PHYTOCHEMICAL, PHARMACOLOGICAL AND FORMULATION STUDIES OF *Andrographis paniculata Nee Acanthacae*

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA, IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICS

DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

JULY, 2010

DECLARATION

I declare that the work reported in this thesis entitled "**PHYTOCHEMICAL, PHARMACOLOGICAL AND FORMULATION STUDIES OF *Andrographis paniculata Nee Acanthacaea*"** has been carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology under the supervision of PROF. P.F. OLURINOLA, of the Department of Pharmaceutics and Pharmaceutical Microbiology and PROF. K.S. GAMANIEL, Dean of the Faculty of Pharmacy University of Maiduguri and PROF.M.A. IBRAHIM of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Jos. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been presented for another degree or diploma at any university.

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CERTIFICATION

This thesis titled "PHYTOCHEMICAL, PHARMACOLOGICAL AND FORMULATION STUDIES OF *ANDROGRAPHIS PANICULATA NEE ACANTHACAEA"* by MSHELBWALA KUBILI meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY (Pharmaceutics) of Ahmadu Bello University Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation

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DEDICATION

To the Almighty God, the King of kings and Lord of lords.

To my late loving husband, Dr. Arhyel Sikumta Mshelbwala

our children

Hannah

David

Philip

Surhyel and their spouses

To our grandchildren born and yet unborn

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ABSTRACT

The leaves of *Andrographis paniculata acanthaceae* is used locally in Northern Nigerian for the treatment of hypertension without quantification or dosage standardization. This work was primarily set out to find justification for the local medicinal use of this plant and to scientifically study the pharmacological activity of the plant and formulate it into a suitable tablet dosage form, as a means of standardization of the dosage regimen.

Phytochemical, pharmacological and formulation studies of the leave, aqueous and Methanolic extracts of the plant leaves were carried out using standard methods. Phytochemical screening as described in official monograph was carried out to detect the presence of secondary metabolites. Extracts were tested for antihypertensive activity in cats by monitoring changes in blood pressure following administration of the extract. The antihypertensive activity was compared with propranolol (standard drug). Toxicity study was also carried out by determining the LD_{50} of the extract in rats.

In formulation studies, micrometric properties of the extracts and granules such as size distribution, moisture content, angle of repose. Carr's Index etc., were determined using different binders (maize starch, polyvinyl pyrollidone (PVP) and gelatin). Disintegrants (maize starch, pregelationised starch, microcrystalline cellulose, heavy magnesium carbonate and dicalcium phosphate) and diluents (Lactose and heavy magnesium carbonate) were also included in the formulations. Different methods of formulations were carried out, but only the best three of the Methanolic extracts were used for this work. Properties of the resulting tablets such as weight variation, crushing strength, disintegration properties, friability and dissolution profiles were determined and analyzed. Effects of storage condition (such as humidity and temperature) on the tablet properties were also determined. Stability studies on the extract were carried out using TLC technique and Differential Scanning Calorimetry (DSC).

Results of the Phytochemical test showed that the plant extract contained alkaloids tanning, cardioglycosides, flavonoids and terpenes. The plant is relatively safe, with an LD_{50} of 1.90 x 10^4mg/kg body weight which is much higher than those of 0.81mg/kg body weight taken by the local people. Pharmacological studies showed that the leave extract possesses significant antihypertensive activity, comparable to Propranolol with methanol extract exhibiting greater activity.

Qualitative and pharmaceutically acceptable tablets could only be produced from Methanolic extract using the wet granulation method. When excipients such as pregelatinzed starch (disintegrants), heavy magnesium carbonate (diluents) and 2.5% w/v gelatine binder was used. The thin layer chromatography and DSC studies showed that the crude extract contain three distinct components. The stability studies showed that the Methanolic extract tablets were stable under proper storage condition even after 4 years.

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ABBREVIATIONS

1- PY = I/K K is the slope

2- A = Intercept

3- D_A = Densification that result from interparticulate bending

4- BD = Bulk Density

5- TD = True Density

6- DB = is the densification as a result of particle movement and rearrangement

7- W = Wt of Material (granules or tablet)

8- A = weight of specific gravity bottle + xylene

9- Y = weight

10- D_A = $1 - e^{-A}$ (e = 2.7) constant

11- D_O = $\frac{BD}{TD}$ = Packing fraction

12- DB = $D_A - D_O$

13- TD = $\frac{w}{(a+w)-y} = x\ S.G.of\ xylene$

14- Y = of bottle + xylene + tablet or granules

CHAPTER ONE

INTRODUCTION

1.1 *ANDROGRAPHIS PANICULATA*

Andrographis paniculata (AP) commonly known as "King of Bitters", is a member of the plant family *Acanthaceae* and has been used for centuries in Asia to treat gastrointestinal tract and upper respiratory tract infections, fever, herpes, sore throat and a variety of chronic and infectious diseases. In India it is called 'Kalmeh' It is found in the Indian Pharmacopoeia and is prominent in at least 26 Ayurvedic formula. In traditional Chinese medicine (TCM), it is an important "cold remedy" herb. It is used to rid the body of heat as in fevers and also dispel toxins from the body (Chung, 1979). In the Scandinavian countries it is commonly used to treat and prevent common colds. Researches done in the 80s and 90s have confirmed that AP properly administered has a surprisingly broad range of pharmacological effects, some of which are extremely beneficial. It has abortion properties (can abort pregnancy); although in Ayurvedic tradition it is allowed to be taken for a short duration during pregnancy. However it is advisable to avoid its use during pregnancy as a precaution. AP has been shown to have very low toxicity (Chung, 1979).

1.1.1 Morphology

AP is an annual branched erect plant. It runs to ½ - 1 meter in height and grows abundantly in South Eastern Asia, India, Sri Lanka, Pakistan and Indonesia. It is also cultivated extensively in China and Thailand (Sandberg, 1994), East and West Indies and Mauritius (Gupta *et al.*, 1990). AP is ubiquitous in its natural areas; it grows in pine, evergreen and deciduous forest areas and along the roads in villages. It grows in all types of soil, even where almost no other plant can be cultivated

particularly “serpentine soil”, which is relatively high in aluminium, copper and zinc. This ruggedness of the plant accounts for its wide distribution. It is therefore not surprising to find it growing in some parts of Northern Nigeria and West Africa. Specifically in Nigeria, it is found in Adamawa and Kaduna states, and the Federal Capital Territory (FCT), Abuja. The plant is known to have proven biological activities and that may be the basis of its wide use for various ailments in different parts of the world. Details of these biological activities will be given later, especially its antihypertensive activity, which is one of the main focus of this research project.



Plate 1: A mature shrub of *Andrographis paniculata* grown in a garden in GRA Zaria, Nigeria.

1.1.2 Biological/Pharmacological Actions

a) Biological Activity

It has been demonstrated that extracts of AP can increase the time it takes for clots to form, thus decreasing the risk of blockage of blood vessels (stenosis) seen after angioplasty procedures. Researchers at Tongi Medical University in China have demonstrated that AP given to dogs one hour after development of myocardial infection decreased the damage that occurred in the heart muscle (Zhao and Fang 1990).

An extract of AP given to hypertensive rats by Zhang and Van (1997) also showed that the AP extract reduces the blood pressure of anaesthetized rats. Mshelbwala *et al.,* (1998) further support this finding. This substantiated its traditional use by the natives in Garkida, Adamawa state of Nigeria. The natives takes ten to fifteen of the fresh leaves of *Andrographis paniculata* to chew once or twice a day with a glass of cold water and this dose is enough to lower blood pressure continuously.

Some studies have shown that 80% of administered dose of andrographolide is removed from the body within eight hours and excretion rates of more than 90% of the compound within forty eight hours have been reported (Jean Barilla, 1999).

b) Anti-microbial activity

Extracts of AP have also been evaluated for anti-malarial activity against *Plasmodium berhei,* one of the agent or parasites that transmit malaria. The extract was found to produce considerable inhibition of multiplication of the parasites (Misra, *et al., 1992*). Pre-treating animals for 15-21 days with the extracts prior to exposure as well as after infection was found to be more effective than treatments started only after infection. The treatment effects were better than that of chloroquine (commonly used anti-malarial drug), after infection.

AP extracts are also effective in dogs, killing filarial worms that obstruct lymph channels in the body, leading to gross swelling termed elephantiasis. Since no toxic effect was observed, researchers believe that it would be safe for human use as well although no plant has previously been shown to have anti filarial action till now (Misra, *et tal*, 1992)

***c) Anti-diarrheal and Intestinal** Effects*

Experiments on animals show that AP can prevent or stop diarrhea. Extract of AP has been shown to have significant effect against the diarrhea associated with *Escherichia Coli* (*E. coli*), (Gupta *et al.,* 1990*)*.However its mode of action against bacterial dysentery and diarrhea could not be confirmed.

d) Effects on the Respiratory System

AP extracts have been used to treat tonsillitis, respiratory infections and tuberculosis. In one study, AP was used to treat 129 cases of acute tonsillitis. Sixty-five percent of patients responded positively to the therapy (http://www.altcancer.com/andcan.htm, 2003). The same review reported the use of A.P. extracts to treat pneumonia, in which thirty five (35) cases were found to show positive changes and nine patients completely recovered.

Tuberculosis is usually treated with the antibiotic Rifampicin and it results in 22.5% mortality, but when a solution of 2.5% w/v water extract of AP was given parenterally to provide 50-80 mg/kg body weight for two months, the results were improved(i.e. less mortality). Seventy cases of tubercular meningitis. 30% of patients were cured with 8.6% mortality. Combination of AP extracts and Rifampicin resulted in a 2.6 fold decrease in fatality rates (http://www.altcancer.com/andcan.htm, 2003).

e) Protection on the Liver and Gall Bladder

In Ayurvedic medicine (a system used in India), there are 26 different formulations containing AP that are used to treat liver disorders. AP extracts were tested for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride (a cleaning solvent), alcohol or other toxic chemical which damage the liver (Kapil *et al,* 1993). When extracts of AP compound were given to these animals three days before toxic chemicals, there was significant protective effect in the liver. This effect is due to antioxidant property of AP compounds. Studies have also shown that, the extracts when given to animals produced significant increase in bile flow (Skukla *et al,* 1992) which aids digestion. A drug like paracetamol, normally decreases bile production. However when extract of A.P. was given concurrently, the result was, increased bile production (Shukla, 1992).

Infective hepatitis is an acute inflammatory condition of the liver. This normally follows liver cirrhosis, which may eventually lead to coma and death. In India where ancient physicians used AP to treat similar liver ailments, a study was conducted to evaluate the effect of administering AP to cases of infective hepatitis. There was marked improvement in the majority of patients tested when given as a decoction or infusion of AP (Http://www.altcancer.com/andcan.htm Review, 2003).

f) Common Cold, Fever, Inflammation

The prevention of common cold with an extract of AP was shown in a pilot double blind study in Sweden. Students were given a formulation of AP by the Swedish Herbal Institute and were observed for the presence or absence of difference between the groups that were administered the AP formulation, and the placebo. The rate of

absences of cold in the AP treated group was 30% whereas for those who took the placebo it was 62%. This is due to the presence of the component of the extract of AP which has immuno stimulant effect (Burgos and Caceres, 1994).

1.1.3 Safety and Toxicity

In Traditional Chinese Medicine (TCM) systems of healing in Thailand and India, it was observed that AP has long been perceived as safe. Mshelbwala, *et al (*1999) using in-traperitoneal (IP) route, the LD_{50} was found to be 1.90 x 10^4mg/kg (body weight). Toxicological studies on animals and in human clinical trials confirmed that none of the mice that received oral AP extracts 10g/kg body weight once a day for seven days died (Chung, 1979). This is an indication that AP has a low toxicity. At a dose of 10mg/kg, there were no abnormal cardiovascular responses, liver enzyme, heart, kidney and spleen functions were normal in these animals (Guo, *et al., 1988*). At dose of AP taken orally at 1g/kg oral for seven days, did not affect the blood count, liver function or kidney function or other important organs and no weight lost was observed (Akbarsha, 1990; Yin and Guo, 1993*)*. All evidence to date indicates that andrographolites, which are component of the extract of AP, are naturally occurring compounds with low toxicity when used appropriately. However, the use of AP has been associated with allergic reactions ranging from minor skin rashes to more serious anaphylaxis, which is a potential problem at high doses. Whether or not these reactions are due to AP or other matter in herbal preparation is not clearly understood.

Herbal medicines as the major traditional remedy have been used in medical practice over many centuries even long before. The use of herbs for treatment of diseases has increased drastically in the last few decades. Use of traditional medicine is associated with developing countries, where it is more difficult to obtain orthodox medicines

which are more expensive and are out of the reach of most of the populace. Therefore they resort to traditional medicines, which are easily obtainable and less costly (Naoemesi, 1996). The World Health Organization (WHO) has since recognized herbal medicine as a valuable and readily available resource for primary health care and therefore endorsed its safe and effective use Akerele *et al.,* (1986).

However, in recent times, even developed nations are now resorting to the use of herbal medicines as a safe method of treatment (Fansworth and Morris, 1976). This is due to the fact that herbal medicines are natural. They are close to natural food and sometimes less toxic over prolonged use than the orthodox medicines, which can be made of synthetic substances that have toxic side effects. This evidence is seen by the various types of herbal preparations both preventive and curative produced locally by traditional medicine healers and also by multinational companies.

1.2 STATEMENT OF OBJECTIVES

1.2.1 Statement of Problem

Hypertension is a common disease that cut across all races especially among the blacks and the cost of treatment is beyond the reach of the majority of the population in developing countries. Hypertension is a silent killer among the most vibrant and productive class of the society. Herbal preparations are most assessable to the majority of the population who cannot afford orthodox drugs.

1.2.2 Justification of Research

70% of herbal production is used by most populace.

Herbal drugs may be cheaper to produce thus making the herbal production more accessible.

Crude herbal preparations may be very toxic and their scientific formulation may help in minimising their toxicities.

The herbal production is screened for toxicity and the appropriate dosage form could be made available for use to the public.

1.2.3 Specific Objectives.

The aim of the study is to confirm and establish the claim of activity of AP by the natives and justify the use of the plant in the management of hypertension

The specific objectives of the study are to:

a) determine the best solvent for extraction of the plant constituents, which will produce the best pharmacological activities.
b) carry out tests on the pharmacological activities of the extracts which will confirm the assumption stated.
c) to determine the toxicity of the plant and LD_{50}
d) to formulate both the extract and the powdered leaves into solid dosage form suitable for use orally.
e) to carry out some stability studies on the best formulation.

CHAPTER TWO

LITERATURE REVIEW

2.1 HERBAL TRADITIONAL MEDICINE

One of the earliest records of the use of herbal medicine is that of Chaulmogra oil from species of *Hydnocorpus goctran*, which was known to be effective in the treatment of leprosy. This was recorded in the pharmacopoeia of Emperor Shen Nung of China between 2730 and 3000 BC.

The traditional healers are called by various names depending on the countries they come from. These methods are based on social, cultural and religious backgrounds as well as the knowledge, attitude and beliefs that are prevalent in the community regarding physical mental and social well being and causes of disease and disability. In India, traditional health practitioners are called "indigenous healers". In China they are called "barefoot doctors" not because they go barefooted; in fact most of them wear shoes, but to signify that they are also farmers. In Cuba, they are called "curann deros". In Nigeria, they are called several names depending on the community they come from. Some of them are called "native doctors", some even go as far as calling them 'witch doctors'(Sofowora, 1993). Each culture has its names for traditional healers, emphasizing their close association with the community in which they live and practice. In some countries especially African countries some incantation is part of the treatment.

In this respect the more convenient traditional healer which may be acceptable is the "Herbalist". The herbalist specializes in the use of herbs to treat various ailments. He is expected to be highly knowledgeable in the efficacy, toxicity and dosages of herbs (Sofowora, 1993).

The main disadvantage of the herbal preparation by the traditional healers is that, the doses are not accurately quantified. Nearly in all cases the LD_{50} and the toxicity tests are not carried out. Phytomedicines can be defined as medicinal products containing as active ingredients, parts of plants or plant materials or combinations in whatever form whether crude or processed. These lead researchers in the field of plant medicine like, Ayurveda, state that, higher plants constitute living chemical factories that provide a vast display of unusual chemical structures with variety of biological activities. They also stated that there are up to 200,000 – 300,000 currently known higher plant species and only a small percentage has been investigated phyto-chemically (Sofowora, 1993). Only a small percentage of this has been subjected to biological and pharmacological screening. This is very necessary because the use of herbal medicine has become very relevant to orthodox medicine.

In recent years the use of traditional medicine as substitute or complementary to modern medicine has been documented by several authors (Harrison, 1976 and Kerharo, 1974), and a host of others.

The World Health Organization (WHO) supports the appropriate use of herbal medicines and encourages the use of the remedies that have been proved effective. As much as possible, the use of herbal medicine should not be discarded. WHO has therefore published guidelines with the main purpose of defining the basic criteria for evaluation of quality, safety and efficacy of herbal medicines and thus assist researchers and others to undertake an assessment of the documentation in respect of such products. This would result in standardization of phyto-medicines, which Elujoba (1996) defines as the process of recommending a set of pharmacognostic, chemical, biological and pharmacological standards adequately controllable. This will provide the monograph for the proposed plant preparation for the purpose of ensuring quality, safety, efficacy and

reproducibility of the preparations. In general, a drug is hardly administered in its pure form, whether as an orthodox medicine or as herbal preparation. They are always administered as a sort of dosage form as simple or complex mixtures, containing the active drugs and other additives. This is even more so with phyto-medicines where traditional medicines prepared are always bulky, unpalatable and prepared generally under unsanitary conditions. These do not meet the standards for substances for consumption. The only way the phyto-medicines can eventually be accepted for administration is to standardize the quality, safety, efficacy and reproducibility of the medicines. This can be guaranteed by formulation of the products into appropriate dosage forms. The choice of the type of formulation depends on the physical character of the medicine, the plant part and the stability of the products concerned. Naomesi (1996), has stressed that ethno-medical information on the mode of drug administration should form the basis of deciding the formulation method to be used for phytomedicines and complex extraction processes should be avoided.

a) Ayurveda: There is also another form of traditional medicine called Ayurveda. It is an indigenous system of medicine in India. Ayurveda, meaning the science of life, is reported to have been practiced for over 3000 years. Its history being divided into four periods:

(i) Vedic period

(ii) The original research and classical period

(iii) A period of compilation of Ayurvedic methods and periods of Rasa Tantras Sidhas (Chemists and Physicians).

(iv) Period of stagnation and eventual recompilations.

Ayurveda considers the human being as a miniature universe and properties found in the universe are believed to be also present in the human body. Similarly, the principles found in human body are to be found in the universe. A healthy man is thus defined as one who possesses a balance of humours, proper functioning of all body elements and one who has a pleasant disposition of mind, soul and body organs.

b) Acupuncture: Acupuncture is another method of traditional medicine as used in China. This type of traditional system of medicine represents perhaps, the most widely employed traditional medical system in the world, with a wide usage of herbs prescribed and used in a standardized manner. It is a traditional medicine system practiced by many countries in the East. Attention is drawn to acupuncture as one of the peculiarities of Chinese medicine. Chinese medicine is a holistic form of medicine in which various factors such as the constitution of the patient are taken into consideration in the treatment of disease. Acupuncture has been practiced in traditional medicine for over 2000 years.

2.1.1 Status of Traditional Medicine and the Use of Medicinal Plants in the World Today

An investigation throughout the world into the status of traditional medicine generally, and the use of medicinal plants in particular shows that in some countries there is collaboration in the orthodox and traditional medicine in the way of health care whereas in others there is confrontation. But the World Health Organisation (WHO) encourages their collaboration.

In the African regions traditional medicine has become a part of the people's culture despite the fact that this form of medicine is not well organized like in India and China.

2.1.2 The Americas

In the Americas more is heard of traditional medicine and its practitioners in the South and among the Amaridians than in North America and Canada. In North America and Canada however between 1959 and 1973 research conducted showed 25% of all prescriptions dispensed in community pharmacies to contain drugs extracted from higher plants (Farnsworth and Morris, 1976). This trend has not changed much since then.

2.1.3 The South - East Asia

This part of the world constitutes the area where traditional medicine has been integrated into the official health care system and where formalized and informal training of practitioner exists. Formalized use of traditional medicine include Ayurveda, Yoga, Tibbi modified Chinese and the Ameni system (a Tibetan system of medicine). New formalized training of practitioners of indigenous medicine also exists in this region including that of herbalists, bone setters, practitioners of Thaad element system home remedy and spiritualists. In India alone there are some 500,000 indigenous medical practitioners and 115 recognized institutions of indigenous medicine of which 98 colleges are involved in training of personnel in all aspects of traditional medicine excluding Ayurveda (Kurup, 1978).

2.1.4 Europe

Europe apparently has little use for traditional medicine. Many countries disapprove of the WHO's promotion of traditional medicine perhaps because these countries have such highly developed health care for their people. For example, in Britain the days of using concoctions of some plants to treat dropsy have long gone and now the active component, digitatis is used in standardized tablet form. Also middle level health personnel replace the traditional healers of other region of the world. Traditional medicine however is practiced in some remote areas; it is difficult to separate them from quackery or chiromancy. Practitioners of fringe medicine which include herbalist, homeopathy, osteopathy, and chiropractice – are classed somewhere between traditional healers and graduates of recognized medical schools. Official and professional attitude in Europe towards an extensive use of traditional practitioners is to say the least cautions. Traditional practitioners and their successors should receive modern training from biological and scientific point of view.

2.1.5 Nigeria

In Nigeria, documentation has not been done accurately, although the use of herbal medicine has long been in existence.

It has also been proposed that knowledge of medicinal plants was gained by accident, although this theory has been refuted by a number of traditional medicinal practitioners who claim that information on such plants was communicated by their ancestors in various ways (Akpata 1979; Lambo, 1979). Most information about herbal medicine has been kept secret in Nigeria. It is only disclosed to a few members of the family. This information is passed on from generation to generation. Some families

specialize in the treatment of certain diseases. Proper documentation has been very difficult and in some cases the healers die with their knowledge if a successor is not appointed in time.

Generally traditional healers are treated with great respect in Nigeria. A lot of the population – about 70% even among the elite still resort to traditional medicine in the treatment of some diseases.

2.1.6 The Activities of International Organizations in the Field of Traditional Medicine

The World Health Organization (WHO) in its coordinating role has established a programme for traditional medicine in Geneva. Regional Directorate of WHO also have some assignments on traditional medicine programme, which vary with whole overall interest, generated in the particular region. The WHO traditional medicine programme has the following as its objectives:

(i) To foster a realistic approach to traditional medicine in order to promote and further contribute to health care;

(ii) To explore the merits of traditional medicine in the light of modern science in order to maximize useful and effective practice and discourage harmful ones;

(iii) To promote the integration of power valuable knowledge and skills in traditional medicine;

The traditional medicine programme is guided by an expert advising panel. It maintains links with United Nations International Development Organisation (UNIDO), United Nations Development Programme (UNDP), United Nations Educational and Scientific Organization (UNESCO) and the World Bank, for extra budgeting financing

of its activities. A recent link was forged between the World Federation of Proprietary Medicine Manufacturers (WFPMM) and the activities of Traditional Medicine Programme at Geneva. Most of these resolutions recognize the fact that many drugs used in modern medicine come from plants or animal sources.

Traditional medicines or herbal medicines have come to stay. Even developed countries now are laying more emphasis on herbal drugs rather than the orthodox drugs (Farnsworth and Morris, 1976). The use of herbal preparations as preventive rather than curative has gained ground all over the world.

2.2 Hypertension

Hypertension is prevalent disease condition all over the world. Hitherto it was thought to affect only the developed countries, due to the pressure of life and the demand on the populace. It was also thought to affect only the middle aged and the old people. This assumption was proved wrong. Recently it has been proved that hypertension affect even more of the developing countries especially those of the black race (World Hypertension League). It is not only confined to the middle age, but even children sometimes are found to be hypertensive.

- The WHO has defined hypertension as systolic blood pressure above 140mmHg. The above definition was adapted to harmonize the cut off levels of blood pressure (BP) to that of WHO grades, the classification of BP to that of WHO grades. The optional B.P. classification is <120/80mmHg as normal, <130/85mmHg as mild and 130-139/85-89mmHg as high. There are three grades of hypertension:
    1. 140-159/90-99 mmHg
    2. 160-179/100-109 mmHg
    3. 180/110mmHg

A detailed population study was carried out in the U.S. and 160/95mmHg was deemed abnormal and 140/90 to 160/95mmHg was regarded as borderline (World Hypertension League). There is hardly any distinction between normal and abnormal. Blood pressure varies within population and also between different races. It is said that the African region has a higher pressure than the Caucasians (World Hypertension League, 1996). The distinction for systolic and diastolic blood pressure is similar and the risk of mortality and morbidity rises continuously. The level of blood pressure can be said to be abnormal when it is associated with clear increase in morbidity. The level varies with age, sex, race and country.

2.2.1 Causes of hypertension

The incidence of hypertension among African American is greater at every age beyond adolescence and tends to induce more vascular damage in blacks than whites (Perry *et al*, 1994).

Hypertension is divided into categories:

i. Essential or (primary) which constitutes 90-95% of cases

ii. Secondary, which takes up the remaining 5-10%.

In a survey done in Africa, cases of hypertension range from 5-15% of the population whether urban or rural. The commonest cardiovascular condition in Africa is hypertension (McMahnan *et al,* 1990).

2.2.2 Factors that Are Associated with Hypertension

a) Sodium Chloride and Blood Pressure (BP): This factor has been studied extensively and it was shown that there is a relationship between sodium chloride and hypertension because sodium is associated with water retention. Although Sodium chloride plays a very important part in our diet, yet it is one of the commonest things that encourage hypertension. Sodium chloride that is consumed is more than needful. Some experts suggests that craving for excess dietary sodium may be genetically determined (Intersalt, 1986).

b) Stress and Blood Pressure (BP): The relationship between stress and blood pressure has been established, although with controversial findings. The finding is that hypertension is positively related to stress although it is difficult to quantify and measure.

c) Potassium and Blood Pressure: The Intersalt study (McMahnan *et al, 1990*) has confirmed a negative relationship between potassium and blood pressure across a large number of societies especially in the elderly Probably Na+/K+ ions will determine the risk of developing hypertension (Folknow, 1995). Potassium is said to aid sympathetic vasoconstriction and higher level of K+ will lead to natriusesis. This will result in increase in blood pressure (BP).

d) Educational Level: A negative association between education and hypertension was reported in the U.S. in both black and white male and female (Anderson. 1984). The educated have better knowledge of health behaviour like exercise and diets, which lead to better coping of strategies for successful management. In Nigeria however, the opposite was noted.

