

**CARDIO PROTECTIVE ACTIVITIES OF N-HEXANE EXTRACT
OF *DESMODIUM VELUTINUM* STEM ON ALBINO WISTER RAT**

BY

**NWANKWO ONYEKACHI OKWUN
BC/2009/281**

**SUBMITTED TO THE
DEPARTMENT OF BIOCHEMISTRY
FACULTY OF NATURAL SCIENCES.
CARITAS UNIVERSITY, AMORJI-NIKE ENUGU
ENUGU STATE**

AUGUST, 2013

TITLE PAGE

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A PROJECT RESEARCH

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**A RESEARCH PROJECT PRESENTED IN PARTIAL FULFILMENT
OF THE REQUIREMENT FOR THE AWARD OF BACHELOR
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AUGUST, 2013

CERTIFICATION

This is to certify that this report titled Cardioprotective activity of N-hexane extract of *Desmodium Velutinum* stem on Albino Wistar Rat submitted by Nwankwo Onyekachi Okwun BC/2009/281 is a borne-fide record of the project work carried out by her under my supervision.

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.....
Mr. Ezenwali Moses
Head of Department

Date.....

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External supervisor

Date:

DEDICATION

This project is dedicated to God almighty for giving me the strength and wisdom, knowledge and understanding to accomplish this great work.

ACKNOWLEDGEMENT

It is my profound gratitude to give thanks to the almighty God for his grace and gratitude throughout my project research and also for giving me the wisdom and intellect which has helped me through my stay in CARITAS UNIVERSITY.

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ABSTRACT

This study evaluates the cardio protective effect of N-hexane extract of *Desmodium Velutinum* stem on albino wister rat. Rats used in this study were grouped into four and each group was fed differently. Rats in group one, three, and four were fed with the aid of a syringe without needle with 6ml of the lipoprotein food mixture containing 3.6g of the cow's brain twice a day for seven days. Rats on group two was fed with only grower's mash and water for seven days; rats in group three was later administered orally 2ml of dissolved vasoprin drug once in a day for 3 days. Rats in group four were also administered orally 0.5ml of the liquid drug extract of *Desmodium Velutinum* stem once a day for 3 days. During these three (3) days, the rats were given grower's mash and water. After the feeding period, the rats fed with the cow's brain showed a significant increase in the activities of marker enzymes such as creatine kinase (CK), creatine kinase MB (CKMB), Aspartate transaminase (AST), Alanine transaminase (ALT), and lactate dehydrogenase. This study shows that N-hexane extract of *Desmodium Velutinum* stem possesses a cardio protective effect on albino wister rats than the known drug (vasoprin) if only one can increase the dosage of the extract for a period of time.

CHAPTER ONE

1.0 INTRODUCTION

The heart is a hollow muscular, cone-shaped organ, lying between the lungs in a block of tissue called the mediastinum (Khader, 2004). The heart begins beating some few weeks following conception and beats throughout life (Khader, 2004). It is also the strongest muscle in the human body and functions to maintain a constant circulation of blood throughout the body, it also acts as a pump and its action is composed of a series of events known as the cardiac cycle. The heart is composed of three layers of tissue which are; the pericardium, the myocardium and the Endocardium. The heart acts as a pump which drives blood into and through the arteries, but the right and the left side of the heart functions separately from one another some of the diseases associated with the cardiovascular system includes coronary heart diseases (CHD) which is the most common of heart diseases and it occurs when the arteries supplying blood to the heart narrow or harden from the build-up of plaque (Gina, 1996.), Ischemic heart disease, cerebrovascular disease (CeVD), peripheral vascular disease, Heart Failure etc and some of the pharmacological treatment includes Angiotensin commonly prescribes includes Benazepril, Captopril, Moexipril, Beta Blockers which includes, Acebutolol, Betaxolol, etc.

The heart marker enzymes includes creatine kinase(CK-MB)which is the enzyme used as the definitive serum marker for the diagnosis or exclusion of acute myocardial infarction (Andreas,2009).

