN NIGERIA

Mr Vice-Chancellor Sir, the entrance of ethnopharmacology from the results presented here today has not only shown the great potential in our flora but has also revealed the pharmacological efficacy, mode of action, toxicity and or safety of our indigenous medicine. This has no doubt giving rise to a value added product that can now be exploited for commercial purposes for the prosperity of this nation.

Herbal Global Market

- Today the herbal global market is estimated to be 90 – 100 billion US$ dollar growing at the rate of 10-15% annually and is expected to be over 5 trillion US$ dollar by 2030.
- However, only China and India are the two key players in this huge market.
- The share of Nigeria in this market is almost nothing to write home about. Which is in no way match with the kind of rich biodiversity and traditional knowledge the country possesses.
- Why then have we not been able to develop a novel drug from our rich forest?
- Why is Nigeria not a key player in the herbal global market?

Issues and Challenges

- Drug development is a risky,resource-intensive and time-consuming process.
- Standardization (weak or lacking).
  - Quality
  - Safety
  - Efficacy
  - Stability
- Weak regulation and legislation on the herbal product.
- Poor conservation strategies in the face of a wild collection of plants.
- Lip service to R&D and poor research capacity of scientists.

Suggestions

(1) Standardization: This is a mandatory requirement in the herbal global market. Therefore all modern tools and definite analytical protocols must be used to achieve the standard.

- The government must ensure adherence to GACP, GMP and GLP guidelines.
- Africa must set up a regional quality specification in line with WHO template
- The government must also establish a national pharmacovigilance/ADR centre for monitoring the safety of medicinal plants.

(2) National Assembly, NAFDAC, and other stakeholders must come up with a definite legislation in the regulation of herbal medicine in Nigeria. The National Expert Committee on verification of herbal cure claims (NECVHLC) was only set up in February 2013 by NAFDAC and only a draft of the guideline was adopted.

(3) A well-articulated conservation strategy is the best way to conserve our medicinal plants. This will involve in situ and ex situ strategies.

- In Situ: The forest protection act 2006 must be reviewed to create a legislation to preserve and protect the plants in their natural habitat.
- Ex Situ: Because of our forest that is threatened by the indiscriminate wild collection of plants, selected plants species can be saved from extinction by;

  - Establishment of botanical/herbal garden nationwide/schools.

  - Deliberate plan to develop seeds, germplasm, and DNA banks to preserve these valuable herbs.

  - Farmers should also be encouraged to go into cultivation of medicinal plants.

(4) Mainstreaming of indigenous medicine with orthodox must be encouraged by government as currently practiced in countries like India and China.

(5) On poor funding/R&D, the India initiative can serve as a catalyst in the drug development in Nigeria.

- Soft loan for pharma-industrial R&D projects. For example in India, the government provides unsecured loan up to 70% of the project cost.
- Grant-in-aid to the pharma industry, this is to support drug trials in the development of drugs for specific/neglected disease in the country.
- Establishment of a national facility. The government can establish a state of the art infrastructure on drugs and pharmaceutical R&D in institutions/academic organizations.

- Collaborative R&D project must be encouraged among the public institutions, industry and the government to facilitate drug development in the country.

(6) Mr Vice Chancellor, Sir, apart from the role of government in providing infrastructure and fund for research, the University of Abuja in its own must come up with definite research policies/ yearly targets, establish a functional research centre and also create an enabling environment for its staff and student development. This is the hallmark of a university that aspires to be a centre of excellence in the area of innovations.

(7) My current research work interest.

My visit to the centre for Physiology and pharmacology in Vienna, Austria has opened my eyes to the field of cyclotide isolation from our local plants as a basis for drug design and its importance to food security. The cyclotide field is a relatively new, but rapidly developing field.

I have been able to establish a working relationship with Prof. PulokMukhlerjee, the director, school of national product studies, India, Dr.Ashafa, the assistant Dean Faculty of Natural and Agricultural Science, University Free State South Africa have both given me unfettered access to their laboratory and equipment for cyclotide research studies.

It is gratifying to note that one of our staff, Dr. Apollo Adeniran is currently in the same university of Free State for his Ph.D. degree.

Concluding Remarks

Mr Vice Chancellor Sir, in the beginning, God planted man and his animal in the Garden of Eden with access to tree fruits, leaves (herbs) for man and grasses for animal and there was a complete homeostasis (wellness).

- Today, man is continually going away from the garden, the more he moves away from the green vegetables (plants) in the garden, the more the sickness (with new one emerging) despite the advancement in medical research.
- It is, therefore, my humble submission that it is time to return to the garden, let the food (herbs) be our medicine or else medicine (drug) will be our food.
- My effort over the years in the area of ethnopharmacology is not only to inject science into the art of healing that is practiced by indigenous people for their health care need, but it is also to bring out the economic potential of this neglected medicinal plants to man. This is the story of my professorship.

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