Body mass index and blood pressure is a stronger factor in the U.S. (Ogunlesi *et al,* 1991). In the U.S., obesity may account for roughly 20% of the population and attributed to risk of hypertension among blacks, but less or modest in the Africans (Stambler *et al,* 1975). There is lower prevalence of obesity than in the white in the sub-Saharan Africa.

e) Alcohol and blood Pressure: It is linked to other risk factors like obesity, urbanization, socio-economic factor, smoking, diet and age. Several studies show non-linear relationship between alcohol consumption and blood pressure (Kumanyika, 1989).

2.2.3 Principles of Management of Hypertension

When managing hypertension not only the managing of the blood pressure but the whole individual is considered. The consequences of and the failure to identify hypertension have been known for at least 50 years. Severe hypertension has a more aggressive nature in most black communities and variably involves the major organs of the body (Akinkugbe, 1972). Hypertension is one of the most studied areas of medicine. It is easy to define theoretical treatment, but in real life it is not easy to adhere to them. WHO has brought a guideline on hypertension management. The diagnosis and management of high blood pressure should be based on accurate blood pressure measured using standard equipment. There should be the patients good medical history, physical examination of the patient include monitoring other underlying disease like diabetes mellitus.

There are non-pharmacological measures to lower blood pressure and reduce cardiovascular risk. The following should be recommended to all hypertensive individuals:

- Salt restriction
- Weight loss
- Alcohol consumption should be stopped
- Increased exercise
- Smoking cessation

The patients to be treated medically include those having the following;

i. Sustained diastolic blood pressure, despite pharmacological intervention in excess of 99mmHg or between 90-99mmHg.

ii. Sustained systolic blood pressure despite no pharmacological intervention in excess of 160mmHg

iii. Blood pressure should be reduced to target levels (e.g. <140mmHg systolic and <90mmHg diastolic) and lower levels in higher risk patients e.g. diabetics and coronary artery disease patients.

iv. Regular follow up is recommended with treatment and blood pressure measurement at least every 6 months.

Orthodox drugs used for the treatment of hypertension for example propranolol at various dosage form e.g. 40mg and 80 mg. Likewise herbs such as *Adrographis paniculata* are also employed to treat mild hypertension at dose of 500mg of the crude leaf which is equivalent to 60 - 70 mg per daily dose.

2.2.4 Prevention of hypertension

The prevention of hypertension involves the population and individual approach (WHL Technical Report Series, 1996). The prevention of complication resulting from high blood pressure in any population requires reducing the risk of developing high pressure in the population as a whole. Individual approach involves identifying the individuals who are at increased risk of developing complications.

Population approach includes increases in the population awareness that elevated blood pressure is a major health hazard although it may not be apparent. Help detect the individuals who have hypertension or may be at risk of developing hypertension and advocate life style which minimize, if not eliminate the controllable risk factors of hypertension. Individual approach counts because many people with established hypertension may not respond to non-pharmacological intervention. It is important to treat hypertension and modify life style (WHL Technical Report Series, 1996) by the following;

- Health education is necessary
- Enriching natural foods with potassium rich fruits

- Regulation of food industry to provide low salt and low fatty foods
- Provide facilities for outdoor recreational sports
- Control tobacco smoking, etc.

It is important to note that the population and individual approach is a comprehensive strategy for the prevention and control of hypertension, and the two have synergic effect.

2.3 TECHNOLOGY OF TABLET PRODUCTION

2.3.1 Tablets

Tablets may be defined as solid pharmaceutical dosage forms containing drug substances with or without suitable diluents and prepared either by compression or moulding methods (Musa, 1999). Tablets have been in use for more than one thousand years. Tablets represent the most popular dosage form among the contemporary methods of drug administration (Bangudu, 1992). Tablets have remained the most popular dosage form of drug administration especially in the United States of America, where the demand for large amount of pharmaceutical supplies increased drastically during the war (Armstrong, 1993). Also the solid dosage form afforded some advantages both to the manufacturer and the consumer. Some of which include;

(i) Ease of administration and therefore more convenient to the patient, with high precision of the dose;

(ii) Ease of handling and transporting at any time;

(iii) Ease in storage, handling and the tablet is more stable at varied temperatures.

(iv) Method of production can be automated. It therefore reduces the cost of production and less cost for the patients;

(v) It also affords the simplicity and economy of production, accuracy of dosage compactness, portability, blandness of taste (Akande, 1988);

The objectives of tablet production for most pharmaceutical manufacturers is to produce tablets of high quality that is efficacious at the lowest cost. It is important to produce tablets which can withstand the mechanical abrasion or friability, that will rapidly disintegrate and release the correct active ingredients at the right time in the appropriate quantity. The correct selection of compression pressure and balance of

excipients materials for each active ingredient combination in a tablet formulation to get the desired formulation is not a simple goal to achieve (Musa, 1999).

Tablet formulation and design may be described as the process whereby the formula ensures that the correct compression pressures is selected in order to produce good quality tablets and the amount of drug in the right form is delivered at or over the proper time at the proper rate and in the desired location, while having its chemical integrity protected to that point. Tablets can have many shapes. There is no specific shape to be adhered to; any shape may be selected depending on the discretion of the company. They may also differ in weight depending on the amount of drug substance incorporated in it and method of administration.

Tablets can either be high dose (macrodose) containing drug of 500mg or above or microdose (low dose) containing less than 50mg of drug. There is no tablet by convention that weighs less than 50mg, the normal weight of tablets for the microdose range from 50mg to 75mg. Where the dose is 2mg per tablet, excipients will account for the remaining weight of the tablet. However Nabintu (2000) has shown that a drug content as low as six percent (6% w/v) offsets the compactional characteristic of the pure excipients. Allagh, *et al* (1994) also showed how folic acid content of two percent (2% w/v) affected the binder uptake volume.

2.3.2 Tablets Manufacture

a) Wet Granulation Methods and Mechanism: Powders, either the active drug or excipients are not directly compressible. Techniques must be found to make them compressible. This technique is called granulation i.e. forming granules out of the powder. Strong bonding are formed during the compression which hold the granules together.

b) Massing and Screening: Wet granulation involves the massing of the powder mix using a solvent. The solvent containing the binder used must be volatile, so that it can be removed by drying. It should also be nontoxic. The typical solvents include water, ethanol and is used either alone or in combination with binders. The disadvantage of water as a solvent is that it may adversely affect drug stability causing hydrolysis for example acetyl salicyclic acid.

This is the oldest and widely used tableting technique which involves the preparation of freely flowable granules(Balami, 1999). Wet granulation takes time, space machinery and personnel (Armstrong, 1993) and is not suitable for thermalabile active ingredient.

c) Dry Granulation Method: Dry granulation method involves granulation of powder mixture by compression without the use of any solvent. It is embarked upon when the other methods of granulation i.e. direct compression or wet granulation cannot be employed for example the granulation of aspirin and effervescent products. Basically the procedure involves forming a compact by compressing the powder mixture and then milling the pellets into granules. The process is called compaction and communation.

There are two methods of dry granulation. The more widely used method is slugging, where the powder is precompressed and communated . The other method the powder is precompressed with pressure rollers (Musa, 1999). Sometimes the slugs are screened again to confer more granulate properties on the material.

i) Advantages of Dry Granulation: Some advantages include the use of less equipment and space. It eliminates the need for liquid binder solutions, heavy

and costly granulating equipment and the process of drying for wet granulation. Some of its advantages include;

(a) It is useful for moisture sensitive materials and thermalabile products.

(a) It also improves disintegration since the powder materials are less bonded together.

(c) There is improved solubility for anhydrous soluble materials that would otherwise tend to set when wet.

(d) It also improves uniformity of drug content, since there is no migration or segregation of active ingredients that occur during the drying of wet granulation. It is one of the best method for thermalabile products

ii) Some Disadvantages of Slugging. It requires specialized heavy duty tableting machine to form the slugs. It does not achieve uniform colour distribution as can be achieved with wet granulation, where the dye can be incorporated into the binder liquid. A pressure roll, such as the chilsonator cannot be used with insoluble drug since this may retard the dissolution rate. The method creates more dust than wet granulation and increases potential for cross contamination, include the risk of explosion.

2.3.3 Direct Compression

Direct compression are superior to those methods employing liquids, since dry methods do not require the equipment and handling expenses required in wetting and drying procedures and can avoid hydrolysis of water sensitive drugs. Some drugs like (aspirin) acetylsaliylic acid can be tableted without further treatment, but the vast majority of drugs require the addition of a direct compression vehicle to aid compression.

In recent years great interest in direct compression has developed. This has resulted in a wide range of direct compression tablet formulations being introduced together with direct compression vehicles. These vehicles are inert substances, which can be compressed with no difficulty and even when large quantities of drugs are mixed with it.

Materials currently used as direct compression diluents can be divided into three groups according to their disintegration properties and their flow characteristics, namely:

a) Disintegration agents with poor flow e.g. microcrystalline cellulose and directly compressible starch.

b) Free flowing materials which do not disintegrate, di-calcium phosphate.

c) Free flowing powders which disintegrate by dissolution, e.g. spray-dried lactose, anhydrous lactose, spray-crystallized maltose, dextrose, sucrose, mannitol and amylose.

Tablets are produced by mixing the drug with the compression vehicle in a blender. The powder mix is then compressed directly on a tableting machine.

Direct compression is now used to define the process by which tablets are compressed directly from powder blends of the active ingredient and other excipients (including diluents disintegrants and lubricants) which will flow uniformly into the die cavity and form firm compact. The advent of direct compression was made possible by the commercial availability of directly compressible tablet vehicles which possess flowability and compressibility and characteristics (Armstrong, 1993) Examples include; Avicel, celluloses and index, starch x 1500. The commercially available binders or vehicles are however more costly than lactose, starch, sucrose, etc. Hence this granulation method is preferred to save cost. In the case of direct compression

(DC) of macrodose tablets, vitamin C and sodium bicarbonate tablets, the knowledge of tablet ingredient compressibility and flowability are necessary. For microdose of strength of 50mg and less such as folic acid 5mg tablet, the choice of the diluent must be such that it is non-reactive, compressible with inherent binding action and have good flowability.

a) Advantages of Direct Compression

i. Economy – Saving is achieved through reduced process of production. The cumbersome method of wet granulation and drying is cut off. There are fewer manufacturing steps, less equipment, and space and lower consumption of power (Batuyois 1966; Radley, 1976; Augsburger and David, 1977).

ii. Moisture and heat are eliminated therefore serve as an appropriate method for thermalabile and moisture sensitive drugs (Lamberson and Raynor 1976).

iii. Prime particle dissolution. It is one of the recent recognized advantages of direct compression which is the optimization of tablet disintegration. In which each primary drug particle is taken from the tablet mass and is available for dissolution (Batuyios, 1966 and Radley, 1976).

iv. It also allows for particle size uniformity and proper mixing of the powder and uniform formulation of the active ingredients.

b) Properties of Direct Compression Excipients

i. High compressibility: should be highly compressible producing non-friable tablets with good crushing strength (Armstrong, 1993).

ii. Should be compatible with drug material, colourless and odourless.

iii. Good flowability: should be free flowing, i.e have good flow rate.

iv. Economy: should be readily available and cheap (Radley, 1976).

v. Bioavailability: the tablets they produce should have prompt disintegration to allow dissolution and release of active drug (Batuyois, 1966).

vi. Should be stable: Should be able to mix well without affecting the stability of the active ingredients (Armstrong 1993).

c) Limitation of Direct Compression

i. The choice of excipients is extremely critical in formulating direct compression tablets.

ii. They often cost more than other excipients or diluents used in granulations

iii. Many active ingredients are not directly compressible either in their crystalline or amorphous forms. Therefore it is important to consider the dilution potential of the major diluent i.e. binder the proportion of active ingredient that can be compressed into an acceptable compaction using the diluent. Because of this mentioned limitation, wet granulation method is preferred provided the active ingredient can withstand the moisture and heat during the process.

2.3.4 . Fluidized Granulation

This is another method of mixing the powder. It uses a fluid e.g Aeromatic Glatt.

Heated air is blown or sucked through a bed of unmixed powders to fluidize the particles and mix the powders. Granulating liquid is pumped through a spray nozzle over the particles and this liquid causes them to adhere when they collide. Sufficient liquid is added to produce granules of the required size, which are then dried in heated fluidizing air stream.

2.3.5 Diluents

Diluents or bulking agents are inert substances which are added to the active ingredients in sufficient quantity to make a reasonably sized tablet. If the dose of the drug is sufficient, excipients may not be necessary. Generally, tablets should weigh at least 50mg, therefore very low dose drugs will invariably weigh 50mg. The principal substance employed as diluents is lactose. It has a pleasant taste, rapidly dissolves in water, absorbs little water and it is inert. Starches are used as diluents and also as binding agents. They are available as fine powders and they aid disintegration.

Microcrystalline cellulose (MCC) is another diluent commonly used. It is normally used as a direct compression vehicle. It has disintegrating properties and requires less lubricant in the formulation than other diluents. Dextrose has been used as a bulking agent, but the granules produced are much softer and not very white in addition dextrose absorbs water. Sucrose is another diluent, but it is very hygroscopic, it becomes sticky on exposure to moisture. Its pleasant taste makes it useful in the formulation of lozenges. Mannitol is another diluent, which is also sugary, very expensive, and because of its high solubility it is used to formulate tablets that should be dissolved before use.

2.3.6 Disintegrants

Disintegrants are always added to tablets to promote breakup of the tablets when placed in an aqueous environment. The objective of a disintegrants is to cause the tablet to disintegrate rapidly so as to increase the surface area of the tablet fragments and so promote rapid release of the drug. Wagner (1977) proposed a scheme, which related tablet break up to drug dissolution and absorption.

Disintegrants can act by swelling in the presence of water to burst the tablet. Starch is the commonest disintegrant which acts by swelling. There are other disintegrants that do not swell up in the presence of water. The mechanism by which this disintegrants acts is controversial. It is believed that the disintegrants that do not swell exert their disintegrating action by capillary action. Liquid is drawn up through capillary pathways within the tablet and ruptures the inter particulate bonds. This action serves to break the tablets apart. Lowenthal (1972) has discussed in detail the various mechanisms of disintegration.

Incorporation of disintegrants into the formulation can be done in various ways. The whole disintegrants could be added at once during the wet granulation, this is called intragranular disintegrants. Alternatively, about two thirds of the disintegrant can be added during the wet granulation and the remaining one third is added to the dry granules. The second addition of the disintegrant to the dry granules is called extra granular disintegrant or exogenous disintegrant.

As for starch the second method of addition is not popular because dry starch is incompressible and too much starch between the granules inhibit their binding. Other common disintegrants include cation exchange resins, cross-linked polyvinyl pyrrolidene (Poly plasdone XL), modified starches (sodium starch decolatell) and cellulose material Avicel. Recently however, pregelitinised starch has been found to be a good disintegrant.

2.3.7 Binders

These are substances that act as adhesives to bind powders together in the wet granulation process and are known as binders. They also help to bind granules together

during compression. If too little binding agent is used in a formulation, soft granules result. Conversely, too much binding agents produces large, hard granules.

Binding agents can be added in two ways.

a) As a powder in the formulation as in slugging or in dry granulation methods.

b) As a solution to the mixed powders as in wet granulation.

Common binding agents include starch mucilage and gelatin solution. Starch is a good and popular binder and needs to be present in an amount equal to 2% w/v starch which is incorporated as mucilage. When dry the starch binder is insoluble in water, unlike gelatin which remains soluble in dry state. Gelatin is often used as a binder in lozenges.

Among the most popular binders are polyvinyl pyrollideone (PVP). It is both soluble in water and alcohol. It releases drugs faster than other binders (Rubinstein *et al*, 1993).

It was observed that T_{50} values in drug release profiles varies between 3.65 minutes with PVP to 118 minutes with starch mucilage. This shows how the choice of binder affects dissolution. Other less common binders include hydrolyzed gelatin, derivatives of sea weed such as alginic acid, sodium, alginate and calcium alginate and cellulose derivatives for particular ethylcellulose and hydroxy propyl methyl celluloses.

Common binders used in wet granulation process are

Acacia mucilage:	Up to	20% yields very hard granules
Gelatin:		Up to 5-20% forms gel when cold. Therefore warm solution is used in lozenge granules
Glucose:		Up to 50% strong adhesive, hygroscopic, tablet may weaken in moist conditions

Polyvinyl pyrdlidene:	(PVP)2-10% soluble in water and in some organic solvents; therefore can be used for anhydrous granulation.
Starch mucilage:	5-10% often used warm, a very commonly used adhesive
Sucrose:	Up to 70% hygroscopic tablets may harden on storage

2.3.8 Glidants and Lubricants

Glidants are materials which are added to tablet formulations in order to improve the flow properties of the formulations. They act by reducing inter-particulate friction. The most commonly used and effective glidants is fumed or colloidal silica. Flow of granules can be dramatically improved by the addition of less than 0.1% w/w of this material to powders and granules. Fumed silica is thought to act by lodging in the irregular surface of the particles or granules, which effectively smoothens the particle surface.

2.3.9 Lubricants

These agents are required to prevent adherence of the granules to the punch faces and dies. They also ensure smooth ejection of the tablet from the die and many lubricants enhance the flow properties of granules. Talc and magnesium stearate appear to be more effective as punch lubricants than steroid acid, which is more effective as a die lubricant. Magnesium stearate is the most popular lubricant used and is normally effective on its own as both die and punch lubricant. It is incorporated by blending with the dry granules prior to compression, up to a concentration of about 1% w/v.

A thin layer of magnesium stearate around the granules is just as effective as a thick layer from the lubrication point of view, but increased magnesium stearate content increases the disintegration time, retard drug dissolution, reduce the binding forces between granules and this results in soft tablets. The reduction in drug release properties

is due to the hydrophobic nature of magnesium stearate preventing drug dissolution. It has been shown (Bolhuis *et al,* 1975) that the extent of mixing time greatly affects the distribution of magnesium stearate around the tablet granules.

An increase in mixing time produces a more uniform distribution of lubricant, but adversely affects tablet hardness and dissolution.

During compression the granule and the resulting tablets are subjected to two forces. The granules are subjected to residual force, which acts against the die wall. This force is perpendicular to the ejection force, E, the force exerted by the lower punch to eject the tablet out of the die during the ejection phase of tableting cycle. The two forces are related by E = MF where M is the frictional coefficient. The main reason for the addition of lubricant to a tablet is to reduce the magnitude of F. the f-residual force which arises as the results of compression of the granules in the die. This force acts against the dye wall. When granules are not properly lubricated, resulting tablets may cap (i.e. the crown will separate, or fall off from the remainder of the tablet). Sometimes cracks may appear on the wall of the tablets. When poorly lubricated the tablet may bind up in the die defying ejection, and the tablet press will stop operating. Lubricants are defined as those agents that act between surfaces in relative motion to prevent friction and wear (Gunsel *et al,* 1970).

Lubricants are added just before compression since they must be on the surface of the granules and in between the granules. The addition of lubricants to granules prior to compression into tablets is very important. The quantity added, the duration of the mixing the granules are very crucial. These processes can affect the inter and intra-batch properties such as dissolution, hardness and friability (Jarosz Parrot, 1984 and Bolhuis *et al*, 1987). As a result of thorough mixing, granules are coated with a hydrophobic film of lubricant (Bolhuis *et al.*, 1975).

Desai *et al* (1993) found that increased mixing time resulted in decreased drug dissolution rate. They also found that among the three hydrophobic lubricants studied, magnesium stearate caused maximum slow down in dissolution, followed by zinc and calcium stearate. The optimum concentration of magnesium stearate for lubrication that would not adversely affect dissolution is 0.25% w/w. These workers employed the hypothesis of Poorest *et al* (1983), which says that the hydrophobic film of magnesium stearate does not envelop the granules completely. This may explain their observation that the presence of 1% magnesium stearate did not affect dissolution when super disintegrants were used since their swelling is not inhibited once they come in contact with dissolution medium.

Desai *et al* (1983) observed that the effects of over mixing of granules with hydrophobic lubricants could be overcome by compressing them into tablets. The lubricant film is disrupted and the granules fragmented during compression, which result in the formation of new, fresh, uncoated surfaces, with unhindered dissolution. Magnesium stearate is the most common glidant or lubricant and a lot has been studied about its mode of action.

Gold *et al* (1968) have proposed that magnesium stearate reduces the adhesion due to vander waals forces between particles of a powder bed. However, other workers have reported the significant reduction of the forces of adhesion due to hydrophobic nature of the material (Ziman, 1982). Deryaguin *et al* (1978) reported that an optimal magnesium stearate concentration i.e. the concentration which would improve powder flow most can be found when a complete film has been formed surrounding such individual particles, as reported by Gold *et al* (1968) as well as Irono *and Pilpel*(1982).

Above the optimal concentration when the film formed increases in thickness or when some fine particles exist, there is a sharp drop in flowability, which cannot be attributed only to a weakening of attractive forces between the host particles.

Tabor (1981) has identified the three basic elements of friction as:

i. The area of true contact between the sliding particles

ii. The type and strength of the attractive forces between the contacting surfaces.

iii. The shearing and rupture of the materials at the contact points and the surrounding area during sliding.

For lubricants such as magnesium stearate, which form a film around the particles and resulting in reduced frictional forces proposed by Bowden *et al* (1964) and Halling (1981), concluded weakening of the interparticulate bonds between the drug particles.

2.3.10 Colourants

Colourant are means of distinguishing one tablet from the other. In some cases, the whole tablets may be coloured. The reasons for using colourants are varied.

a) protection of ingredients from the environment particularly light.

b) masking the bitter or unpleasant taste of the drug. Coloured coated tablets makes tablets easier to swallow.

c) coloured coated tablets aid in the rapid identification of product by the manufacturer, dispensing pharmacists and the patients.

d) coatings often centres on added mechanical strength to the tablet core.

2.4 Compaction characteristics of pharmaceutical powder

The compaction behaviour of pharmaceutical powders has engaged the attention of investigators for a number of years. Train (1956) related applied pressure to relative volume of powder. He stated that elastic compression of the solid powder occurs at high

compression force, here the compact is sufficiently strong to support the applied load with no further reduction in volume. The more important point about elastic compression should be that the material can recover when the pressure is removed

Various authors, namely Huffine *et al* (1962), Heckel, (1961), Cooper *et al* (1962), have discovered the different stages during the compression of powders in dies. These include the

(i) Filling and rearrangement of particles in the die,

(ii) The elastic deformation and plastic deformation of particles,

(iii) The fracture of particle fragmentation and bonding;

It is believed that several of these processes occur simultaneously. Some authors reviewed a large number of equations that have been proposed to describe compressional behaviour of powders to differentiate the various stages in a compression process. The Heckel's constants K and A were found to have valuable physical significance and could be used to draw conclusions concerning the effect of the characteristics of the material and particle size on the densification process.

York and Pilpel (1973) as well as Kurup and Pilpel (1978), have also agreed that the Heckel equation appears to be the most sensitive in distinguishing the various process for some soft pharmaceutical materials.

Paronen and Justin (1983), have also drawn the same conclusion from their work on the compressional characteristics of various starches. The Heckel's equation is given as:

$$\ln\left(\frac{1}{1-D}\right) = KP + A \quad \text{.....equation 1}$$

where D is the relative density at applied pressure, P, K and A are constants.

The reciprocal of K is the mean yield pressure Py which is an important compressibility index that describes the tendency of a material to undergo total and

plastic deformation. It also relates to the rate of deformation, this is important because tableting machines have short dwell times. A is a function of the original compact volumes and represents two stages of consolidation, the first due to the initial relative density of the powder and the second due to densification by particle rearrangement. From the value of A the relative density D_A, can be calculated using the following equation of Roberts and Rowe, 1986.

$D_A = 1\text{-}e^{-A}$ …….. eqn 2

D_A which is obtained from the ratio of loose density of powders to effective particle density is the relative density of the powder bed when the applied pressure, P is zero. Itiola (1991) used it to describe the initial rearrangement phase of densification as a result of die filling. The D_A describes the phase of rearrangement of particles during the initial stages of compression. The extent of this phase depends on the theoretical point of densification at which deformation of particles begins.

D_B is obtained from the equation:

$D_B = D_A - D_O$ ……..eqn 3

D_B is relative density which describes the phase of rearrangement of particles during the initial state of compression. It is observed from the relationship $D_B=D_A-D_O$.

D_O is obtained from the ratio of loose density of powders to effective particle density. It is also relative density of powder when applied pressure is zero. It describes the initial rearrangement phase of densification as a result of die filling (Itiola, 1991)

From the Heckel equation, various workers have identified two types of powder compression behaviour (Hersey and Rees, 1971; Summers et al, 1976) as powder compaction behaviour type A and type B. Type A behaviour has a linear relationship between $\ln\left(\frac{1}{1-D}\right)$ and p which is observed at all applied pressures indicating densification apparently only by plastic deformation. An example of a material with

such behavior is sodium chloride. The type B behaviour, has an initial region, usually curved, followed by a linear region. The initial region represents brittle fracture or particle fragmentation, followed by plastic flow or deformation. Hard, brittle materials, such as lactose usually display these characteristics (Armstrong and Lowudes 1984).

The type of powders requires initial consolidation by fragmentation to form a consistent (denser) packing. Type A curves usually exhibit a steeper final slope than type B indicating a lower mean value. In practice, hard brittle materials are more difficult to compress than soft yielding ones. Fragmentation with subsequent percolation of fragments is less efficient than void filling by plastic deformation. A third powder compaction behaviour type C behaviour was proposed by York and Pilpel (1973).