Troponin 1 Rapid test which is a lateral flow chromatographic immunoassay for the qualitative detection of cardiac Troponin 1(CTn1)and its complex in human serum or plasma at the level equal or higher than 1ng/ml. Myoglobin which is also designed for qualitative determination of myoglobin in human whole blood serum or plasma as an aid in the diagnosis of myocardial infarction (MI) and myoglobin is also a low molecular weight cytoplasm protein which is released into the blood stream when muscle cells are damaged and this protein is released into the blood stream more rapidly than any other myocardial marker and elevated levels can be detected as early as one (1) hour after the onset of AMI(Penttila,2002). *Demodium velutinum* has been suggested to have some therapeutic effect in the treatment of cardiovascular disorder and in the reducing of high cholesterol content in the body. *Demodium Velutinum* is one of the few shrubs species that has been identified as a well adapted to acid tropical soil and a good nutritive value(Schultze-Kraft, 2002).Every culture has relied on the variety of natural medicines found in healing plants for the therapeutic properties

(Armstrong,2004).Researchers find that food and their individual constituents perform similar fashion to modern drugs and sometimes better without the dreaded side effect. The leaves and young stems are rich in crude protein and mineral elements and can provide higher levels of some nutrient (Bakker, 1994). Natural plants have been valuable sources of mineral agent with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents(Guyton, 2002).Hence potentially useful drugs can often be recognized from their relative importance and uses in folk medicine. Extract of *Desmodium velutinum* stem are used traditionally in some diseases condition particularly headache and may be a source of a pharmacological active agent useful in the treatment of aches and pain(Anowi,2012). This work aimed at investigating any hypocholesterolemic activity in the N-hexane extract of *Desmodium Velutinum* stem.

CHAPTER TWO

LITERATURE REVIEW

2.0 The Heart

The human heart is the size of fist. However, it works relentlessly from before birth to death. The heart being beating by 21 to 28 days after conception and beats throughout life. It is also the strongest muscle in the human (Anaya 1996).The average heart beats about 100,000 times over a seventy year lifetime. With each beat, the heart pumps blood through the blood vessels or arteries to all parts of the body. It beats approximately 70 to 80 times a minute; this rate can double during exercise or at time of emotion.

General structure of the heart

The heart is composed of the three layers of tissues

- The pericardium;

This is the outer part that covers the heart and consist of two layers or sacs- the outer layer or fibrous pericardium is securely attached to the diaphragm, the outer coat of the great vessels and the posterior surface of the sternum and therefore maintains the heart in its position. Its fibrous nature prevents over distension of the heart. The inner layer the serous pericardium consist of two layers the outer or parietal layer lines the outer fibrous sac and the inner or visceral layer covers the heart muscle.

The serous membrane secretes serous fluid into the space between the visceral and parental layers that allows smooth movement between the layers, when the heart beats.

- Myocardium;

This is the middle layer of the heart and is composed of specialized muscle tissue called the cardiac muscle, on which the circulation of blood depends. It varies in thickness, being thickest in the left ventricle, thinner in the right ventricle and thinnest in the atrium.

- Endocardium

The inner lining of the heart is thin, smooth, glistening membrane consisting of flattened epithelial cells which is continuous with the valves and with the lining of the blood vessels.

Interior of the heart.

The heart is divided into a right and left side by a muscular partition called as the septum. The two sides of the heart have no communication with each other. Each side is sub-divided into an upper and lower chamber, the upper chamber on each side is called the auricle or atrium and is the receiving chamber on each side is called the ventricle and is the discharging chamber from which the blood is driven into the arteries.

Each atrium communicates with the ventricles below it on the same side of the heart through an opening, guarded by a valve called atrio-ventricular valve. The valve separating the right atrium from the right ventricle is known as the right atrio-ventricular valve (tricuspid valve) and is made up of three flaps or cusps. Similarly, the valve separating the left atrium from the left ventricle is called left atrio-ventricular valve (mitral Valve) and is composed of two flaps of cusps.

Functions of the heart

The main function of heart is to maintain a constant circulation of blood throughout the body and also to act as a pump and its action is to compose of a series of events known as the cardiac cycle.

Cardiovascular system of the heart

The blood from the heart travels from the left side of the heart and is rich in oxygen. It travels via arteries of ever-decreasing size till it reaches the narrowest of arteries called capillaries in all the organs and parts of the body and having delivered its oxygen and nutrients and having collected waste products, blood is brought back to the right side of the heart through a system of progressively enlarging vein called circulating system or cardiovascular system. Cardiovascular system literally means cardio or heart

and vascular or a system or network of blood vessels (American heart Association, 2009).

2.0.1 Cardiovascular Disorder

Cardiovascular disorders (CVD);this includes dysfunctional conditions of the heart, arteries ,and veins that supply oxygen to vital life-sustaining areas of the brain, the heart itself, and other vital organs. if oxygen doesn't arrive the tissue or organ will die. Ischemic heart disorder is the technical term for obstruction of blood flow to the heart. In general this results because excess fat or plaque deposits are narrowing the veins that supply oxygenated blood to the heart(Matyal,2008). Excess buildup of fat or plaque is respectively term arteriosclerosis and atherosclerosis.

2.0.2 Diseases associated with cardiovascular system are.

- **Coronary Heart Diseases (CHD)** is the most common form of heart disease. It occurs when the arteries supplying blood to the heart narrow or harden from the build-up of plaque. Plaque is made up of fat, cholesterol and other substances founding the blood(WU,2002). This plaque build-up is known as atherosclerosis and the site of the plaque determines the type of heart disorder such as.

- **Coronary artery disease;** this is the build-up of plaque in the arteries supplying blood to the heart.
- **Peripheral artery disease,** This is the build- up of plaque in the arteries supplying blood to the arms and legs.
- **Carotid artery diseases;** This is the build-up of plaque in the arteries that supply blood to the brain.

Coronary heart disease can be caused due to risk factors like high blood pressure, high blood cholesterol, tobacco use, obesity, unhealthy diet, physical inactivity, diabetes advancing age (Nissen, 2005).

- **Ischemic heart disease.** It refers to problems with the circulation of blood to the heart muscle (Armstrong, 2004). A partial blockage of one or more of the coronary arteries can result in a lack of enough oxygenated blood(ischemia)and the symptoms includes;
 - Angina (chest pain) and dyspnea(shortness of breath)
 - A complete blockage of an artery causes necrosis (damage to the tissues) or a myocardial infarction commonly known as heart attack.
- **Peripheral vascular disease.** It affects the circulation primarily in the legs, patients with this diseases typically complain of pain in their calves especially when walking. Peripheral heart diseases occur when fat and

cholesterol deposit or plaque buildup in the peripheral arteries which are the blood vessels outside the heart. This build up narrows the artery walls, restricting the amount of blood flows to the body's tissues. Depending on the arteries, where the blockage occurs, this can lead to stroke, heart attack .etc.

- **Heart failure;** This occurs when the pumping action of the heart cannot provide enough blood to the rest of the body as it is needed and it can be as a result of damage to the heart muscle for example for a heart attack or from excessive consumption of alcohol or because of a heart muscle disease also called cardiomyopathy and the symptom includes shortness of breath and swelling of the legs.
- Rheumatic heart disorder; this diseases begins with a bacterial infection in childhood, affecting joints and heart valves. Then the heart problems appear many years later. Often the valves have to be replaced by an operation.

2.0.3 Some of the Risk Factors for Cardiovascular Disorder

The most important behavioral risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol (Gastaldelli, 2010). Behavioral risk factors are responsible for about 80% of

coronary heart disease and cerebrovascular disease. The effects of unhealthy diet and physical inactivity may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids and overweight and obesity. These intermediate risks factors can be measured in primary care facilities and indicate an increases risk of developing a heart attack, stroke, heart failure and other complications.

Cessation of tobacco use, reduction of salt in the diet, consuming fruit and vegetables ,regular physical activity and avoiding harmful use of alcohol have been shown to reduce the risk of cardiovascular disease. The cardiovascular disorder can also be reduced by preventing or treating hypertension, diabetes and raised blood pressure

2.1 Causes of Cardiovascular Disorders:

- **Your age.** Men over 45 and women over 55 are more likely to develop heart disease than their younger counterparts. The American Heart Association (AHA) states that more than 83 percent of people who die of coronary heart disease are 65 or older. The older you get, the more likely you are to have damaged arteries and/or a weakened heart muscle. Most people have plaque buildup in the arteries by the time they reach their 70s.