They reported that the effect of compression of fatty acids and lactose-fatty acid mixtures bring about the type C behaviour. This type of curve exhibit initial steep linear region which flatten out as the applied pressure is increased. This is believed to be due to the absence of a rearrangement phase and densification due to plastic deformation and melting. It was noted that type C behaviour fit was a typical type A curve except that the initial densification was observed to be slight due to the nature of the mixtures, the yield pressure was found to increase with increase in the melting point of the fatty acids.

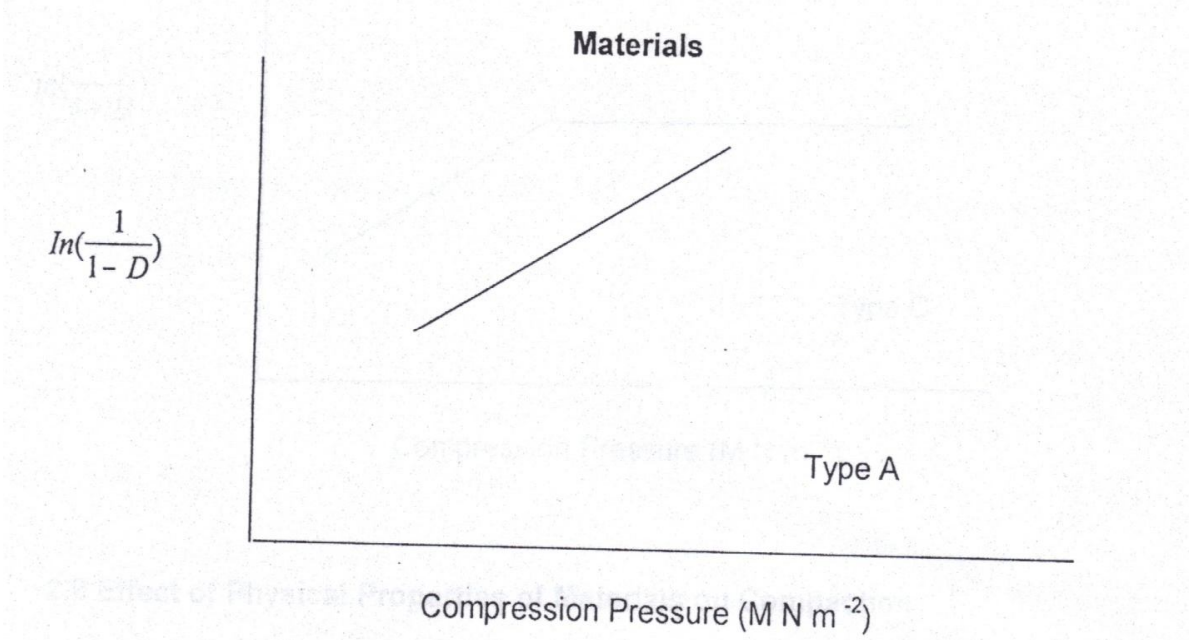


Figure 2.1: A schematic representation of the Heckel Plots for Type A materials

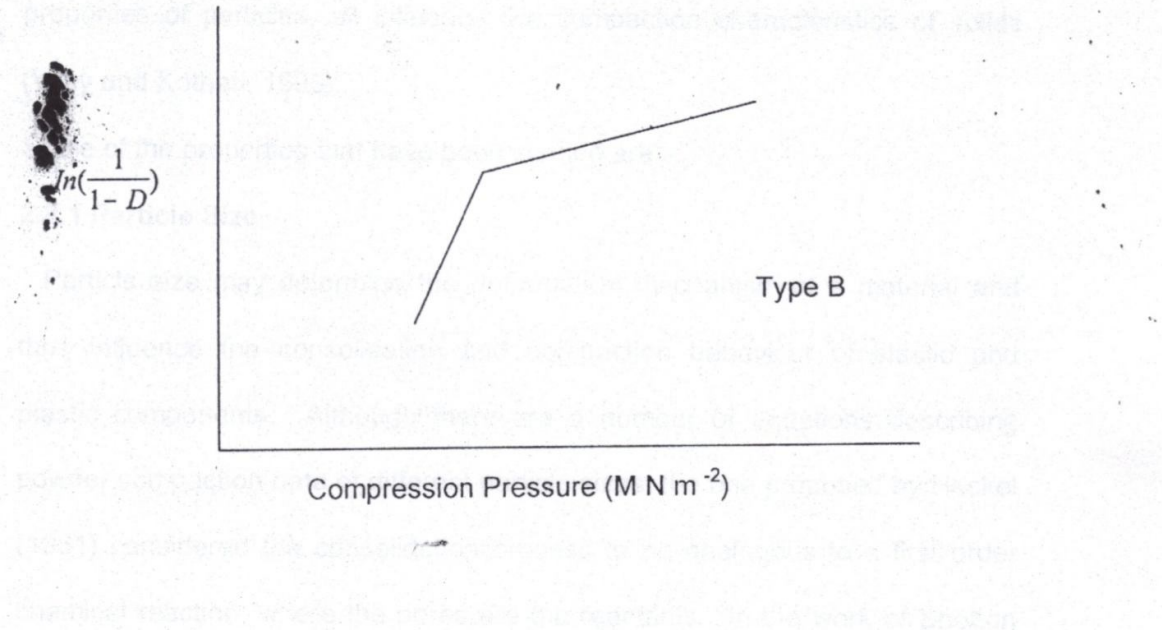


Figure 2.2: A schematic representation of the Heckel Plots for Type B materials

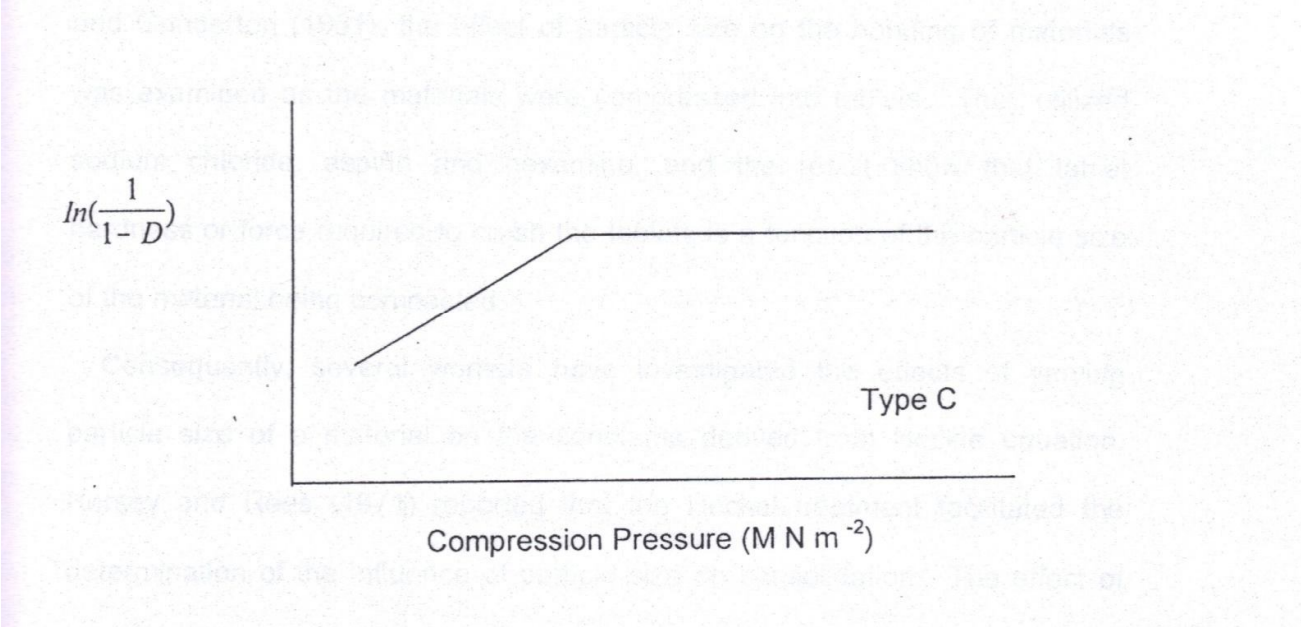


Figure 2.3: A schematic representation of the Heckel Plots for Type C materials

As earlier mentioned the Heckel plot has been used in various compaction studies. The results of the various workers tend to give different pictures. Rue and Rees (1978) observed non-linear Heckel plots for a granular microcrystalline cellulose, and suggested the use of areas under the Heckel plots for different punch contact time (York, 1979), demonstrated that the numerical values for yield pressures obtained by various workers, for crystalline lactose, were dependent on experimental conditions such as degree and type of lubrication of punches and die, punch diameter, compaction rate and method of determining relative density. Other workers have also found that compression time (Rue and Rees, 1978) and time of measurement i.e. whether density measurement is made during compression or after ejection (Feel and Newton, 1971) affect the Heckel plot.

Paronen (1983) evaluated Heckel plots as indicators of elastic properties of pharmaceutical powders. Tablet-in-die and ejected tablet methods were used to determine values for Heckel functions.

The total elastic recovery was obtained using the reciprocal of the difference of the Heckel upward slopes obtained from the two methods used in the study. The degree of densification obtained during the compression process at a compaction pressure to about 170mpo was shown to decrease in the order, modified starch> microcrystalline cellulose> sodium chloride > dicalcium phosphate dihydrate. The tendency of the materials to deform both plastically and elastically was described by the reciprocal of the Heckel plot slope obtained, using the tablet-in-die method. The tendency of the material to deform plastically was shown to be the reciprocal of the slope of the Heckel plot obtained by the ejected tablet method, the order of the degree of densification of materials according to this permanent densification tendency was sodium chloride, microcrystalline cellulose modified starch and dicalcium phosphate dehydrate. The

difference between the order of the materials obtained by the two methods was ascribed to the difference in the tendency of the materials to recover elastically.

The Heckel equation which is used to treat most powder compression data is only applicable at pressures above the yield strength. Carstersen et al (1990) proposed an equation based on the indication that powder void volume decreases exponentially with pressure and tends towards a value different from zero within pharmaceutical pressure range, suggesting that the final compressed solid always possesses some level of porosity.

2.4.1 Powder compression Equation

Kawakita and Ludda (1970) when considering powder compression concluded that Heckel equation though it has deviated from linearity slightly at low compression pressures was the most informative on mechanism of powder consolidation. York and Pipel (1973), Kurup and Pilpel (1978) also agreed that Heckel equation appears to be the most appropriate to explain what happens during tablet compression.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals/Powders

The following chemicals and powders were used as received unless otherwise stated.

- Chloroform (Prolab. 12 Rue Pelle, Paris,France)
- Petroleum ether (Hopkins and Williams, Chadwell Health, Sussex, U.K)
- Ethanol (BDH Chemicals Ltd., Poole, U.K.)
- Tween 80 (BDH Chemicals Ltd., Poole, U.K).
- Concentrated Hydrochloric acid (BDH Chemicals Ltd., Poole, U.K.)
- Lead Acetate (BDH Chemicals Ltd., Poole, U.K.)
- Lactose (May and Baker, Germany)
- Heavy Magnesium Carbonate (May and Baker, Germany)
- Maize starch (MS) BP (May and Baker, Germany)
- Gelatin (GLT), BP, (May and Baker, Germany)
- Polyvinyl pyrrolidone (PVP) (BDH, Chemicals, Ltd., Poole, England)
- Magnesium Stearate (Hopkins and Williams, U.K.)
- Microcrystalline cellulose (Avicel) (FMCS Biopolymer, 1301, Ogle Town Road, Newark DE, 19711, U.S.A.)
- Pregelatinised starch (PGS) locally prepared, Pharmaceutics Laboratory, Ahmadu Bello University, Zaria, Nigeria.
- Lactose powder (BDH Chemical Ltd, Poole, England.)

- Talc powder BDH(Chemical Ltd ,Poole England)
- Powdered leaves of *Andrographis paniculata* from Garkida, Adamawa State, Nigeria and cultivated; in Zaria, Nigeria
- Mature male cat (Felis catus) 2.498kg, Zaria, Nigeria.
- Rats ((132 +3.0)g male and female), bred in the Animal House of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
- Methanol (BDH Chemicals Ltd., Poole England.)
- Heparin C P Pharmaceuticals Ltd Wrexham ,L L 13 ,9UF,UK,
- Distilled water from the laboratory, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.
- Physiological solution (normal saline 0.9% w/v)
- Thiopentane 1g Biocheme ,Vienna ,Austria.
- Methyldopa tablets, 250mg (Merck, Sharp and Dohms)
- Propranolol tablets B.P. 40mg(Vardhman Export, Mumbai India)
- Adrenaline (Sigma Chemical Company, USA)-
- Methanol (BDH chemicals Ltd., Poole, England)
- Ketamin Rotex medica (GMBH) 50mg/ml(Embassy Pharmaceutical and chemicals Ltd., Lagos, Nigeria.)
- Acetylcholine (Ach) 10µg/ml (Sigma Chemical Company, USA)
- Methanolic extract of (AP) - 10mg/ml freshly prepared
- Aqueous extract of (AP) - 10mg/ml freshly prepared.
- Ferric chloride solution (BDH Chemicals Ltd., Poole, England)
- Mayers reagent (BDH Chemicals Ltd, Poole, England)
- Vanillin Sulphuric Acid spray reagent BDH Chemicals Ltd England
- Dragendoff's reagent (BDH Chemicals Ltd, Poole, England)

- Fehling's solution A and B (BDH Chemicals Ltd, Poole, England)
- Distilled water
- Potassium carbonate
- Sodium Chloride (Dangote Nigeria)

3.1.2 Equipment/Instrument

These are the major pieces of equipment and instrument used;

- Moulinex, Blender Mill 2 (France)
- Gallen Kamp hot plate (Philip Harn's Ltd, England)
- Sieves of various sizes and collection pan, 75µm, 90µm, 150µm, 250µm 500µm(Endecotts Text sieve shatter). Endecott LTD London SW10 England.
- Single punch tableting machine (Erweka AR 400, West Germany)
- Monsanto Table.tting machine (Erweka AR 400, West Germany)
- Friablility tester (Erweka TA – 3 Friabilator Heusenstanin Knofter Bash, West Germany)
- Monsanto tablet hardness tester (Philip Han's Ltd., England)
- Micrometer Screw gauge (Moore and Wright, Sheffield, England)
- Carver hydraulic hand press, model C USA, Carver Inc. USA
- Water bath (Gallen Kamp No. N-BSE-=800-0106, England)
- Hot air oven (Gallen Kamp Size 3BS, 2648, England)
- Disintegration apparatus (Erweka type 2T3, No. 41728)
- Dissolution apparatus equipped with beaker and magnetic stirrer Erweka DT .80 Copley ET 2001(West Germany).
- Electronic balance (Denver Instrument Company, U.S.A.).

- UV – Visible Recording Spectrophotometer (Shimadza UV – 1604, Japan)
- Ugo Basile recording microdynomometer model No. 7050 (Comeriovarese, Italy)
- Dissecting set (Gold Cross)
- Flask shaker
- Digital Weighing balance (OHAUS Coperation Pine Brook USA)
- Oxygen transducer (Canon Electric ,Germany)
- Soxhlet apparatus Quickfit made in England
- Canula (size) Intramedic (Eastermedi Kit, Ltd, India)
- NETZSCH DSC 204 F1 Instrument for differential scanning Calorimentary (made in Germany)

3.2 METHODS

3.2.1 Plant Material Collection and Identification

The original sample of AP was brought from Garkida village, Hawul Local Government Area, in Adamawa State, Nigeria. Due to the requirement of the project, some samples were cultivated in a garden in Zaria, Sabon-Gari Local Government Area, Kaduna State, Nigeria.

The plant was identified at National Institute for Pharmaceutical Research and Development (NIPRD), Idu, the Federal Capital Territory Abuja, Nigeria. The herbarium number is NIPRD/H/5558.

3.2.2 Plant Preparation

Fresh leaves were plucked and dried in a Gallen Kamp hot air oven for 24 hours at 40^{o}C. The leaves were micronised using pestle and mortal and further size reduced with Moulinex Blender. This was sieved through 250µm using Endocott sieve shaker for 15 minutes, and then stored in an airtight container until further use.

3.2.3 Preparation of Extracts

a*)* ***Aqueous Extraction*****:** Powdered leaves weighing about 250g was socked in hot distil water for 24 hours with occasional stirring and then filtered. The filtrate was brought to complete dryness. The fraction was micronized and sieved through 250µm sieve and stored in an airtight container until further use.

The percentage yield was calculated as follows: $\frac{\text{weight of extract}}{\text{weight of leave powder}} \times 100\%$ .....eqn 4

b) *Methanolic Extraction*: Five hundred gram (500g) leaf powder of *Andrographis paniculata (AP)* was extracted with 1.5 litre of 96% methanol using the soxhlet apparatus. The extract was dried over hot water bath and brought to complete dryness using Gallen Kamp hot air oven set at 20^{o}C. The fraction was size reduced and sieved through 250µm sieve size and stored in an airtight container until further use.

Organaleptic Properties: Physical properties such as odour, the taste and colour of the powdered leaves as well as the dried aqueous and Methanolic extracts were observed.

3.2.4 Phytochemical Tests

***a) Test for Cynogenic Glycosides*:** Two (2) mls of the aqueous extract was placed in a test tube and distilled water added. A prepared moist sodium picrate paper was suspended in the neck of the test tube by means of a cork. The tube was placed in a water bath for an hour and observed.

b) Test for Anthraquinone Glycosides

i) <u>Borntragers Test for Free Anthraquinones:</u> The powdered leaves (300mg) was taken in a test tube and 10ml of chloroform was added and shaken for 5 minutes. The extract was filtered and allowed to cool. The filtrate was added to equal volume of 10% v/v ammonium solution and shaken and observed for colour change.

ii) <u>Test for Presence of Free and/or Combined Anthraquinones:</u> Five hundred milligrammes of the leaf powder was boiled with 10ml of 10% w/v hydrochloric acid for 20-30 minutes. The extract was filtered while hot and allowed to cool. To the filtrate was added an equal volume of chloroform. The test tube was inverted a couple of times to avoid vigorous shaking. The solution was transferred to a separating funnel and the two layers allowed to separate. The lower chloroform layer was poured into a clean test tube and 10% w/v ammonium solution added and shaken. The two layers were again allowed to separate and observed for colour change.

c) Test for Saponin Glycosides

i) Froth Test: One gram of the powdered leaves was added to 2.5ml of 95% ethanol and boiled. The mixture was filtered while hot and cooled followed by the dissolution of 2.5ml of the ethanol extract in 10ml of distilled water in a test tube. The test tube was corked and shaken vigorously for 30 seconds and allowed to stand for an hour and observed.

ii) Haemolysis Test: The powdered leaves (500mg) was extracted with 10ml warm water, filtered and the filtrate retained in one tube while the residue was also retained in another tube Two(2) ml of 1.8% w/v sodium chloride solution was added to each of the two test tubes. To one of the test tube, 2ml of distilled water was added and to the other filtrate was added 2ml of the extract. Blood was obtained by pricking the human thumb at the base of the finger-nail and blood was drawn into a small pipette. Five drops of blood were added to each of the test tubes. The tubes were stoppered and inverted gently to mix the contents and observed.

iii) Keller-Kiliani Test: One of the chloroform residues from above was dissolved in 1ml of glacial acetic acid containing a trace of ferric chloride solution. The solution was carefully poured on the surface of 1ml sulphuric acid already contained in a test tube and observed for any colour change.

iv) Kedde Test (for Free or Combined Cardenolide Aglycone): The reserved filtrate from the extraction was treated with 1ml of 2% v/v solution of 3, 5-dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5% sodium hydroxide and observed for colour change.

v) Legal Test (for Cardenolide Aglycone): The second reserved residue from the extraction process was dissolved in a few drops of pyridine and few drops

of 2% sodium nitroprusside was added followed by a drop of 20% w/v sodium hydroxide solution and observed for colour change.

d) General Test for Tannins

0.1g of extract was added to lead subacetate solution and observed.

d) Test for Terpenes and Steroids

i) Lieberman-Burchard **Test(for unsaturated steroids and Triterpenes):** To 5ml of ethanol extract, chloroform was added and filtered. A portion of chloroform was mixed with 1ml acetic anhydride followed by the addition of 1m of concentrated sulphuric acid and poured down the wall of the test tube to form a layer underneath and observed.

ii) Solkowski's Test: A second portion of the solution was mixed with 2ml of concentrated Sulphuric acid carefully so that the Sulphuric acid formed a lower layer and observed.

***e) Test for Flavonoids*:** Two (2)g of the powdered leaves was completely retained (Segelman *et al,* 1971*)* with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered while hot. The filtrate was collected and used for the following tests.

i) **Lead Acetate Test:** To five millilitre of the methanolic extract was added 3-5 drops of lead sub-acetate solution and observed.

ii) **Ferric Chloride Test:** Two millilitres of the ME was diluted with distilled water in the ratio 1:4(i.e. ME: water) a few drops of 10% ferric chloride solution was added and observed.

iii) **Shinoda Test:** To 5ml ethanol extract of the leaf was added few pieces of magnesium filling followed by few drops of concentrated Hydrochloric acid and observed.

f) Tests for Alkaloid **:** About 500mg of methanolic extract was stirred with 5ml of 1% v/v aqueous hydrochloric acid on a steam bath, 1ml of the filtrate was treated with a few drops of Mayers reagent, another portion was treated with Dragendorffs reagent and a third portion with Wagner's reagent and observed.

g) Thin Layer Chromatography(TLC): The solvent system was mixed in the ratio Hexane: Ethyl Acetate : Methanol as indicated below

Hexane:	Ethyl Acetate	:	Methanol
2	: 2	:	1

The system was thoroughly mixed together and left for 10 minutes. Pre-coated TLC plate was spotted 1cm from the bottom with the dissolved Methanolic extract using glass pipette. The spots were allowed to dry for few minutes after which it was placed in the glass tank containing the solvent system. The plate was left in the system for sometime and observation of the length to which the solvent system had travelled up the pre-coated plate was marked and measured. The pre-coated plate was removed and allowed to dry after which it was sprayed with vanillin in sulphuric acid. It was placed in the oven at 120^0C for 2 minutes. After cooling, observation for any spot was made on the pre-coated plate. The R_f values were calculated and it is the ratio between the distance travelled on the pre-coated plate by the component of the test solution and the distant travelled by the solvent. Under standard conditions this is a constant for the particular compound (Evans W.C., 1996).

i.e. $R_f = \frac{a}{f}$ where a = distance travelled by the spot from the extract

f= distance travelled by the solvent front.

3.3 PHARMACOLOGICAL INVESTIGATION

3.3.1 Preparation of Extract Solution.

a) ***Water Extract:*** A concentrated solution of the water extract (WE) was prepared by dissolving 100mg of WE in 10ml of normal saline to obtain a 10mg/ml stock concentration.

b) Methanolic extract: A Concentrated solution of Methanolic extractME wa sprepared by dissolving 100mg of ME in 10ml of normal saline to obtain a 10mg/ml stock concentration

3.3.2 The Determination of effect of the extracts(ME & WE) on cat blood pressure.

The cat was stabilized in the laboratory for 24 hours before the experiment. The cat was anaesthetized with pentobarbitone sodium BP (Vet) 60-mg/ml at a dose of 27.5mg/kg body weight for the general anesthesia. 1.35ml of the 60mg/ml was administered. The injection was given intraperitoneally.

Heparin sodium 6.8 mg/ml and 0.2 ml which was equivalent to 1.3 mg/1.8kg body weight was given to prevent blood clot. The anaesthetide cat was placed on the dissecting board and secured in a supine position to the board using threads. A midline incision was made in the neck and the vagus vein and right carotid artery were separated and short ligatured. An incision was made in the carotid artery large enough to insert a cannula. The cannula was held and secured in the artery by a second ligature. The cannula from the artery was connected to the one end to a microdynamometer for the measurement of the mean arterial blood pressure.

Another incision was made in the left femur, the left femoral vein was exposed, cannualated and ligated as was done for the carotid artery. The cannulated femoral vein was the site for the administration of drug and test samples. Blood clotting was

prevented by the injection of heparinised normal saline (N/S) intravenously into the femoral venous cannula.

After about 30 minutes equilibrium period the control blood pressure was recorded after which 2ml of normal saline was administered and the mean arterial blood pressure reading made.

The following studies were carried out on an anaesthetised cat and the point of administration was the femoral vein.

i. The effect of Acetylecholine (Ach) at the dose of 2.0×10^{-4} mg/kg and 4.0×10^{-4} mg/kg were administered respectively and the response on the blood pressure was observed.

ii. Adrenaline of 8×10^{-3} mg/kg was also administered and the response on the cat blood pressure was observed.

iii. Comparison of the effect of Methanolic extract and Water extract on the cat blood pressure was also observed at a dose of 0.041mg/kg, 0.083mg/kg, 0.165mg/kg, 0.330mg/kg, 0.412mg/kg, 0.826mg/kg and 1.651mg/kg of the Methanolic extract was administered respectively. Likewise the water extract (WE) at the dose of 0.041mg/kg, 0.083mg/kg and 0.165 mg/kg were administered and the response of both ME and WE on the cat blood pressure was observed.

iv. The antagonistic effect of the ME against Atropine was also observed at dose of 4×10^{-3} mg/kg, and followed immediately with dose of 1.615mg/kg of ME and the response on the cat blood pressure was observed.

v. The comparison of ME with standard drug, propranolol was observed on the cat blood pressure. The same doses of both the ME and Propranolol were administered at 0.041mg/kg, 0.083mg/kg, 0.165mg/kg, 0.330mg/kg,

0.412mg/kg, 0.826mg/kg and 1.651mg/kg. The response of the drugs on the cat blood pressure were observed.

3.4 TOXICOLOGICAL STUDIES

3.4.1 Determination of LD_{50}

Male and female rats weighing 132±3.0g were used. They were obtained from the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria and were fed on standard feeds and water freely. The rats were acclimatized in the laboratory for one week before the experiment.

In determining $LD_{50,}$ eight groups of rats comprising 12 rats per group were placed in different cages. The various doses of AP water extract was administered intraperitoneally. The water extract was reconstituted using distilled water to form the appropriate concentrations ranging from 1.0×10^3 to 5.0×10^3.

3.4.2 Autopsy

Autopsy was carried out on the group 2, 3, 4, 5, 6, and 7 where death was recorded. The organs examined were the kidneys, livers and hearts.

3.4.3 Histopathology

Histopathology was also carried out on all the organs mentioned in the autopsy and also on the control group. The photomicrographs were taken immediately after which the slides were read.

Method of Histopathology*:* Tissue sections of the liver, kidney and heart were taken and fixed in 10% buffered neutral formalin for 72 hours. The tissues were then processed routinely using standard methods and stained with Haematoxylin and Eosin. The slides were examined under a light microscope at x40 objective.

3.5 FORMULATION STUDIES

3.5.1 Preparation of Pregelatinised Starch

Twenty grams (20g) of maize starch was weighed and placed in an empty clean container. Fifty millilitres (50ml) of distilled water was added to form a paste. One hundred and fifty millilitre (150ml) of boiled water was subsequently added with continuous stirring to obtain a translucent mucilage.

The mucilage was spread on a stainless steel tray and placed in the oven to dry at 40^0C for 48hours. The dried mucilage was size-reduced in a mortar with a pestle. It was further micronized using blender and sieved through 250µm sieve.

3.5.2 Determination of Hygroscopicity

Three grams of PGS was placed in an evaporating dish to dry in atmospheric condition by placing it in the open laboratory space for 24 hours. The final weight was recorded after the exposure and the percentage change in weight was calculated.

3.5.3 Moisture Content Determination

To determine the moisture content of the PGS powder and the various granules used in this work, 3g of each powder sample or granules were taken in separate evaporating dishes of known weights, which were placed in a Gallenkamp oven and dried at 105^oC to constant weight. The moisture content was calculated as percentage loss in weight of the original weight which was then recorded

$$MC = \frac{100(Iw-Fw)}{Iw}\% \quad \text{.....Eqn 5}$$

Where;

MC = Moisture content
Iw = Initial weight of the powder or granules
Fw = Final weight of the powder/granules after drying to constant weight.

The moisture contents of all the granules samples were also determined in the same way.

3.6 TYPES OF FORMULATIONS THAT WERE COMPRESSED INTO TABLETS

a. *Granulated Powdered Leaves*

Table 3.1 Formulae for studying the effect of disintegrants type and binder concentrations on tablet properties of powdered leaves of Ap

No.	Powdered Leaves (mg)	MS Intra Dist (mg)	MS Extra Dist (mg)	PGS Intra Dist (mg)	PGS Extra Dist (mg)	Lactose	Binder w/v % (amount per tablet)	Talc (mg)	Magnesium stearate (mg)	Wt per tab (mg)
F_i	250.0	12.5	12.5			12.5	5% w/v (7mg)	5.0	0.5	300.0
F_{ii}	250.0	12.5	12.5			12.5	7.5%w/v(7mg)	5.0	0.5	300.0
F_{iii}	250.0	12.5	12.5			12.5	10%w/v(7mg)	5.0	0.5	300.0
F_{iv}	250.0			12.5	12.5	12.5	5%w/v(7mg)	5.0	0.5	300.0
F_v	250.0			12.5	12.5	12.5	7.5% w/v(7mg)	5.0	0.5	300.0
F_{vi}	250.0			12.5	12.5	12.5	10%w/v(7mg)	5.0	0.5	300.0

* Maize starch was used as binder.

Key: CP - Compression pressure 6MT
TD – Tablet diameter 10mm
MG – Method of granulation Wet granulation

b. Methanolic extracts

Table 3.2 Formulae for the effects of diluents types (HMC and Lactose) and binder concentration on tablets of Methanolic extract (ME) of *Adrographis paniculata*

No.	ME (mg)	PGS Intra Dist (mg)	PGS Extra Dist (mg)	MS Intra Dist (mg)	MS Extra Dist (mg)	HMC diluents (mg)	Binder (amount per tablet) w/v %	Talc weight (mg)	Magnesium stearate (mg)	Lactose diluents	Wt per tab (mg)
F1	10.0	20.0	15.6			140.0	Gel 2.5% w/v(10mg)	4	0.4		200.0
F2	10.0			20.0	15.6		MS 5%w/v(10mg)	4	0.4	140.0	200.0
F3	10.0			20.0	15.6		Gel 2.5%w/v(10mg)	4	0.4	140.0	200.0

Key: ME – Methanolic extract (active ingredient)
HMC- Heavy magnesium carbonate
PGS – Pregelatinised starch
CP - Compression pressure 5.5MT
TD – Tablet diameter 10mm
MG – Method of granulation Wet granulation

These formulae were the best formulations of the effects of diluents type (HMC and lactose) and binder concentrations on the tablets of ME of AP as shown in Appendix XI

3.6.1 Production of Granules

Wet Granulation Method: Wet granulation method was used for production of granules for tablets. The powdered leaves or the extracts were mixed with diluents in a mortar and pestle using the doubling up technique. Quantities of the binder suspension were added a little at a time to the mixture and further trituration until a doughy mass which did not stick to pan was formed. This amount of binder used was then calculated. The weight mass was pass through 1.7 mm mesh size, dried at 40^0c in a Gallen Kamp oven for 30 minutes. The partially dried granules were further passed through 1.6mm mesh size and further dried at the same temperature in the Gallen Kamp oven until constant weight was obtained. Other tableting excipients such as gliddant and lubricant e.g. Magnesium stearate and Talc were added and mixed using mortar and pestle.

3.7 DETERMINATION OF GRANULE PROPERTIES

3.7.1 Particle Size Analysis

The sieve method described by Martin *et al* (1983*)* was employed. Ten grams(10g) of the granules from each batch was weighed and placed on a stack of five sieves as the case may be ranging from 75, 90, 150, 250 and 500µm in order of decreasing aperture. The largest aperture was placed at the top and the finest at the bottom next to the collector pan. The stack of sieves was mounted on the Endocott test sieve shaker. The granules were placed on the top most sieve (500µm) and vibrated for 15 minutes. The weight of granules retained on each sieve was recorded. The percentage weight retained was also recorded. The percentage cumulative undersize was plotted against the sieve size (µm) (Macelo *et al,* 1993). The mean granules size (MGS) was calculated as follows:

$$MGS = \frac{\sum(\text{sieve size } (\mu m) \times \% \text{ wt})}{100} \quad \text{.....Eqn 6}$$

3.7.2 Flow Rate

Ten grams(10g) of the granules were put into the funnel of the Erweka TA-3R flow meter. After switching on the machine, the time it took the granules to pass through the orifice was recorded. It was repeated twice and the average was recorded. The flow rate is the amount of granules that flow out through the flowmeter/second i.e. g/sec.

3.7.3 Angle of Repose

The angle of repose is defined as the maximum angle between the base of the powder and the horizontal plane at which the material slides over itself. About 50g of granules were poured into the funnel and white sheet of large paper was placed on the

horizontal surface directly under the funnel. On removal of the piece of paper blocking the orifice of the funnel the granules flowed out of the funnel and formed a heap. The height of the heap was measured as well as the circumference made by the heap. From the circumference of the granules, the radius of the heap was measured. From these data the tangents of the various angles formed by the powder were calculated using:

$$Tan\theta = \frac{h}{r} \text{.....Eqn 7}$$

TOA : Tan θ = opp/adj; $Tan\theta = \frac{h}{r}$

Where;

ϒ = radius

h = height of the heap of the powder

3.7.4 Carr's Index (Compressibility) of the Granules

Fifty grammes (50g) of the granules were poured into 100ml glass measuring cylinder and the initial volume of the powder was recorded. The cylinder was tapped 50 times on a soft surface at tapping rate of 25 taps per minute from a height of 3cm from the horizontal. Thereafter the new volume (tapped volume) was also recorded.

The bulk and the tapped densities were calculated using the equation. Bulk density volume was the volume of the granules at (O) zero tap.

$$\text{Bulk Density} = \frac{\text{Weight of granules}}{\text{Bulk volume i.e. at tap 0}} \text{.....Eqn 8}$$

$$\text{Tapped density} = \frac{\text{Weight of granules}}{\text{Tapped volume i.e. at 50 taps}} \text{ ....Eqn 9}$$

Data from the equations above were used to calculate the Carr's Index.

$$\text{Carr's Index} = \frac{TD - BD}{TD} \times 100\% \text{ .....Eqn 10}$$

Where;

TD = Tapped Density

BD = Bulk Density

3.7.5 True Density of Granules

The method of specific gravity bottle was employed. The bottle was filled with xylene. It was wiped and dried and weighed. One gram of granules was weighed and transferred into the 20ml specific gravity bottle. Xylene was carefully poured into the bottle and allowed 5 minutes to equilibrate at room temperature. The granules were gradually stirred to release entrapped air and allowed to equilibrate for another 5 minutes. This procedure was repeated until all the entrapped air had been released. The bottle was filled with more xylene and the lid was carefully replaced. The bottle was wiped dry and weighed. The density was calculated using the formula (The granules used were insoluble in xylene).

$$\text{Granule Density} = \frac{W \times SG \text{ of Xylene}}{[(a + W)] - b} \quad \text{.....Eqn 11}$$

Where;

W = Weight of granules

S.G. = Specific gravity of xylene

a = Weight of bottle + xylene

b = weight of bottle + xylene + granule

3.8 COMPRESSION TO TABLETS

3.8.1 Compression of Granules

The granules were lubricated with 0.2% magnesium stearate and talc 4% and compressed using Erweka AR 400 single punch tableting machine in all. The 300mg leaf powder tablets were compressed at six metric tonne pressure(6MT) using 10mm punch and die set while 200mg tablet of the ME were compressed at 5.5MT using 8mm punch and die sets.

3.9 EVALUATION OF TABLET PROPERTIES

3.9.1 Weight Variation:

A total of 10 tablets were used per batch. The tablets were weighed individually and collectively. The mean weight and standard deviation for each sets tablets were determined.

3.9.2 Crushing Strength (Hardness Kgf)

The crushing strength of the tablets was determined using a Monsanto tablet hardness tester. The mean of six readings was calculated for each batch.

3.9.3 Friability:

Ten (10) tablets were subjected to abrasion in a Roche friabillator at 25rpm for 4 minutes. The tablets were weighed before and after the test.

The percentage weight loss was calculated which was the indication of percentage friability.

$$\frac{X-x}{X} \times 100\% \quad \text{.....Eqn 12}$$

Where;

X = weight of tablets before abrasion

x = weight of the tablets after abrasion

In the case of capping or breaking of the tablets the procedure had to be discarded and then repeated. The mean of the three readings were determined for each batch of tablets.

3.9.4 Tablet Thickness/Specific Thickness:

Ten tablets were taken at random from each batch and their thickness was determined using a Vernier Caliper. The mean thickness from the batches was determined. The specific thickness is the ratio of the mean thickness to mean weight:

$$\text{Specific thickness} = \frac{\text{Mean thickness(mm)}}{\text{Mean weight (mg)}} \quad \ldots\ldots\ldots\ldots \text{Eqn 13}$$

3.9.5 Disintegration Time of Tablets:

The disintegration times of the tablets were determined according to the BP procedure using Erweka disintegration tester. The apparatus was thermostatically maintained at $37^{o}C \pm 0.5$ and the disintegration medium was water. Six tablets were placed in the tubes of the tester of which the lower end was fitted with a gauzed disc. The apparatus was calibrated to operate at thirty cycles per minute. For each batch of tablets the experiment was repeated and the mean calculated.

3.9.6 Tablet Dissolution Test:

Since this is a new drug where the actual wavelength of maximum absorption is not known, a full spectral scan was carried out. The extract was dissolved in a medium of a ratio of 1:10 methanol to water. Methanolic extract (M.E) 0.1 mg was dissolved at first in 2ml of the solution and after complete dissolution, it was made up to 10ml volume with the stock solution. The scan was carried out using a spectrophotometer . A consistent peak at 313nm was observed (see appendix 11a)

Various concentrations of the extract were obtained ranging from 0.0001 to 0.1mg. Using the 313nm as wavelength a calibration curve was constructed, see appendix 11b. Dissolution test was carried out of the formulated methanolic extract in which the absorbance of withdrawn samples and concentration were taken at the wavelength of 313nm.

The dissolution medium was 900ml of 0.1N hydrochloric acid (HCl) which was maintained at $37^{o}C \pm 0.5$. The dissolution apparatus has the capacity of running up to six samples at a time. The tablets were placed in the various compartments in the dissolution medium attached to the electronic stirrers. The stirrers were run at 100rpm. Two (2ml) of the samples were withdrawn from each compartments and replaced with 2mls of the dissolution medium. The samples were withdrawn at 2, 4, 6, 8, 10, 15, 20, 30 minutes. From then on, the samples were withdrawn at 10 minute intervals up to 120 minutes. The drug content was determined by using the UV-visible spectrophotometer . With reference to the calibration readings, the machine gave the concentrations of the samples from their absorbance

3.10. Moisture Sorption Capacity on F1, F2and F3

Moisture Absorption:

Saturated salt solutions to provide the various relative humidities were prepared.

(After Winston and Bates 1960) as shown in Table 3.2.

Table 3.3 Saturated salt solution used to provide various humidity environments (after Winston and Bates 1960).

Saturated salt solution	Relative Humidity
K_2CO_3	43%
NaCl	74%
H_2O	100%

These saturated salt solutions provided the required relative humidity for the determination of moisture sorption capacity at 29.5^0C. Five tablets (1g) weight was dried in the oven at 105^0C to constant weight i.e. until all the moisture had been driven off. One gram(1g) of each sample was kept in a container and equilibrated under 100%, 74% and 43% relative humidities for one week. The final weight of each sample was then recorded. The increase in weight was calculated as the amount of moisture absorbed. A graph of the moisture absorbed was plotted against the relative humidity. The dissolution rate of the tablets at the various humidity levels was also carried out.

***Moisture Desorption Capacity*:**

The equilibrated samples were kept under 0% humidity i.e. silica gel for one week and the percentage moisture loss was calculated and the graph of moisture desorption was drawn.

3.11 EFFECT OF STORAGE ON F_1, F_2 AND F_3

The best tablets of methanolic formulations was stored on the shelf in an airtight container and also in desiccators for 4 years at a temperature of 29.5^0C and the drug content and physicochemical tests were carried on these tablets after the 4 years.

3.12 THE DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The differential Scanning Calorimetry of the crude extract was carried out using NETZSCH DSC 204 F1 machine.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 PHYSICAL PROPERTIES OF *ANDROGRAPHIS PANICULATA*.

4.1.1 Organoleptic Properties of the Powdered AP Leaves

The colour of the dried powdered leave was green and the taste of the leave was very bitter. That is why it was named "King of Bitters" (http://www.altcancer.com/andcan.htm, 2003) and the powder was odourless.

Physical Properties of the Aqueous and Methanolic Extracts of AP

The colour of the extracts was brown and the taste of the extracts was very bitter. The bitterness was much more than the leaf. The extracts were odourless, but very sticky to touch. They were very hygroscopic.

4.2 PHYTOCHEMISTRY

The Phytochemistry results revealed that, the leaves of AP contained a lot of common constituents e.g. tannin, flavinoid, alkaloids, saponin, anthraquinones etc., as shown in Tables 4.1. The most important are the secondary metabolites i.e. terpenes and the steroids. These are the sources of the most active component of the leaves which contain the andrographolide. The yield for Methanolic Extract was 7.8% w/v and that of aqueous was 6.8% w/v. This was the crude extract. This findings is supported by the report that the isolated content of the andrographolide from the leaves is given as 2.3%, (http://www.altcancer.com/andcan.htm, 2003).

Higher yield was obtained with Methanolic extract as solvent, where ME, and WE yielded 7.8% and 6.6% respectively.

The difference in the yield is not much because the polarity of methanol and water are similar. The mean particle size of the aqueous extract were of bigger size therefore the aqueous granules could flow easily. This will cause less friction between the particles and the container (Stainforth, 1982).

Thin layer chromatography

The results showed that three prominent spots were seen on the precoated TLC plate. The Rf values were as follows**.**0.35, 0.56 and 0.67 indicating three components in the "crude" Methanolic extract sample.

Table 4.1: Results of the Phytochemical tests on powdered leaves of AP.

Test	Comments/Observation	Inference
Alkaloids		
Dragendorff	Precipitation was observed for all the three tests	+
Wagner		+
Mayer's Reagent		+
Saponins		
1. Froth test	No honeycomb froth was observed	-
2. Haemolysis test	No haemolysis was observed	-
Tannins		
1. Tannins	Gelatinous precipitated observed	+
Anhraquinones	No observation of bright pink colour	-
Cardiac glycosides		
1. Keller-Kiliani test	A blue colour was observed	+
Flavanoids		
1. Lead acetate test	A heavy ppted was observed	+
2. Ferric chloride test	A blue solution was observed	+
3. Shinoda test	A red colour was observed	+
Test for Terpenes and Steroids		
1. Leiberman- Burchard test	A green colour in the chloroform was observed	+
2. Salkowskis test	A reddish brown colour was observed.	+

Key
+ Present
- Absent

Table4.2 . Some properties of the Aqueous extracts of AP

	Aqueous extract
Percentage yield	6.6%
Moisture content(%)	4.0
Flow rate gm/sec	3.75
Bulk Density (BD) gm/ml	0.517
Tapped Density (TD) gm/ml	0.576
Hausener ratio $\frac{TD}{BD}$	1.114
Compressibility (%)	10.2
Angle of repose (^{0})	20^0
Mean particle size granules (µm)	411.35

4.3 PHARMACOLOGICAL

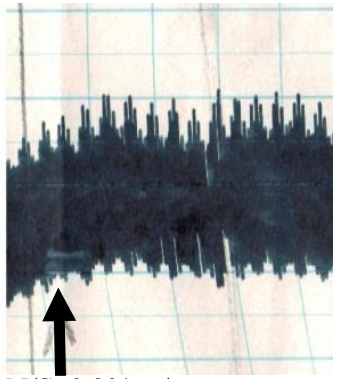
4.3.1: Effect of AP on anaesthetised Cat's blood pressure

The cat blood pressure lowering action of the extract (ME and WE) were dose dependent as seen in appendix VIIIa, VIIIb and Plate 2. Normal saline was used to wash off both ME and WE, and there was no change in the blood pressure when normal saline 0.9w/v was introduced.

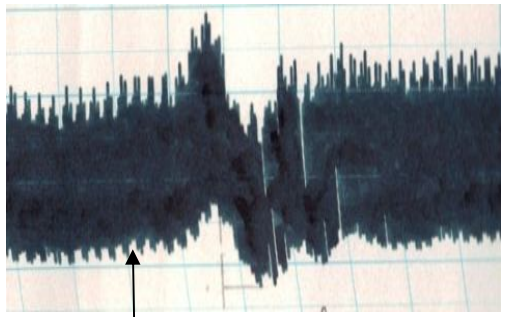
The effects of the methanolic extract and the water extract (WE) on the cat blood pressure (BP) were dose dependent in the lowering of the blood pressure. However, the ME had greater response in blood pressure lowering than the WE as shown in Fig 4.1

Comparing the ME with standard drug Propranolol the two gave graded decrease in the cat blood pressure(BP) up to the highest dose of 1.651mg/kg . This is shown in Appendix VIIIa and appendix VIIIc. The comparison of the aqueous Methanolic extract and the standard drug can also be clearly seen in Fig 4.1. The study suggests that (ME) may be bringing about the decrease in BP through β receptors inhibition since Propranolol is a β receptor antagonist. Atropine could not block ME as could be seen in Plate 5. Therefore ME was not acting through parasympathetic system. It is only a possibility that ME and Propranolol may be acting on the same receptors. Further proof should be investigated.

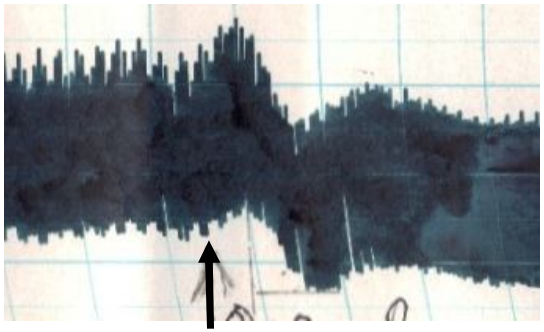
However, ME was more potent pharmacologically than WE (Appendix VIIIa and VIIIb). Plates 2 and 3 showed the reaction of ME and Propranolo on cats blood pressure, lowering. The reactions were dose dependent(Appendix VIIIb and VIIIc). Propranolol act by blocking the antogonizing effect of noradrenoline on β adrano receptors. This account for the lowering of blood pressure observed.



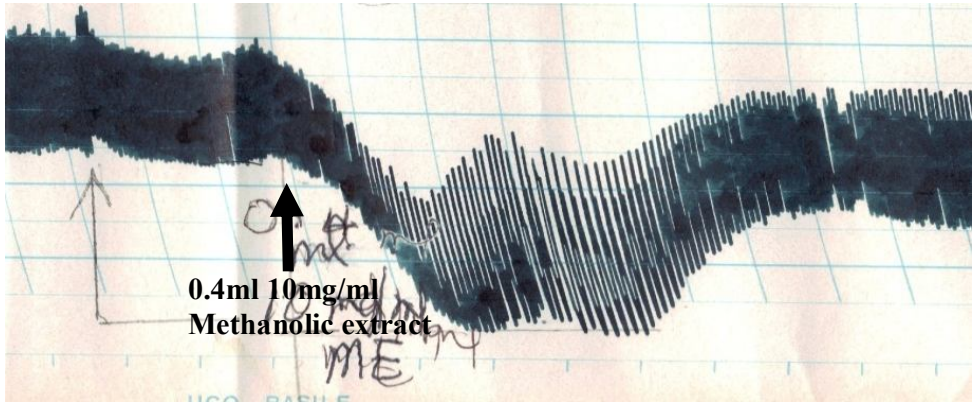
N/S 0.9% w/v percent
(as control)



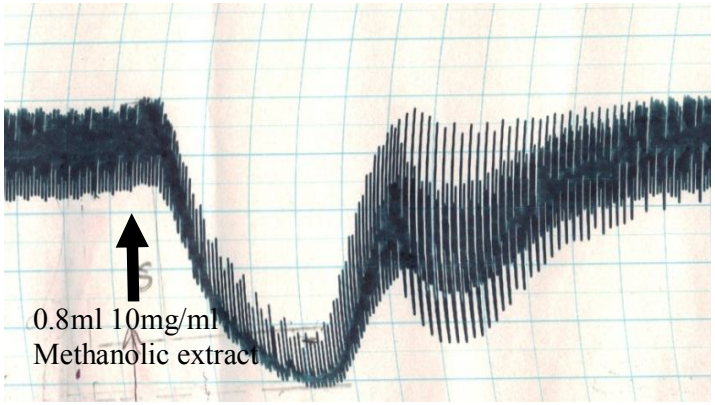
0.1ml 10mg/ml
Methanolic extract



0.2ml 10mg/ml
Methanolic extract



0.4ml 10mg/ml
Methanolic extract



0.8ml 10mg/ml
Methanolic extract

Plate 2 : Effect of the various concentrations of Methanolic extract on cat blood pressure.

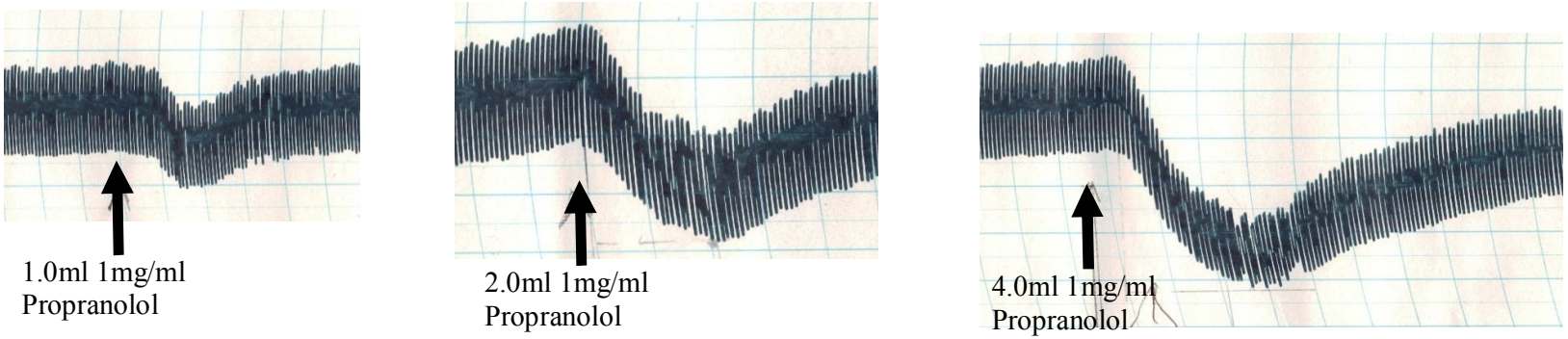
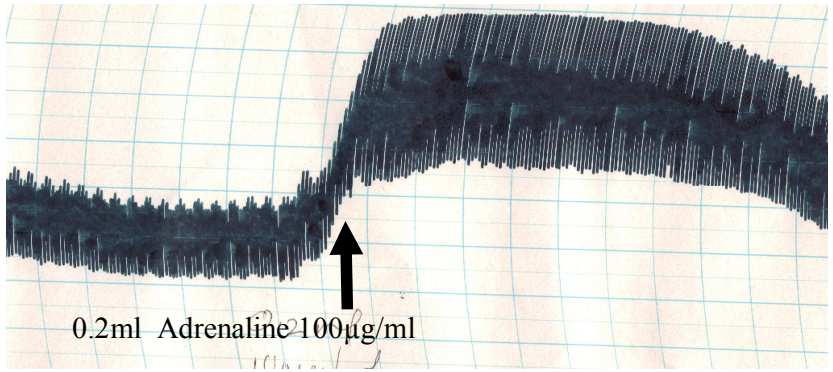


Plate 3: Effect of the various concentrations of Propranol on cat blood pressure.



Plate 4: Effect of the various concentrations of Acetylcholine(Ach) on cat blood pressure.



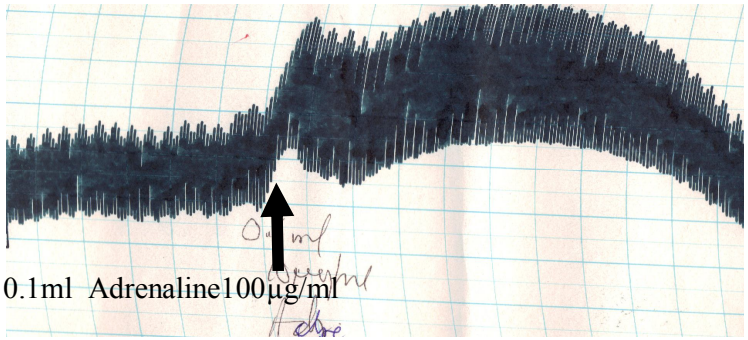
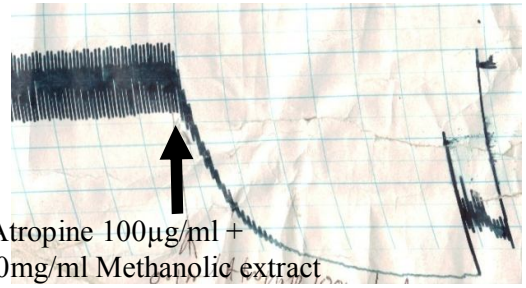


Plate 5: Effect of the various concentrations of Adrenaline on cat blood pressure.



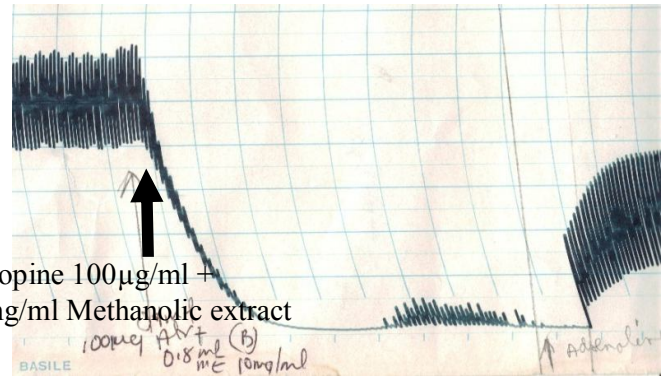


Plate 6: Effect of the concentrations of Atropine and Methanolic extract of AP administered respectively on cat blood pressure.

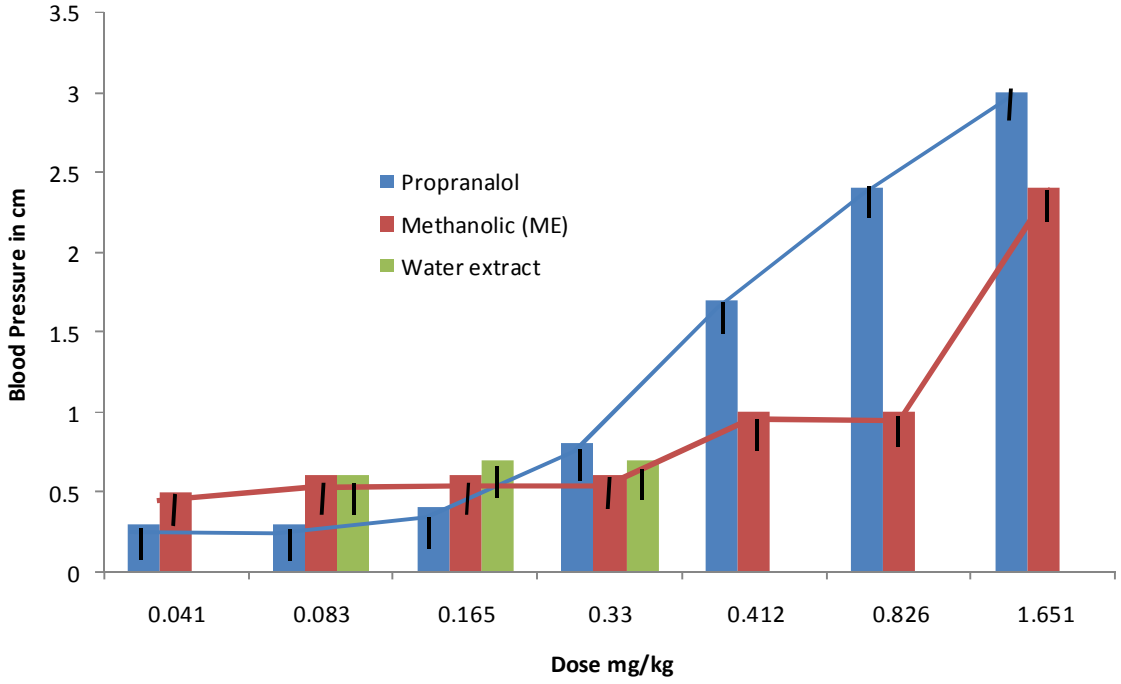


Figure 4.1: Comparison of WE, ME and Propranolol on cat blood pressure (BP)

4.3.2 Toxicological investigation

Various toxicological observations were made during the studies as shown in Table 4.3. In Group 1, no death was observed after 24 hours. There were no significant changes in the behaviours of the rats. While in Group 2, two deaths were recorded within the first 4 hours. The remaining rats were agitated in behaviours, but recovered within 14 days. In Group 3, three of the rats died within 2 hours of the administration with the extract. However, in Group 4, five out of the twelve rats died within 2 hours of injection with the extract. There was an increased breathing rate, ataxia and muscular fatigue. The rats were sluggish and weak.

In Group 5, six (50%) out of twelve died within 3 hours of administration. The rats were weak and had increased breathing rate. Some of the deaths were within one hour of administration with the extract. In Group 6, the behaviour was not different from Group 5, the number of death recorded was six. The rats were weak, fatigue and sedentary, 50% of death occurred within two hours of dosing.

With Group 7 however, within 8 minutes of administration of the extract, one rat died, another died within 10 minutes. Within two hours, nine of the rats had died as a result of the dosing.

Group 8: The reaction to the extract was drastic. Two rats died within 10 minutes, they kept dying subsequently and within four hours all the rats died.

The Group 9, injected with 0.9% of normal saline, were kept as control. None of the rats changed their behaviour and none died either.

Table 4.3: Results of determination of the LD_{50} of aqueous extracts of AP in rats using Probit method.

Group	Dose mg/kg	Concentration Mg/ml	Dead	Survival	Dead %	Corrected %	Probit
1	$5.6x10^3$	$1.0x10^3$	0	12	0	2.08	2.95
2	$1.1x10^4$	$2.0x10^3$	2	10	16.6	16.6	4.026
3	$1.4x10^4$	$2.5x10^3$	3	9	25	25.0	4.326
4	$1.7x10^4$	$3.0x10^3$	5	7	41.6	41.6	4.786
5	$1.9x10^4$	3.5x103	6	6	50.0	50.0	5.0
6	$2.2x10^4$	$4.0x10^3$	6	6	50.0	50.0	5.0
7	$2.5x10^4$	4.5x103	9	3	75.0	75	5.0
8	$2.8x10^4$	$5.0x10^3$	12	0	100	97.91	5.674
9	$6.923\ x10^1$	9.0	-	12	-	-	7.054 -

4.3.3 Autopsy Results

In Group 1, which was used as control group, the autopsy reviewed normal livers, Kidneys and heart. Group 2 revealed some congested livers, kidneys and heart. Groups 3, 4 and 5 all revealed congested livers, kidneys and heart. Groups 6 and 7 revealed severely congested and haemorrhagic livers, kidneys and heart as seen in Plate 8, 10 and 12 under Histopathological studies 4.3.4.

The results indicated that all of extract used were hepatotoxic, nephrotoxic and were also toxic to the heart and lungs only at very high concentration. However these doses were extremely very high as compared with the dose used by the natives to reduce Blood Pressure which was found to be about 0.81mg/kg body weight and the LD_{50} was 1.9×10^4mg/kg body weight. In the central group of Goup 9 no significant histopathological findings were observed in the major organs.

4.3.4 : Histopathological Studies

Table 4.4: Histopathological studies of the various organs of the rats

GROUP TREATMENT	OBSERVATION ON		
	LIVER	KIDNEY	HEART
1 ; 2 3	The liver had diffuse areas of necrosis with mononuclear cellular infiltration, haemosiderosis, dilated sinusoids and congested blood vessels	Necrosis of the glomerulus ;proximal and distal convolued tubules and collecting tubules with mononuclear cellular infiltration there was purplish pigment Ca^{+2} in the medulla of the kidney	Necrosis of the myosiste ;fibrosis and monounclear cellular infiltration myocarditis
GROUP 4	Areas of necrosis with mononuclear cellular infiltration, dilated sinosoids and congested blood vessels	Nucrosis of glomerulus proximal and distal convoluted tubules involving the collecting tubules; mononuclear cellular infiltration and proliferation of fibrous connective tissue fibrosis in medullar and pelvis	Necrosis in the heart with mononuclear cellular infiltration myocarditis
GROUP 5 & 6	As shown plate 8: Had areas necrosis ;mononuclear cellular infiltration, dilated sinusoids and haemosiderosis	Plate 10 had necrosis of glomerulus proximal and distal convoluted tubules involving the collecting tubules with mononuclear cellular infiltration	Had focal areas of necrosis with mononuclear cellular infiltration
GROUP 7&8	The liver of the rats had areas of necrosis, dilated sinusoids, mononuclear infiltration and congested blood vessels	The kidneys had necrosis of the glomeruli and distal convoluted tubules involving the collecting tubules with mononuclear cellular infiltration and congested blood vessels	The hearts in plate 12 had areas of necrosis and mononuclear cellular infiltration
GROUP 9 CONTROL	No significant histopathological findings	No significant histopathological findings	No significant histopathological findings

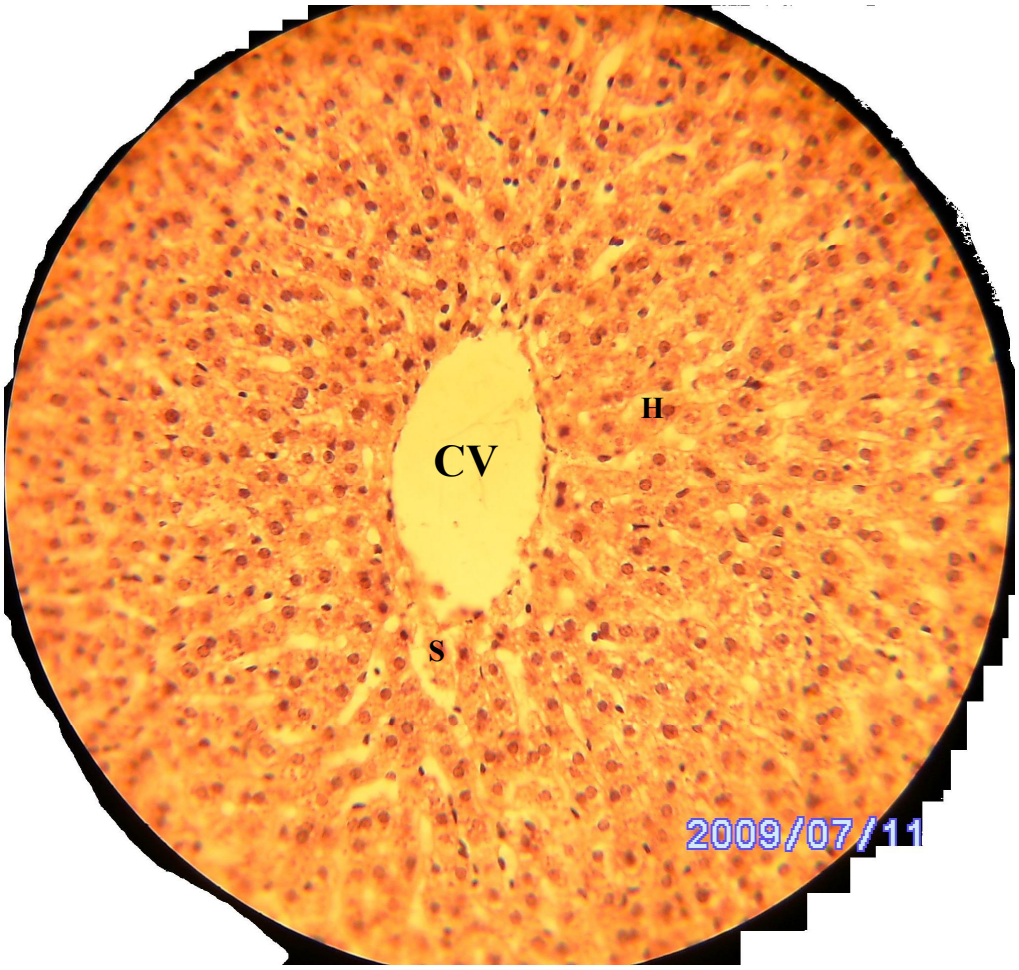


Plate 7: Photomicrograph of a section of untreated liver of rat which served as control.

Note : Note the normal sinusoids (S) and normal hepatic cords (H). H&E stain x400

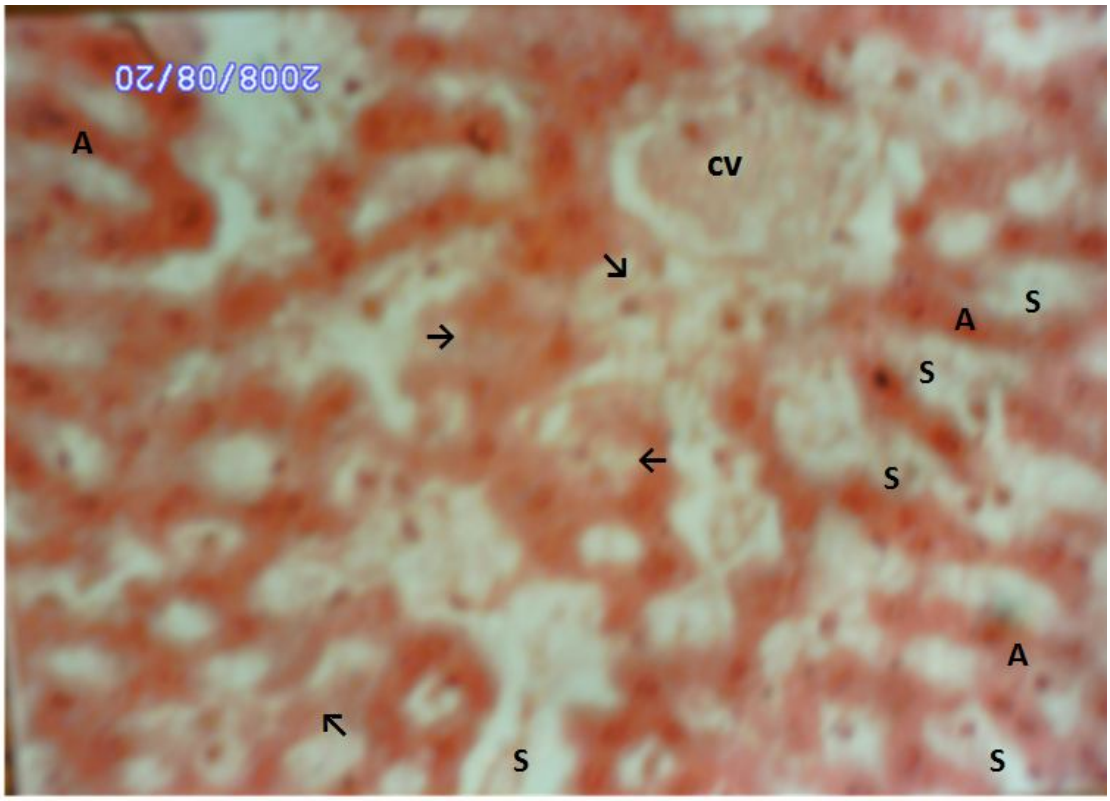


Plate 8: Photomicrograph of a section of a liver from a rat treated with water extract of *Andrographis paniculata* at 2.2 x 10^4 mg/kg for two hours. Note the areas of necrosis of the hepatic cells (arrow heads) the dilated sinusoidal spaces the atrophied (shrinked)hepatic cords (A) and the central vein (CV). H & E stain X 400

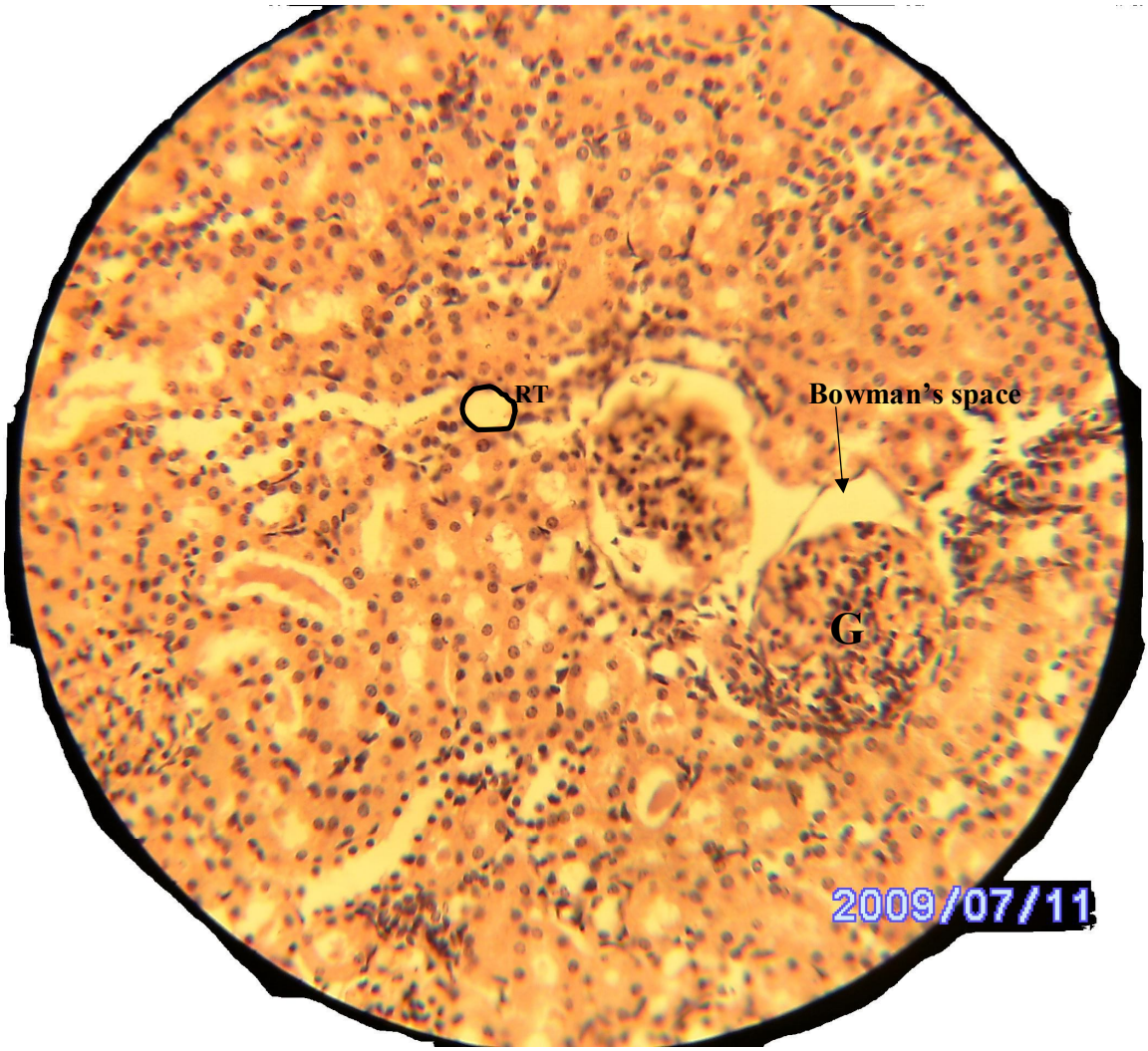


Plate 9.Photomicrograph of a section of untreated kidney of rat which served as control.

Note: The normal glomerular (G) and normal Renal tubular epithelial cells (RT).

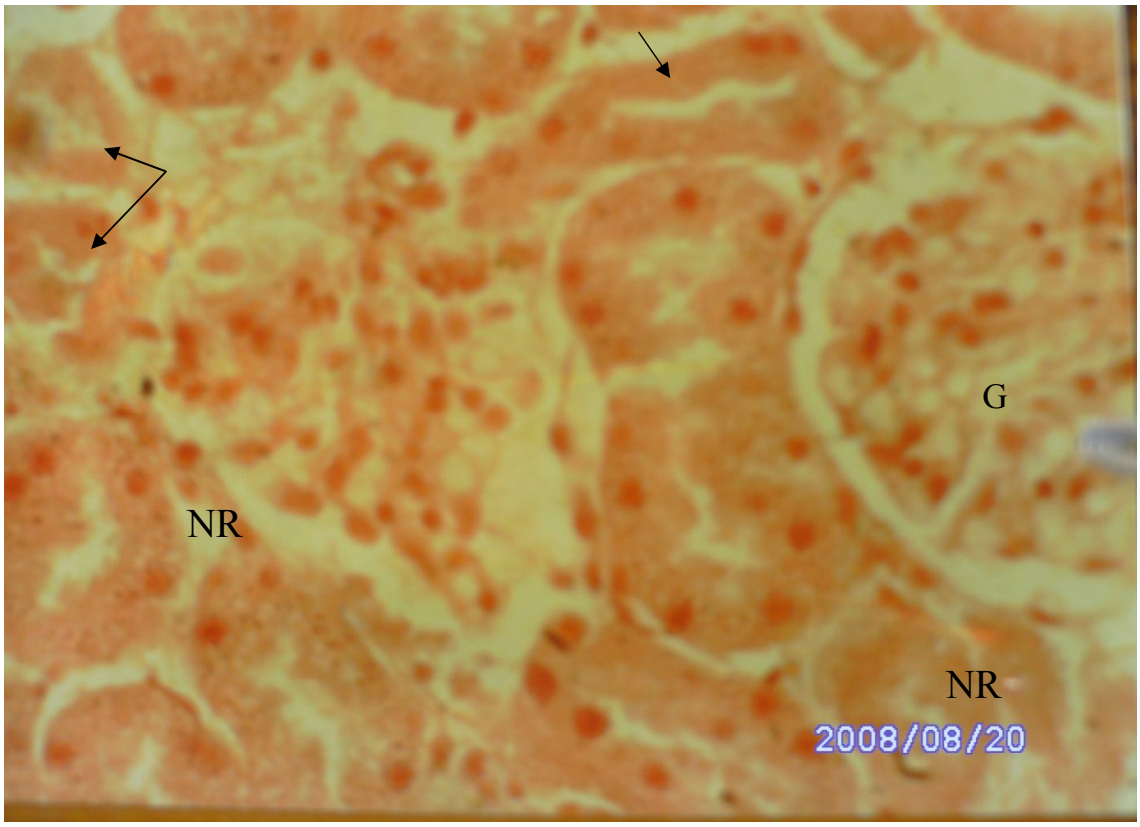


Plate 10.Photomicrograph of a section of the kidney of a rat treated with water extract of *Andrographis paniculata* at 2.2 x 10^4 mg/kg from two hours. Note the glomenular(g) and renal tubular arrow head (necrosis). H & E stain x400

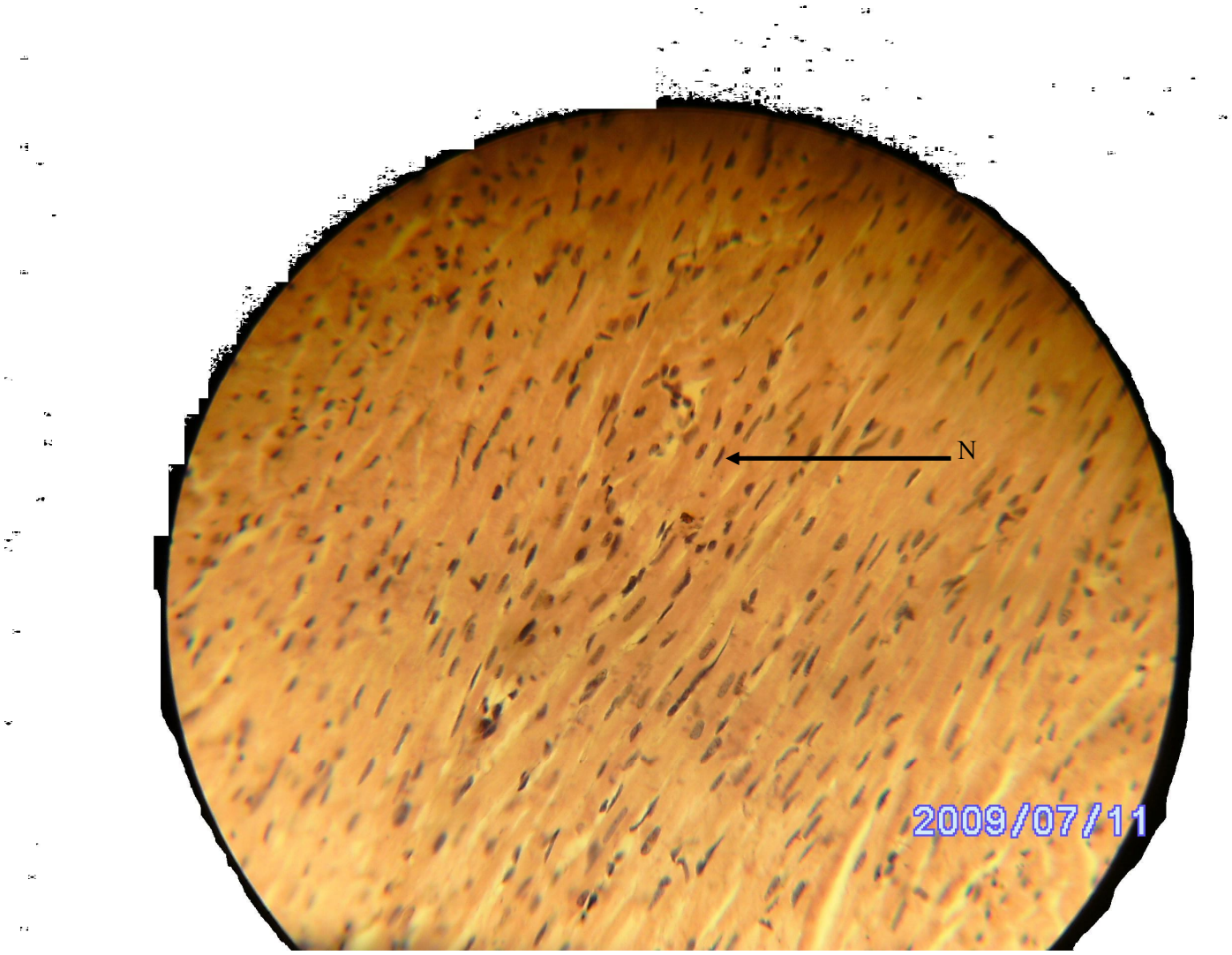


Plate 11: Photomicrograph of a section of untreated heart of rat which served as control.

Note: Distinct nuclei of myocardial cells(N). H & E stain x 400

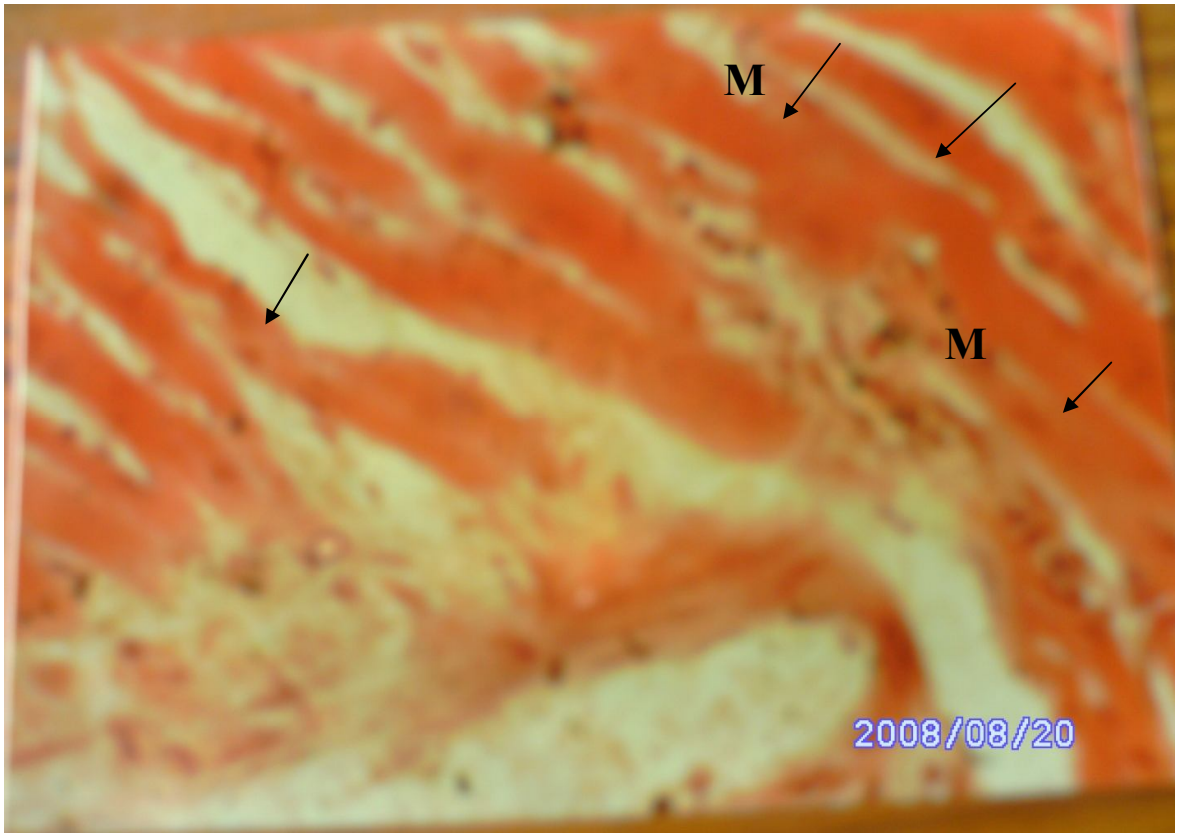


Plate 12: Photomicrograph of a section of a heart from a rat treated with water extract of *Andrographis paniculata* at 2.5 x 10^4 mg/kg from 10 minutes. Note the areas of necrosis of the myocardial cells (arrow heads) and the mononuclear cells (M). H & E stain x 400

4.3.5 Discussion of Pharmacological Results

***a) Effect of AP on blood pressure*:** There was gradual decrease in BP as the concentration of both ME and WE increased, but the reduction in BP for WE was not as significant as for ME. The reduction in BP for ME continued to the maximum of 1.651mg//kg, which recorded the maximum decrease in BP as recorded at this dose. Comparing the reduction in BP with standard drug e.g Propranolol, ME compared favourably in BP reduction on the cat as shown in plate 2 and 3 and appendix VIIIb and VIIIc. The study compared the potency of propranolol and ME. Atropine could not block ME as could be seen in plate 5. Therefore it could mean that ME is not acting through parasympathetic system.

b) Toxicological effect of AP: *Andrographis paniculata* at a dose of $5.6x10^3$mg/kg body weight, no death was observed within 24 hours. These rats recovered after two weeks. At a dose of $1.1x10^4$mg/kg body weight 16.6% death was recorded. At a dose of $1.4x10^4$ mg/kg body weight 25% mortality was observed. At a higher dose up to $2.5x10^4$ mg/kg body weight, 75% mortality was recorded. At maximum dose of $2.8x10^4$mg/kg body weight, 100% mortality was recorded.

The surviving animals recovered from the symptoms of toxicity after 14 days. The active constituent of *Andrographis paniculata,* Andrographilide, has a very short half life. 80% of it is removed within eight hours via the kidney and gastro intestinal tract and 90% is eliminated within forty-eight hours (http://www.altcancer.com/andcan.htm, 2003).

The dose used in the traditional treatment of hypertension involves chewing of 10 to 12 fresh leaves three times a day. This finding has been supported by Zhao and Fang (1990) in studies carried out on rabbits. *Andrographis paniculata* extracts were shown to significantly prevent constriction of blood vessels. Narrowing caused by injury to the inner lining of the blood vessel and high cholesterol level was also decreased by the extract.

Andrographis paniculata also has sedative effect (Deng, 1978). The antihypertensive effect was confirmed by Zhang et al, (1997) and Mshelbwala et al., (1998)

Andrographis paniculata family of compounds have very low toxicity. In an oral toxicity studies using mice, 10g/kg body weight once a day for seven days did not cause death (Chung, 1979). In rabbits given 10mg/kg intravenous andrographolide (a constituent of *Andrographis paniculata*), showed no abnormal cardiovascular response; liver enzyme test, the heart, liver, kidney and spleen were normal (Guo *et al*, 1988). The traditional adult daily dose for the treatment of hypertension in Adamawa State of Nigeria is an experiment of 500mg of dried leaf powder. This is equivalent to 40-50mg dried WE, and can be approximated to 0.8mg/kg. This dose is well tolerated, and has a wide safety margin for use in the management of hypertension. The safety margin is wide because the curative dose taken by the native is 0.8mg/kg body weight and the LD_{50} is 1.9×10^4mg/kg.

Fig 4.2 showed the graph of acute toxicity of the aqueous extract (WE) of AP given intraperitoneally from which the LD_{50} was extrapolated. The LD_{50} 1.9×10^4 mg/kg body weight was quite safe compared to the dose used by the natives which was approximately 0.8mg /kg body weight of the crude extract taken by the natives. This confirmed the findings of Chung (1979) who administered 10 mg/kg to mice for seven days and no death was recorded.

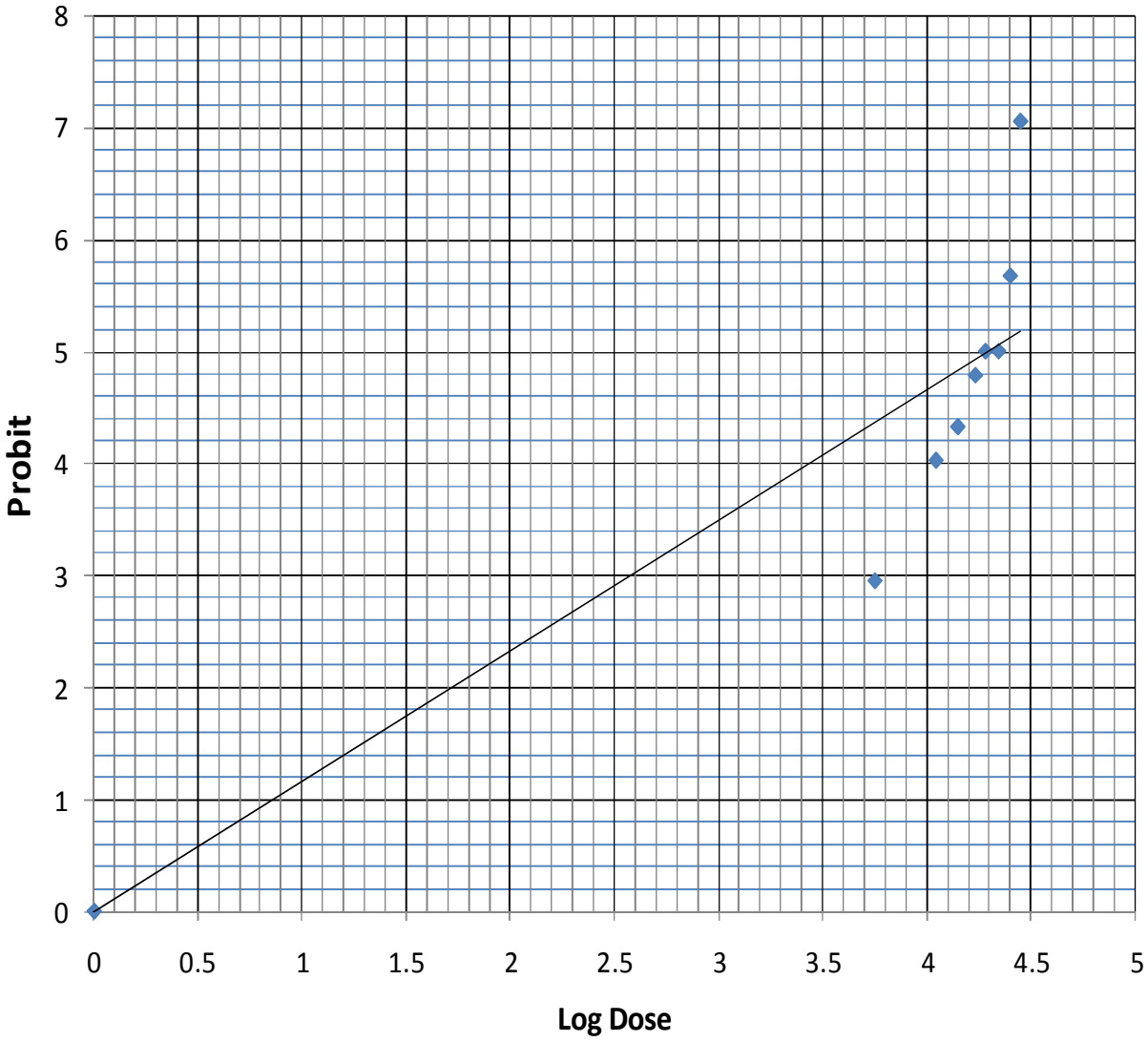


Figure 4.2 Graph of acute toxicity of aqueous extract of AP intraperitoneal route (IP route) of administration

4.4 FORMULATION VARIABLES

Drugs are rarely administered solely as pure chemical substances, but rather as part of a formulation in which it is combined with one or more non medicinal agents that served varied and specialised pharmaceutical functions. Those non medicinal agents often referred to either as adjuncts, excipients, pharmaceutical aids or necessities, and impart unique characteristics such as taste form, appearance, bioavailability etc which enable the drug to achieve its desired activity. The type and amount of the excipients as well as the processing procedure has great influence not only on the physicochemical properties of the active ingredient, but also on the ability of the formulation to meet set down quality control parameters e.g. crushing strength, friability, disintegration, dissolution, drug content and variation in case of tablets.

In the formulation study of the powdered leave of AP and the Methanolic extract, the effect of different types of binders PVP, gelatine and maize starch and their concentration as well as the method of granulation were studied. This was done to enable the choice of suitable formulation composition that will produce the best tablets with the best optimum desired characteristics.

4.4.1 Effect of various binders (MS) concentrations on the granules properties of powdered leaf of AP using (MS) and PGS as disintegrants Fi-Fvi.

The granules were of large sizes. The moisture, contents was low for all the batches. Although lactose which was used as diluents was very soluble in water, most of the water absorbed during granulation was lost during the process of drying due to the formation of large granules. The moisture content for all the batches were below 10% .This value can be considered as acceptable as reported by Ansel H.C. (1981).The Carr's Index, which was the measure of compressibility were all within the 15% which was an indication of granules with good compressibility property. The Hauseners ratio was high well above 1, therefore was an indication that good tablet with high crushing strength would be formed by the granules. The granules had good flow rates, the highest was 2.3g/sec. An indication of granules with good flow rate which will cause less friction between the particles and the container (Stainforth, 1982). The angles of repose were all below the 25^0 which was an indication of granules with good flow property. This will result in uniform flow into the punch and die cavity and will eventually result in tablet of uniform weight. Tablets of uniform weight may invariably result in release of uniform active ingredient and eventually good and uniform therapeutic effect.

Figures 4.3 and 4.4 shows the percentage cumulative weight undersize of granules containing MS and PGS as disintegrants, indicating or exhibiting an almost superimpose effect. An indication that the mean granules sizes were within the same range.

Table 4.5: Analysis of granules of AP powdered leaves containing different concentrations of the maize starch binder.

Property of granules	Binders concentrations					
	F_i	F_{ii}	F_{iii}	F_{iv}	F_v	F_{vi}
Bulk Density (BD) g/cm^3	0.40	0.35	0.34	0.35	0.36	0.35
Tapped Density (TD) g/cm^3	0.44	0.37	0.36	0.37	0.39	0.38
Carr's Index $\left(\frac{TD-BD}{TD} \times 100\right)$ (%)	9.0	5.4	5.5	5.4	7.6	7.8
Hausener Ratio $\frac{TD}{BD}$	1.1	1.06	1.06	1.06	1.08	1.09
Angle of repose (o)	12.5o	14.0 ^{o}	13.4 ^{o}	15.0 ^{o}	14.5 ^{o}	15.6^o
Flow rate g/sec	1.2	1.3	1.35	1.5	1.4	1.6
Moisture content %	3.5	3.0	2.0	3.55	3.5	3.0
Mean granules size	380.0	396.1	370.4	392.4	349.2	366.6

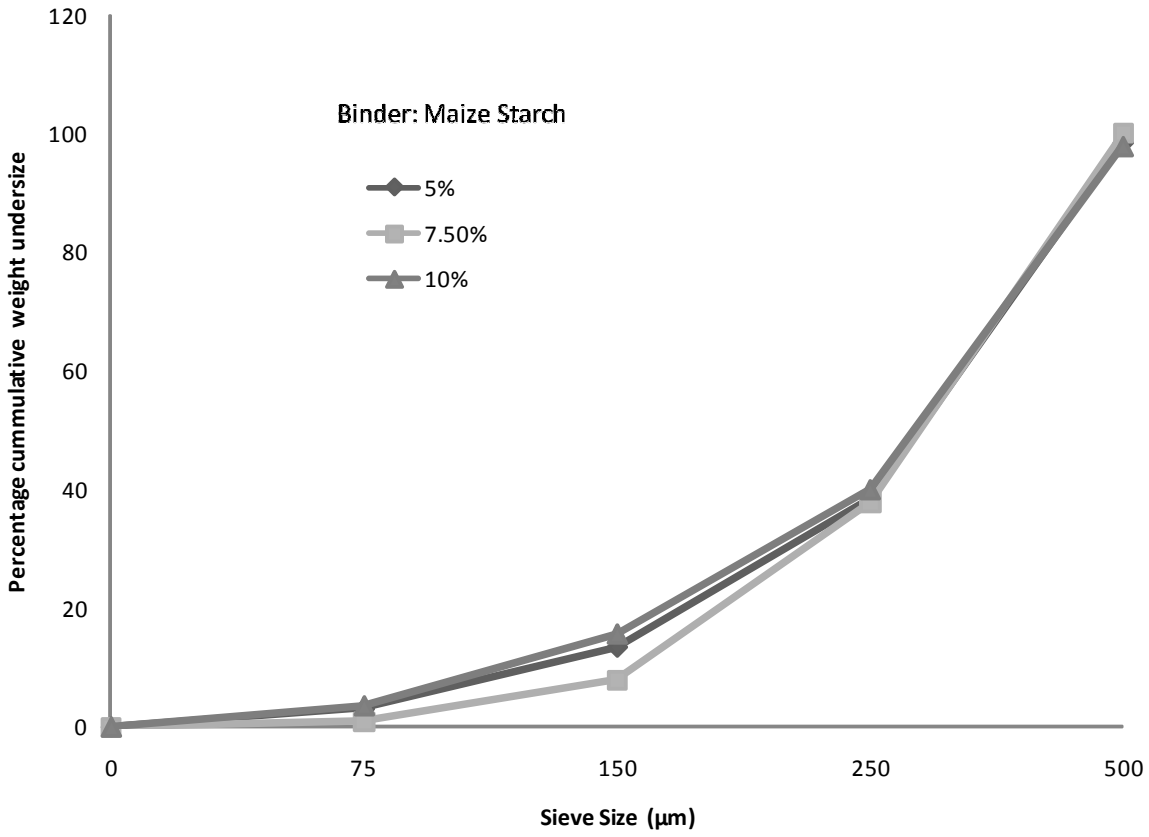


Figure 4.3:Percentage Cumulative weight undersize against sieve size (μm) for granules of powdered leaves of AP containing maize starch as disintegrant.

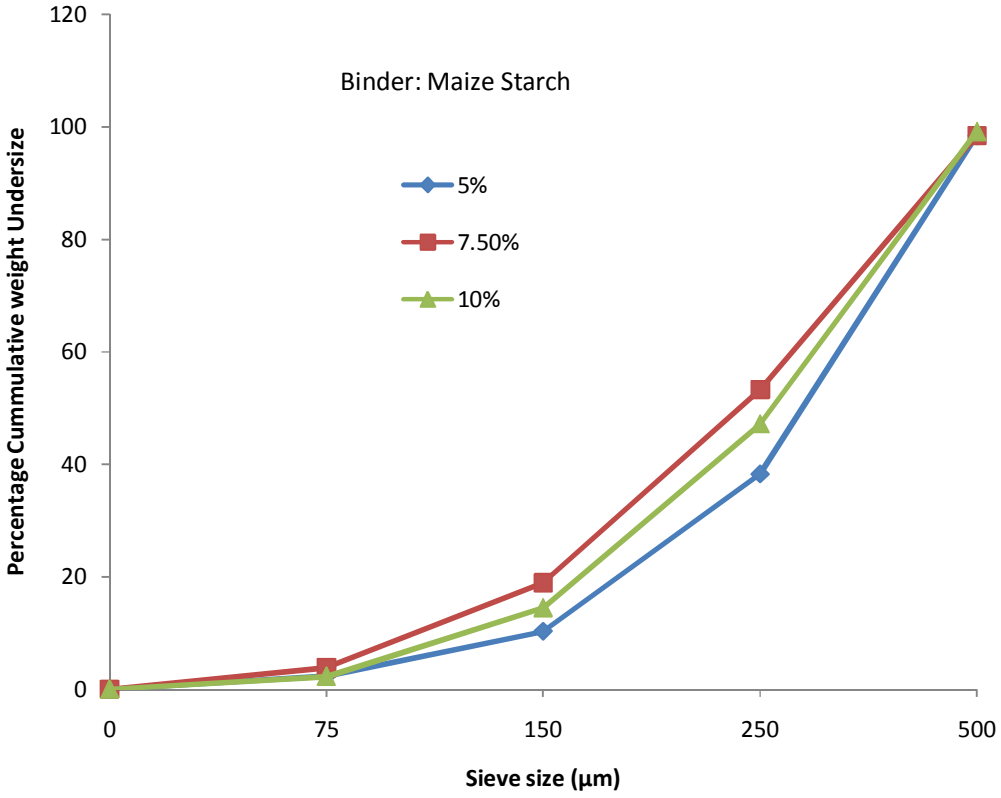


Figure 4.4: Percentage Cumulative weight undersize against sieve size (µm) for granules of powdered leaves of AP containing PGS as disintegrant.

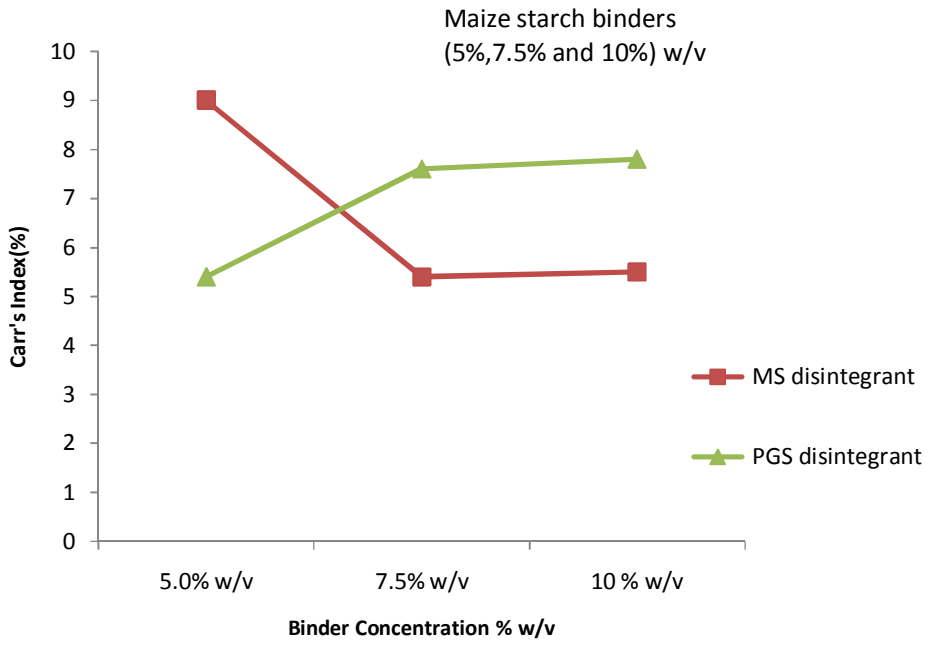


Figure 4.5 Graph of Carr's Index(%) against Binder concentrations (w/v) for the granules of powdered leaves of AP

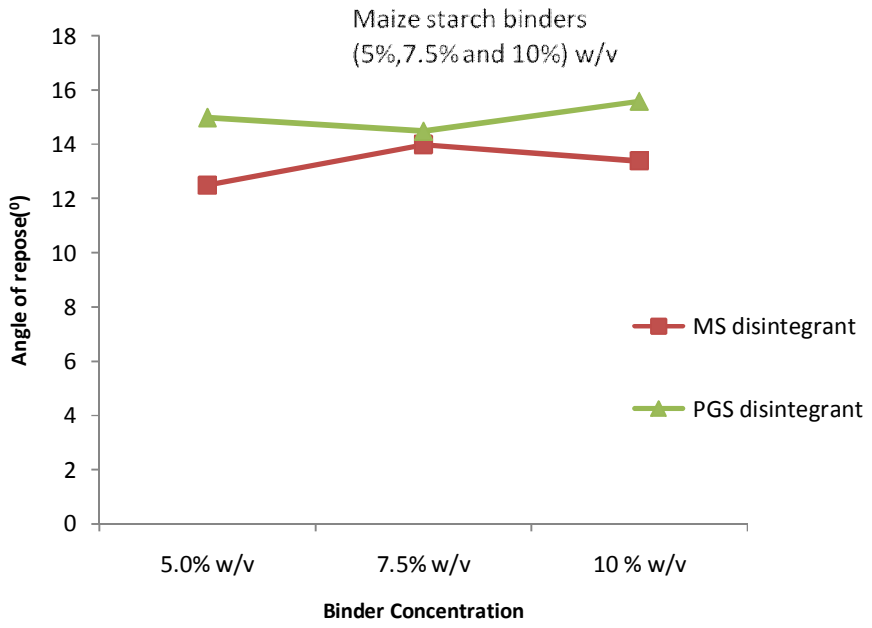


Figure 4.6 Graph of Angle of repose (^{0}) against Binder concentrations (w/v) of the granules of powdered leaves of AP

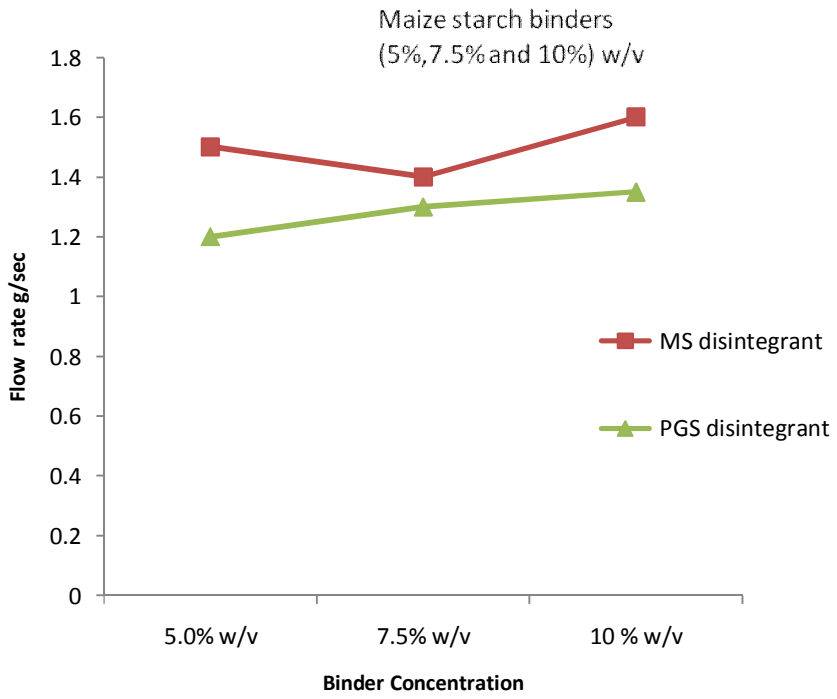


Figure 4.7 Graph of Flow rate (g/sec) against Binder concentrations (w/v) of the granules of powdered leaves of AP

The results shows that as the concentration of binder increased to a certain point, there was increase in particle size. As the concentration of binder increased to maximum of 10% w/v the particle sizes reduced. At lower binder concentration, the binder was less viscous therefore more fluid available to form the granules and subsequent increase in granule sizes. At higher binder concentration, however, the binder mucilage was more viscous and less fluid available to increase the size of the granule.

Mean granule sizes were of medium range as shown in Table 4.5 for the formulation F_i-F_{vi} they were within the 400µm size. The granules sizes increased with increase in binder concentration up to 7.5% w/v and then decreased in granule sizes at maximum binder concentration of 10% w/v.

4.4.2 Effect of Diluents Types on Granules Properties of ME of AP

Lactose produced higher particle size than Heavy magnesium carbonate having granules of 396µm and HMC 371.0µm as shown in Table 4.6. The granules using Heavy magnesium carbonate as diluent also has slightly lower moisture content than those using lactose as diluent. These differences in moisture contents may be attributed to the inherent nature of the diluents as well as differences in particle size. Heavy magnesium carbonate being slightly soluble in water will lose any dissolved water easily when exposed to heat during drying of granules.

Lactose on the other hand is water soluble and water absorbed during granulation will not be lost easily during drying. Granules produced with $MgCO_3$ are smaller, resulting in greater surface area being exposed to drying. This results in greater moisture being lost.

The moisture content of all the granules were above 10%. The forces operating within the granules at this level may be capillary force as indicated by Pilpel 1971.

These forces result in the formation of liquid bridges, attraction is by surface tension and hydrostatic pressure, at the capillary state. It is by surface tension and by hydrostatic pressure at the liquid air surface.

Since the Hausener ratio is high it will form good tablets because high Hausener ratio indicates a good level of compressibility and will result in the formation of good compact tablet with less weight variation. This could be seen in Table 4.8, the tablets compressed had good crushing strength of at least 4.0kgf as indicated as the minimum acceptable crushing strength of tablets by Ansel 1981.

a) ***Carr's Index***

The mean granule sizes for F1 and F2 formulations were within the 400μm size and Carr's index the measure of compressibility of granules is within the accepted 15% value. This is an indication of granules with good compressibility properties, which will form tablets of good compact as reported by Aulton (1988). F3 on the other hand produced granules of smaller sizes and (CI) well above the expected 15% an indication of granules with poor compressibility values: invariably the granules when compressed into tablets would be soft, with low (kgf) values. This is shown in Table 4.8.

Table 4.6 Analysis of Granule Properties of the effect of diluents types and binder concentrations on Methanolic extract of AP.

Properties of granules	F1	F2	F3
Bulk Density (g/ml)(BD)	0.42	0.42	0.37
Tapped Density(g/ml)(TD)	0.53	0.5	0.45
C.I.($\frac{Td-Bd}{Td}$ x 100)%	13.5	16	22.9
Hausener Ratio $\frac{TD}{BD}$	1.26	1.19	1.23
Flow rate (g/sec)	5	3	4
Moisture content (%)	12.0	13.5	12.0
Mean Granule size	371.0	396.9	331.0
Angle of Repose	18.5	14.5	16.8
Packing Fraction	0.3	0.35	0.32

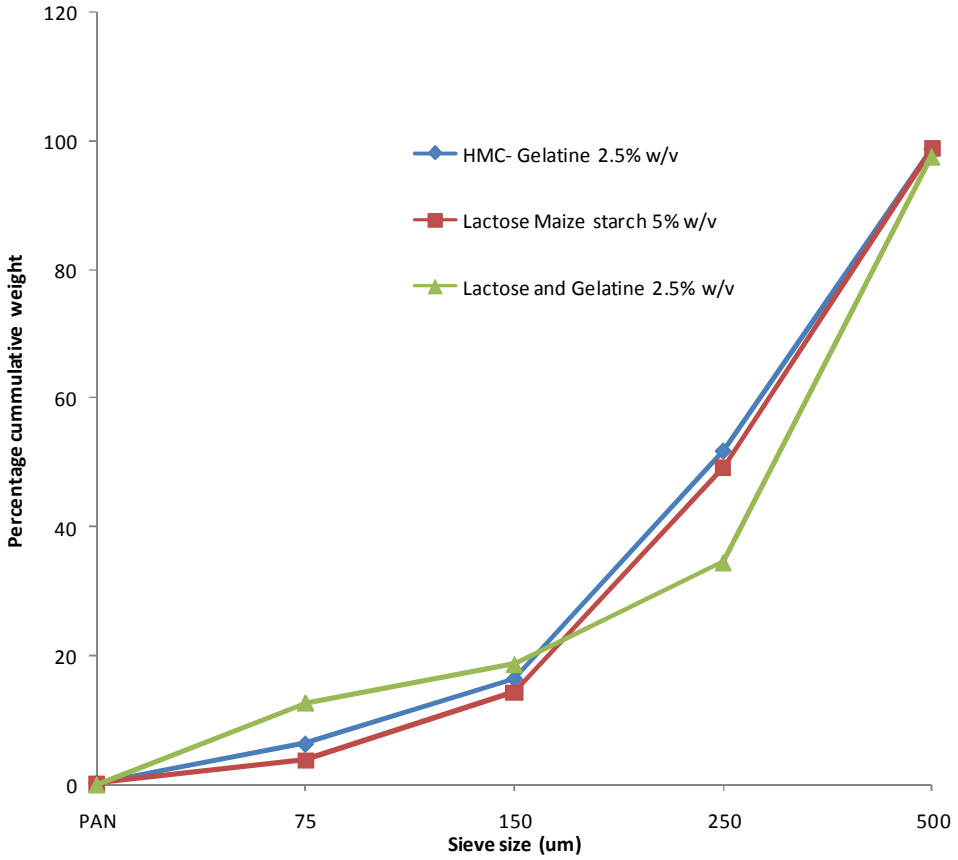


Figure. 4.8 Percentage cumulative weight undersize against sieve size in µm for the effect of various diluents types and binder concentration on granules of ME of AP

***b) Flow rate*:** The flow rate as shown in Table 4.6 indicated that the granules were of larger sizes. The mean granule sizes (MGS) as seen on the Table 4.6 ranged from 300 - 400µm for all the formulations. Therefore it was not surprising that the flow rate was high for all the formulation ranging from 3-5g /sec. This confirmed what Ojile (2000) reported that granules of larger sizes have high and good flow rate. Good flow rate indicates that the tablets would be of uniform mean weight which would result in tablets of the same uniform active medicament and invariably tablets of uniform therapeutic effect.

***c) Angle of repose*:** All the angles of repose fell within the 25^0 therefore, an indication of granules with good flow rate as reported by Aulton, 1988.

The moisture content was quite high for all the granules. There was not much difference between the granules with Heavy Magnesium carbonate as diluents or the lactose.

4.5. PHYSICOCHEMICAL PROPERTIES OF THE TABLETS SHOWING THE EFFECT OF BINDER CONCENTRATIONS ON THE TABLETS OF POWDERED LEAVES OF AP (BY WET GRANULATION METHOD)

As can be seen in Table 4.7, the effect of binder concentrations on the tablet properties did not change the mean tablet weight much as the binder concentrations increased. They all fall within the $\pm$10% weight variation as stipulated in British Pharmacopoeia (1980). The crushing strength however increased with increase in the binder concentration, this has also been reported by Esezobo and Ambujam (1982). This may be due to the interacting forces that came to play during the tablet compaction. Solid bridges might have been formed during the asperitic melting. Those forces have been reported to be responsible for the higher crushing strength values of the tablet (York and Pilpel, 1973).

The relationship between tablet friability and binder concentration varied with the type of binder used. In this case only one binder type was used and as the binder concentration increased the crushing strength increase therefore, the tablet has better value of friability i.e. within the accepted 1% value. This was not surprising, because the tablet crushing strength also increased with increase in binder concentration as is observed in Table 4.7. This applied to all tablets in F_i-F_{vi}. The disintegration time was not affected much with increase in binder concentration.

Considering formulation F_i-F_{vi} for the powdered leaf tablets, formulation F_{vi} produced tablets of the best physicochemical paramaters. Nonetheless dissolution test and stability tests were not carried out.

Table 4.7: Properties of tablets of powdered leaf of *Andrographis paniculata* (AP) using maize starch and pregelatinised starch (PGS) as disintegrants at various MS binder concentrations using a single punch machine Mansanto tableting machine.(F_i-F_{vi})

Batch	Binder Concentration	Binder type	Mean Weight (mg)	Thickness (mm)	Crushing strength (kgf)	Friability (%)	Disintegration Time (Minute)
F_i	5%	Maize starch	300±3.3	4.05	8	0.28	4
F_{ii}	7.5%	Maize starch	301.5±2.5	4.00	8	0.2	4.03
F_{iii}	10%	Maize Starch	300.8±1.7	4.00	9	0.2	4.11
F_{iv}	5%	Maize starch	299.8±0.7	4.00	8	0.10	4.00
F_v	7.5%	Maize starch	300.4±1.7	3.99	8	0.12	4.5
F_{vi}	10%	Maize starch	300.5±1.7	4.00	9	0.0	4.5

4.6 PROPERTIES OF TABLETS EVALUATED FROM METHANOLIC EXTRACT (ME)

4.6.1 Effect of diluents properties on ME Tablets

All the tablets had good crushing strength and were all within the accepted minimum of 4.0kg as stipulated by Ansel 1981. This was expected because the Hausserner ratio was high and this result in the formation of good compact tablet with good crushing strength. The type of diluents did not affect the crushing strength of the tablet much. The $MgCO_3$ and Gelatin as a binder and lactose and Gelatin as binder both had crushing strength within the same range. The disintegration time however, were all within 15 minutes as stipulated by British Pharmacopia 1980 for uncoated tablets. However the tablets with lactose and Gelatin as binder had slightly longer disintegration time. The mean tablet weight variations were all within the range 0.5 to 1 which is acceptable by British Pharmacopia 1980.

Table 4.8: Effects of types of diluents on the properties of tablets of ME of AP

Batches	F1	F2	F3
Mean weight (mg)	200±0.7	201±1	201±0.6
Crushing Strength (kg/f)	5	5	4
Friability %	0	0	0
Disintegration Time (min)	0.25	1.5	8
Mean Thickness (mm)	0.019	0.019	0.018

As for the friability, which was the measure of percentage loss, all the formulations had all passed the test as they all had 0% loss. This was expected because the crushing strength was good within the minimum 4kgf crushing strength acceptable of value as reported by Ansel (1981). Therefore the tablets could withstand handling and transportation stress. All the three formulations had crushing strength ranging from 4.0 kgf to 5.0kgf. This fall within the range of the 4.0kgf which was the minimum acceptable value of crushing strength as reported by Ansel (1981)

4.6.2 Dissolution Test

Result of the dissolution test of the formulations as shown in figure 4.9 showed that the drug release was steady. As the time increases the amount of drug released increased until it reached plateau (climax) at about 18 minutes. And the drug release persisted up to 120 minutes. The total drug release for all the formulations was above 95%, which had passed the BP 1980 specification of 70% of the stated labeled amount.

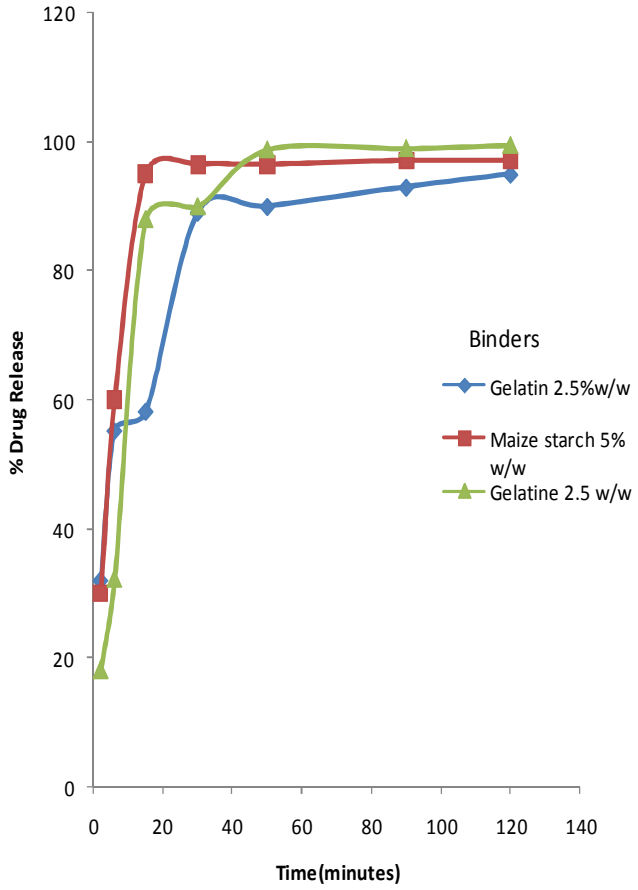


Figure 4.9 Plot of percentage drug release against time (minutes) of tablets of Methanolic Extract of *Andrographis Paniculata.*

4.6.3 Scanning of ME of AP

Scanning

The result of the blind scanning showed that the peak of the wavelength was consistent at 313nm (Appendix IIa). Calibration curve was plotted, but it was not used, because the UV – 160A Shimadza read the concentration directly see Appendix IIb.

4.7 STABILITY STUDIES OF THE TABLETS

4.7.1 Effect of storage on formulations of Methanolic extract

Table 4.9 shows the effect of storage on tablets which was stored at 29.5^{0}C, an average room temperature. All the values were well above 70% drug content as recommended by BP (1980). That meant that the active medicament of Methanolic Extract of AP was stable even after storage for 4 years, both on the laboratory shelf and in the desicator.

Table 4.9 Effect of age on the drug content of the tablets of Methanolic extract of *Andrographis paniculata*

Formulation	Content of active ingredient at time of Production	Drug content After 4 years Storage:	
		Table Top	Desiccators
F1	95.0%	94%	84.6%
F2	96.0%	94.0%	95%
F3	99.5%	92.7%	90%

There were differences in percentage drug content of F1, F2 and F3. This was as a result of mixing. The manual mixing was used therefore it was not possible to have uniform percentage drug content. Nonetheless the drug content was far above the 70% active ingredient as stipulated by British Pharmacopia (1980). Most conventional drugs deteriorate after sometime, but surprisingly the content of ME did not deteriorate – this might be because it is a herbal preparation.

Table 4.10: Comparison of the physicochemical properties of the tablets F1, F2 and F3 at the time of production (0 time) and after 4 years stored at 29.5^{0}C

	F1		F2		F3	
	0 time	After 4 years at 0 Humidity & 29.5°C	0 time	After 4 years at 0 Humidity & 29.5°C	0 time	After 4 years at 0 Humidity & 29.5°C
Crushing strength	5.5	5.5	5	1.5	4	0.75
Disintegration	0.25	0.3	1.5	0.5	8	3.5
Friability	0	0.5	0	0.3	0	0
Mean weight	200±0.7	200±0.1	201±1	200±0.2	200±0.6	200±1.0

Key: (0 Time is time of production)

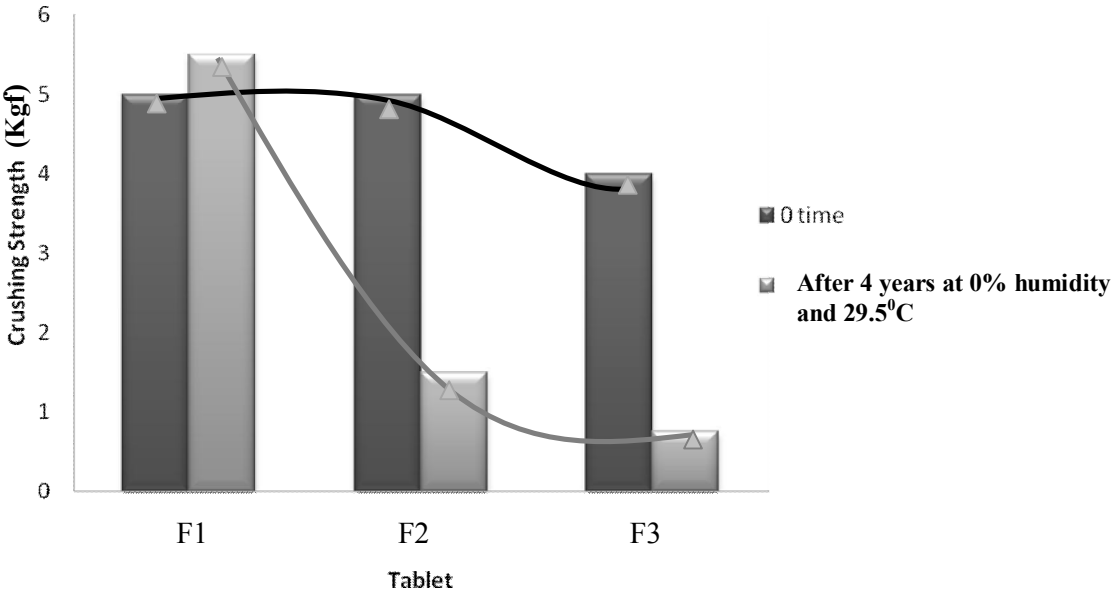


Figure 4.10 Bar charts showing the crushing strength of the tablets F1 F2 and F3 at the time of production and after 4 years of storage

4.7.2 Time of storage had different effects on the tablet properties.

a) Crushing Strength: The crushing strength for F1 was not affected by the storage time. This might be due to the good compressibility properties of the PGS, which was used as disintegrants. This is shown in the value of CI in Table 4.6. Modification of the ordinary maize starch to PGS may have impacted some good compressibility properties to the ordinary maize starch. However, F2 and F3 where ordinary maize starch was used as disintegrants, the crushing strength of the tablet was drastically reduced during storage time. This was not surprising because ordinary maize starch has poor compressibility property and this is shown in the value of the CI in Table 4.6

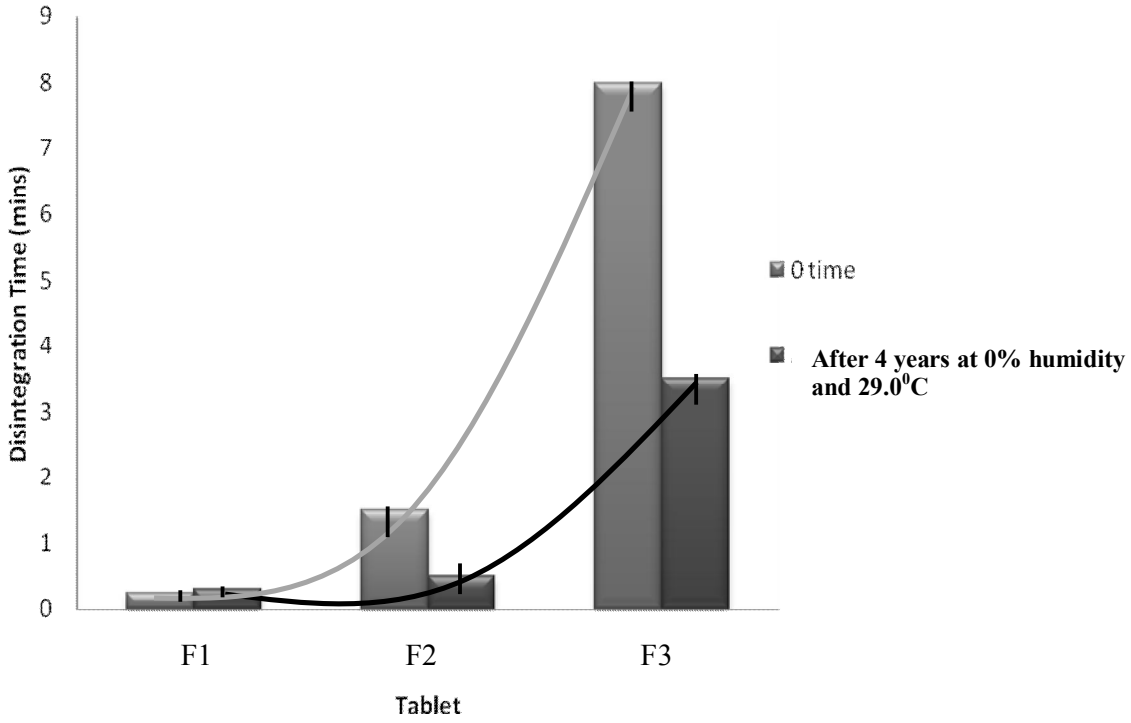


Figure 4.11: Bar charts showing the Disintegration time of the tablets at the time of production and after 4 years of storage

b) Disintegration Time: There was no significant change in disintegration time of F1 even after 4 years of storage whereas F2 and F3 witnessed a significant change in their disintegration time. This was expected because maize starch is a very good disintegrants as compared to PGS. Disintegration properties of the modified starch as in F1 are not affected with age whereas the one of maize starch (F2 & F3) decreased with increased storage time because the maize is a better disintegrant.

4.7.2 Effect of Humidity on Tablets of Methanolic Extract of AP.

As the percentage relative humidity increased the formulation absorbed moisture gradually. The moisture absorbed is proportional to the amount of Relative humidity to which the tablets are exposed Fig 4.12 – Fig 4.14 (see appendix X)

The moisture desorption was also in the similar pattern. The humidity was gradually lost as the saturated tablet F1, F2 and F3 were exposed to the desiccant. The amount moisture lost was very gradual and not much after the one week. This can be seen in Fig 4.12 to Fig 4.14 and appendix X. The moisture desorption did not follow any uniform pattern but on the other hand there were more moisture observed than moisture lost.

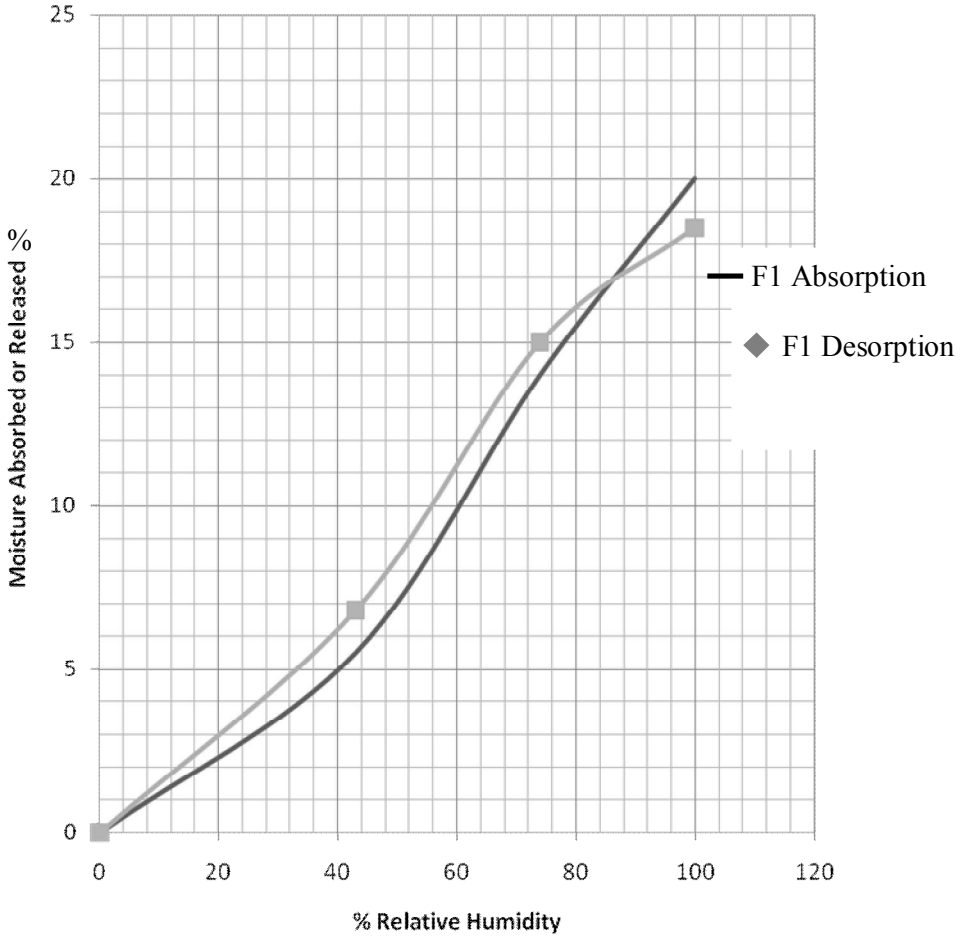


Figure 4.12: Moisture Absorption and Desorption pattern of F 1

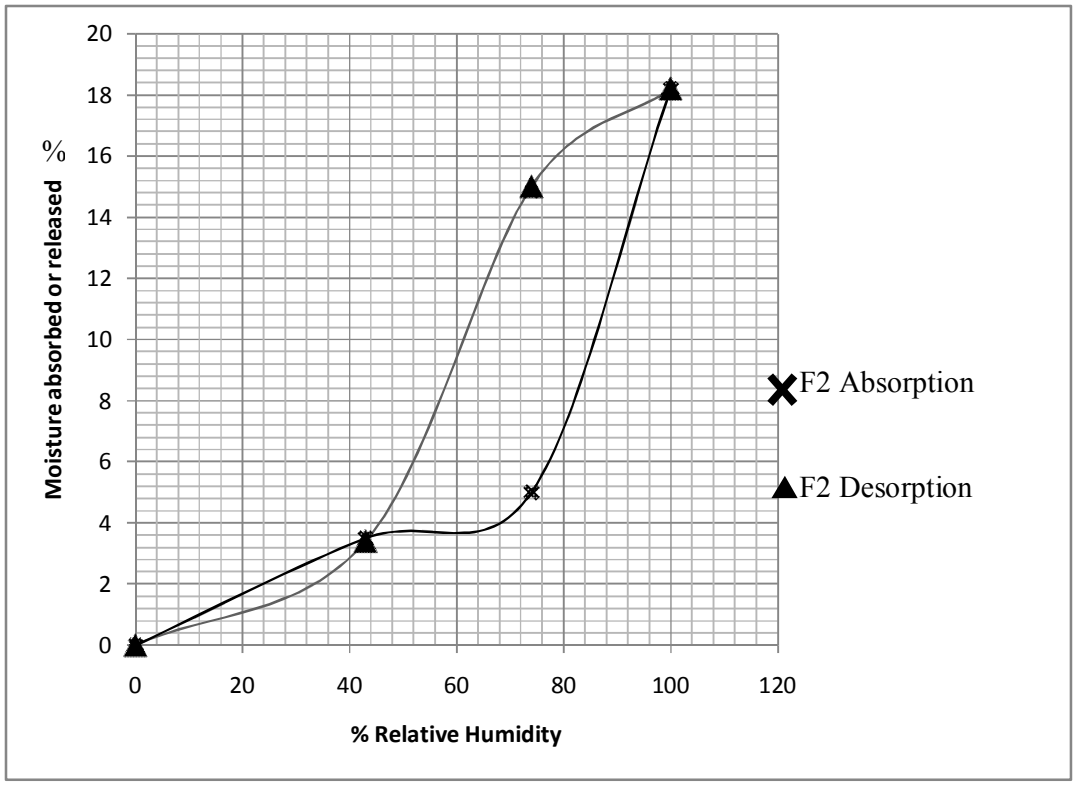


Figure 4.13: Moisture Absorption and Desorption pattern of F2

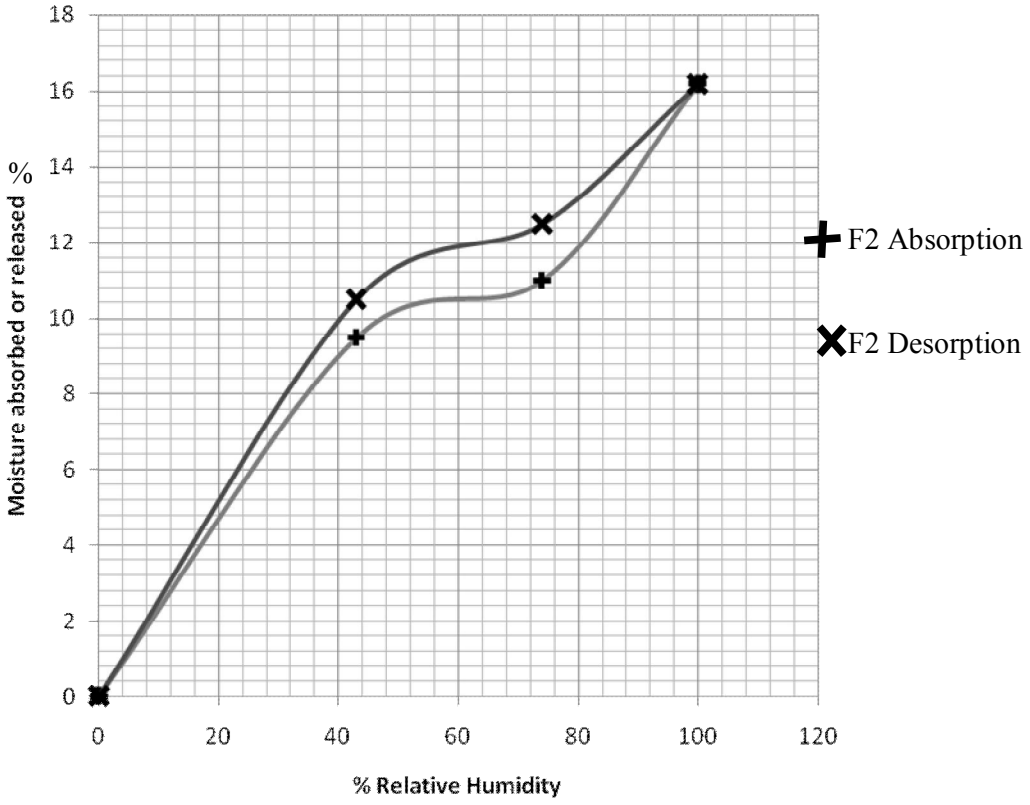


Figure: 4.14 Moisture Absorption and Desorption pattern on F3

Table 4.11 : Effect of Humidity on drug content of tablets of Methanolic extract of AP after 7 days storage

RH	F1	F2	F3
Control	96%	97.0%	97.2%
43%	54.9%	38.7%	63.9%
74%	44.7%	36%	31.5%
100%	29.7%	26%	22.5%

Exposing the tablet to direct moisture had drastic effect on the drug content of the tablets. Moisture absorbed drastically affected the drug content of tablets as could be seen in Table 4.11. As the Relative Humidity increased, the percentage drug released decreases and the lowest results was recorded at 100% humidity. The gummy nature of the Methanolic extract of AP might have affected the release of active ingredient. Due to the absorption of the moisture the physical parameters of the tablets could not be assessed.

4.8 DIFFERENTIAL SCANNING CALORIMETRY (DSC) OF THE CRUDE METHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA*

The thermogram of the pure sample is as shown in Figure 4.15. There are three endothermic peaks indicating three different melting points of three different components. The melting points are as follows A) 120^0C, B) 180^0C and C) 325^0C and A, B and C indicating the three different products with the various melting points. To confirm the DSC results that there are three components, a TLC was performed which gave spots having R_f values of 0.35,0.56,0.67 indicating three components in the crude Methanolic sample. No further examination was conducted beyond this point.

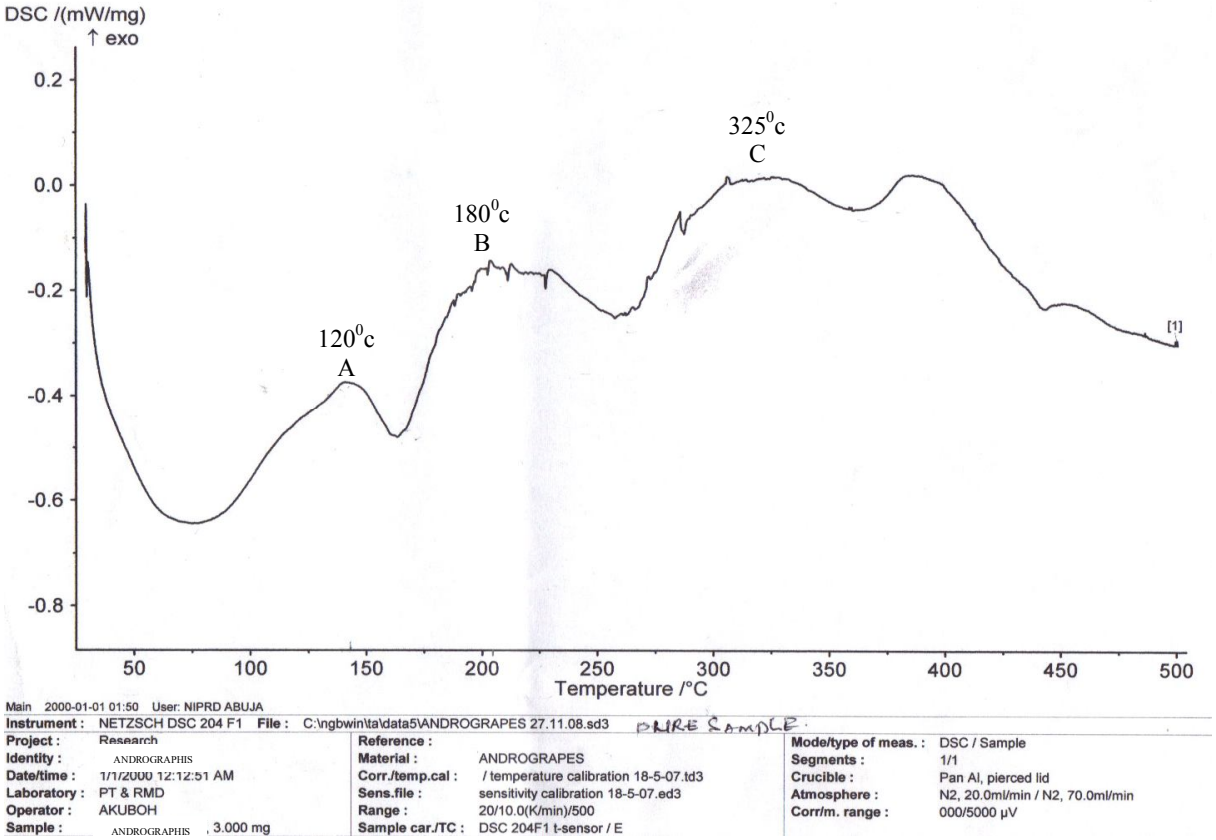


Figure 4:15: Thermogram of Differential Scanning Calorimetry for the pure sample of *Andrographis paniculata*.

CHAPTER FIVE

5.1 SUMMARY

The results from this study have indicated that the plant *Andrographis paniculata* (AP) possesses prominent antihypertensive property.

Phamacological screening using the Methanolic extract revealed prominent blood pressure lowering property of the plant. Both the aqueous extract (WE) and the Methanolic extract (ME) possess this activity. The ME is more potent. Their activity is dose dependent. The potency of the ME compared favourably with Propranolol.

The toxicological studies using rat as an animal model revealed a wide safety margin between the effective dose used in Nigerian traditional medicine and the LD_{50}. Usual administered doses is 500mg daily of the fresh leaves orally equivalent to 0.81mg/kg body weight, while the LD_{50}(in rats) is 1.9×10^4mg/kg body weight.

The main constituents as revealed through phytochemical screening are cardiac glycosides, flavonoids, steroids and especially terpenes which contains the active component, "andrographolide".

AP has been successfully formulated into tablets. Out of all the formulation carried out, the Methanolic extract gave the best tablets that is formulation F1 using PGS as disintegrant and gelatin 2.5% w/v as binder. Stability studies on the ME tablets proved that both the drug content and the physicochemical properties of the tablets were stable under proper storage condition, even after four years. However, direct humidity, affected both the potency and the physical properties of the tablets.

5.2 CONCLUSION

This study has justified the use of *Andrographis paniculata* (AP) by some Nigerian in traditional medicine for the treatment of hypertension, since through it, its blood pressure lowering property has been established.

There is a wide margin of safety between effective treatment dose 0.81mg/kg body weight and the LD_{50} of 1.9×10^4mg/kg body weight.

AP extracts have been successfully formulated into tablets, the quality of which is within BP limits.

Best tablets are produced with Methanolic extract, pregelatinsed starch as disintegrants and gelatin 2.5w/v as binder using wet granulation method.

5.3 RECOMMENDATION

The best Methanolic extract formulation (F1) should be used for in vivo test to establish the use of AP as an antihypertensive. Since the DSC shows three components of the Methanolic extracts, efforts should be made to extract the actual part causing the lowering of blood pressure and used in the tablet formulation.

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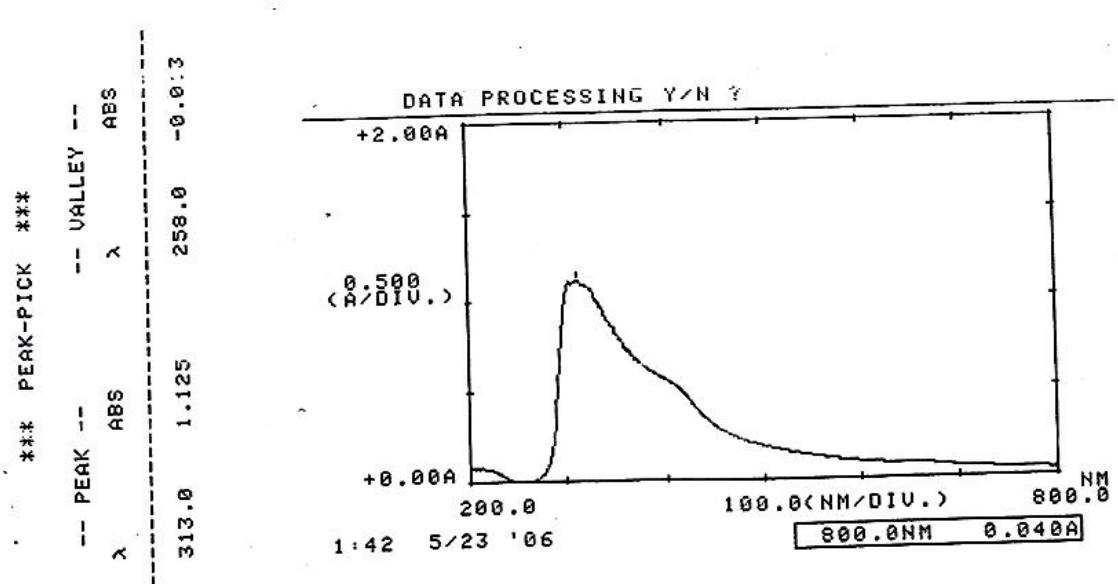
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APPENDIX I

Papers published from the Dissertation.(See Pages 152 --------)

a) Mshelbwala, K., Ojile, J.E and Kanayo, E. (2005). 'A comparative study of the effect of granulation method on the properties of *Andrographis paniculata* tablets', Nigerian journal of Pharmaceutical research vol. 4 No. 1 pp.83 – 88 (September, 2005).
b) Mshelbwala, K., Ojile, J.E, Adikwu, M.U. and Ameh. B.A. (2007). 'Tabletting properties of the aqueous leaf extract of *Andrographis paniculata'*. Nigerian journal of Pharmaceutical Sciences vol. 6 No. 2(October 2007).
c) Mshelbwala, K., Ojile, J.E, Abikoye F.A and Musa H.(2007) 'The Effect of Disintegrants on Granules and tablet properties of Methanolic Extract of *Andrograhpis paniculata* an Anti-Hypertensive' Journal of Advancement in Medical and Pharmaceutical Science,vol.1 N0.3.
d) Mshelbwala, K., Olurinola,P.F., Ibrahim, M.A., and Zumuk, K.K. 'Effects of granule sizes of Pre-Gelatinised Starch (PGS) on the properties of leaf powder tablets of *Andrographis paniculata'.* Nigerian journal of Pharmaceutical research vol. 6 No. 1 pp.70 – 74 (September, 2007).
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APPENDIX IIa



Blind scanning of the methanol extract

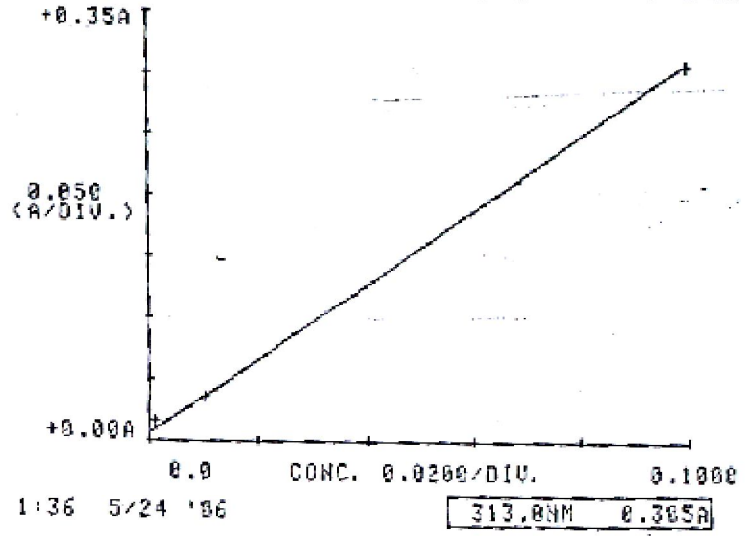
APPENDIX IIb

CALIBRATION WORKING CURVE DISPLAY Y/N ?

STD. No.	CONC.	ABS.
1	0.0000	0.001
2	0.0001	0.009
3	0.0010	0.015
4	0.0100	0.036
5	0.1000	0.305

1:35 5/24 '06 313.0NM 0.305A

WORKING CURVE CONC. MAX. CHANGE Y/N ?



1:36 5/24 '06 313.0NM 0.305A

APPENDIX III

Methods of Preparation of Binder Mucilage. To a weighed amount of starch a little amount of cold water was added to make a smooth paste. Sufficient quantity of boiling water was added gradually by stirring until smooth mucilage was formed.

b) *Polyvinyl pyrrolidone (PVP) suspension* The quantity of PVP powder required to make 2.5, 5.0 and 7.5% w/v paste of mucilage was suspended in cold distilled water and stirred until all the powder went into solution.

c) *Gelatin suspension:* The required amount of the powder to constitute 2.5, 5 and 7.5% w/v Gelatin was suspended in cold distilled water and allowed to hydrate. The suspension was then warmed on a hot water bath until a smooth mucilage was formed. To avoid gelling, the binder was kept warm while it was being used.

APPENDIX IV

Table showing result of sieve analysis of F1-F3 of percentage cumulative weight undersize.

	F1	**F2**	**F3**
PAN	**0.2**	0.1	0
75	**6.3**	3.6	12.7
150	**16.4**	14.2	18.7
250	**51.8**	49.2	34.53
500	**98.7**	98.7	97.53

APPENDIX V

Table showing result of sieve analysis of Fi-Fvi of percentage cumulative weight undersize

	Pan	75	150	250	500
Batch 1	0	3.1	13.5	38.5	98.5
Batch 2	0	0.8	7.8	37.8	99.8
Batch 3	0	3.6	15.7	40	97.8
Batch 4	0	2.3	10.3	38.3	98.5
Batch 5	0	3.8	18.9	53.3	98.5
Batch 6		2.3	14.5	47.2	99.2

APPENDIX VI

Tablet mean weight of Methanolic extract F1,F2,F3

	F1	F2	F3
	200.5	200	200
	199.5	202	200
	201	201	199.5
	199.5	200	200.5
	200	200	201.5
	201.5	201	200.5
	200.5	203	199.5
	200.5	202	200.5
	199.4	200	200.5
	200	201	201
	0.69	1.05	0.63
Mean	200±0.7	201±1	200±0.6

APPENDIX VII

	Fi	Fii	Fiii	Fiv	Fv	Fvi
	305	299.5	300	299.5	300	300
	300	305	302	300	300	300
	299.5	300	305	300	299.5	299.5
	305	300	300	299.5	300	299
	300	299.5	300	300	305	300
	295	305	299.5	300	300	305
	295.5	305	300.5	299.5	299.5	300.5
	300.5	300.5	300.5	300	300	300
	300	299.5	299.5	300	300	300
	299.5	300.5	300.5	299.5	299.5	300.5
MEAN	**300.0**	**301.5**	**300.8**	**299.8**	**300.4**	**300.5**
STANDARD DEVIATION	**3.3**	**2.5**	**1.7**	**0.3**	**1.7**	**1.7**

APPENDIX VIII

a) Effect of Water Extract (WE) on the cat's blood pressure(BP)

S/No.	Extract	Dose	Results	Water extract blood pressure lowering in cm
1	WE	0.041mg/kg	No effect	0 .0
2	WE	0.083mg/kg	Decrease BP	0.6
3	WE	0.165mg/kg	Decrease BP	0.7
4	W.E.	0.330mg/kg	Decrease BP	0.7

b) Effect of Methanolic extract (ME) on cat's blood pressure (BP)

S/No.	**Extract**	**Dose mg/kg**	**Response**	**Decrease in (cm)**
1	Methanolic Extract (ME)	0.041mg/kg	Decrease BP	0.5
2	Methanolic Extract (ME)	0.083mg/kg	Decrease BP	0.6
3	Methanolic Extract (ME)	0.165mg/kg	Decreased BP	0.6
4	Methanolic Extract (ME)	0.330mg/kg	Decreased BP	0.6
5	Methanolic Extract (ME)	0.412mg/kg	Decreased BP	1.0
6	Methanolic Extract (ME)	0.826mg/kg	Decreased BP	1.0
7	Methanolic Extract (ME)	1.651mg/kg	Decreased BP	2.4

c) Comparison of response of the methanolic extract (ME) with propranolol on cat's blood pressure (BP)

S/No	Dose mg/kg	Propranolol blood pressure lowering in (cm)	Methanolic extract blood pressure lowering in (cm)
1	0.041	0.3	0.5
2	0.083	0.3	0.6
3	0.165	0.4	0.6
4	0.330	0.8	0.6
5	0.412	1.7	1.0
6	0.826	2.4	1.0
7	1.651	3.0	2.4

APPENDIX IX

Table 4.9 Percentage drug released for F1,F2,F3.

Time (minutes)	F1	F2	F3
2minutes	32	30	18.0
6 minutes	55.2	60	32.2
15 minutes	58.2	95	88.0
30 minutes	89.1	96.4	90.0
50 minutes	90.0	96.4	98.8
90 minutes	93.0	97	99.0
120 minutes	95.0	97	99.5

APPENDIX X

Relative Humidity(%)	(a) Absorptions %			(b) Desorption %		
	F1	F2	F3	F1	F2	F3
0	0	0	0	0	0	0
43	6.8	5.5	3.4	5.5	3.5	2.5
74	15	14	15	11	5	12.5
100	20	20	18.2	16.2	18.2	16.2

APPENDIX XI

Formulae for studying the effect of diluents types (HMC and Lactose) and binder concentration on tablets of Methanolic extract(ME) of Andrographis paniculata.

No.	PGS Endo Dist (mg)	PGS Exo Dist (mg)	MS Endo Dist (mg)	MS Exo Dist (mg)	HMC diluents (mg)	ME (mg)	Binder w/v %11494+4+.369*	Talc weight (mg)	Magnesium stearate (mg)	Lactose diluents	Wt per tab (mg)	Compression Pressure (mg)	Tablet Diameter (mm)	Method of Granulation
Fi	20.0	15.6			140.0	10.0	MS 2.5% w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fii	20.0	15.6			140.0	10.0	MS 5% w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fiii	20.0	15.6			140.0	10.0	MS 10 % w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fvi	20.0	15.6			140.0	10.0	PVP 2.5% w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fv	20.0	15.6			140.0	10.0	PVP 5% w/v (10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fvi	20.0	15.6			140.0	10.0	PVP7.5 % w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fvii**	20.0	15.6			140.0	10.0	Gel 2.5% w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fviii	20.0	15.6			140.0	10.0	Gel 5.0% w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fix	20.0	15.6			140.0	10.0	Gel7.5% w/v(10mg)	4.0	0.4		200.0	5.5	10.0	Wet gran
Fx**			20.0	15.6			MS 5.0% w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxi			20.0	15.6			MS 7.5% w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxii			20.0	15.6			MS 10.0% w/v(10g)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxiii			20.0	15.6			PVP 2.5% w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxiv			20.0	15.6			PVP 5.0% w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxv			20.0	15.6			PVP 7.5% w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxvi**			20.0	15.6			Gel 2.5% w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxvii			20.0	15.6			5.0 % w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran
Fxviii			20.0	15.6			7.5 % w/v(10mg)	4.0	0.4	140.0	200.0	5.5	10.0	Wet gran

Key

Fvii = F1**
Fx= F2**
Fxvi= F3**

Appendix XII

Effect of diluents type and binder concentration on the tablet properties of ME of AP

Batches	Fi	Fii	Fiii	Fiv	Fv	Fvi	Fvii**	Fviii	Fix	Fx**	Fxi	Fxii	Fxiii	Fxiv	Fxv	Fxvi**	Fxvii	Fxviii
Mean weight (mg)	199.1 ±4.1	202 ±2.7	201 ±4.1	199 ±4.1	200 ±3.5	200 ±3.5	199 ±4.1	202 ±4.1	199 ±2.2	201 ±2.2	199 ±4.1	200 ±3.5	200 ±3.5	198.1 ±2.6	199.1 ±4.0	201 ±2.2	201 ±4.1	198 ±2.7
Crushing Strength (kg/f)	3	5	5	6	6	8	5	4	5	5	4	5	5	5	5	4	4	4
Friability %	0.92	0.94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Disintegration Time (min)	0.5	0.5	0.5	1.7	1.5	1.5	0.25	0.75	0.8	1.5	1.85	2.3	3.3	4.0	5	8	18	18
Mean Thickness (mm)	0.016	0.018	0.019	0.018	0.019	0.019	0.019	0.016	0.016	0.019	0.018	0.016	0.016	0.016	0.018	0.018	0.018	0.018

Fvii = F1**
Fx= F2**
Fxvi= F3**

Appendix XIII

Percentage drug Release for the effect of diluents types and binder concentration on the tablet of ME of Ap

Batches	Fi	Fii	Fiii	Fiv	Fv	Fvi	Fvii**	Fviii	Fix	Fx**	Fxi	Fxii	Fxiii	Fxiv	Fxv	Fxvi**	Fxvii	Fxviii
T_2	20	25	36	30.1	28	30	32	25	30	30	32	22	18.0	38	35	18.0	5	4
T_6	29	30	45	54	45	55	55.2	45	50	60	50	25.0	30.0	50	45	32.2	8	9
T_{15}	32.7	40.9	60.0	69.9	65.7	80.1	58.2	60.8	62.7	95	72.9	53.6	60.2	72.3	80.7	88.0	11.8	11.81
T_{30}	44.0	43.0	62.2	75.9	77.1	80.0	89.1	65.1	60.0	96.4	69.3	75.0	84.0	73.5	90.4	90.0	28.2	12.72
T_{50}	33.5	33.0	63.0	84.3	83.7	86.0	90.0	58.4	65.5	96.4	68.7	76.1	84.5	68.7	74.7	98.8	28.2	26.36
T_{90}			65.0	92.8	89.8	85.5	93.0	65.1	74.5	97	67.5	77.1	85.0	73.5	70.2	99.0	29.0	32.0
T_{120}			67.0	93.8	75.0	89.8	95.0	45.1	65.0	97	66.9	77.1	86.0	50.0	50.0	99.5	20.0	20.0

Key

Fvii = F1**
Fx= F2**
Fxvi= F3**