- **Your sex.** Overall, more men have heart attacks than women do, and they experience them earlier in life, too. While a woman's risk of dying from heart disease increases after menopause, it's still lower than a man's.
- **Your family history.** If people in your family have heart disease—especially close or immediate relatives, your risk of developing it increases. If a parent or sibling developed heart disease at an early age (before age 55 for men, or before age 65 for women), your risk is even higher. Developing heart disease isn't necessarily in your DNA, however. Lifestyle habits (diet, exercise, smoking, drinking, etc.) tend to be passed down from generation to generation, which means that some portion of this risk is controllable.
- **Your race.** Somewhat related to family history, your race can also predetermine part of your risk of heart disease. African Americans, American Indians, Mexican Americans, and native Hawaiians are more likely to have heart disease than Caucasians, but this is partly due to other risk factors that these populations tend to experience, such as diabetes and high blood pressure.
- **Your body type.** Whether or not you become overweight or obese is mostly within your control, but you cannot control your weight distribution, which refers to where your body stores fat. For years,

experts warned that people who tend to carry excess weight in their belly area (known as "apple" shapes) are at a greater risk of several health problems, including heart disease, while "pear" shaped bodies that store more fat in the lower body don't have the same risk

- **Smoking:** Smoking is the leading preventable cause of heart disease and heart attack. People who smoke are 2-4 times more likely to develop heart disease than non-smokers, according to the AHA. Smoking damages the walls of your arteries, constricts blood vessels, and lowers your HDL (good) cholesterol levels (Gastaldelli,2010).
- **Your diet:** A diet that's high in saturated fat, trans sugar, sodium, added sugars, cholesterol can raise your cholesterol and blood pressure levels and increase your risk of heart disease. Some research shows that diets too high in animal-based foods (meat and high-fat dairy products) and too low in plant-based foods like whole grains, fruits, vegetables and nuts can lead to heart disease, too.
- **Activity level:** If you're inactive, you're almost twice as likely to develop heart disease as people who get moving on a regular basis, reports the National Heart, Lung and Blood Institute (NHLBI). Regular exercise naturally decreases the LDL (bad) cholesterol levels in your blood while increasing your HDL (good) cholesterol levels. It also lowers blood

pressure and helps with blood sugar control and exercise strengthens the heart and cardiovascular system so that it is more efficient

- **Weight:** The more excess body fat you have, the greater your risk of heart disease and heart attack—even if you have no other risk factors. Being overweight increases your blood LDL (bad) cholesterol and triglyceride levels, lowers HDL (good) cholesterol, and exacerbates other heart disease risks like diabetes and high blood pressure. Plus, carrying excess weight simply puts additional strain on the heart, forcing it to work harder. Calculating your body mass index (BMI) is one way to determine if you are overweight.
- **Stress:** Experts aren't sure why people with chronic stress have higher rates of heart disease, but they believe that stress (and the hormones it releases) may damage the arteries over time and make blood clots more likely to form. Just one stressful episode can elevate the heart rate and blood pressure for a short period, and even lead to a heart attack
- **Drinking habits:** People who drink moderately (defined as an average of one drink day for women and two drinks daily for men) have a lower risk of heart disease than nondrinkers. Too much alcohol can raise blood pressure and triglycerides, as well as contribute to obesity, irregular

heartbeat, cardiomyopathy, alcoholism, heart failure, cancer, stroke and other diseases.

- **High blood pressure (hypertension)**: Uncontrolled blood pressure can increase the workload of your heart, as well as harden and thicken the arteries, making it harder for blood to pass through. According to the AHA, high blood pressure coupled with other risk factors like obesity, smoking, high cholesterol or diabetes increases the risk of heart attack and stroke several times over. In many cases, high blood pressure can be controlled through lifestyle changes and medications.
- **High cholesterol**: As cholesterol levels rise, so does your risk for cardiovascular disease. High cholesterol (especially high levels of LDL can lead to artery blockage and damage, which contributes to heart disease and can lead to a heart attack.
- **Type 2 diabetes**: People who have type 2 diabetes are twice as likely to experience heart disease or stroke—even if it is well managed. 65% of people with diabetes die of some form of cardiovascular disease.

2.1.1 Symptoms Of Cardiovascular Disorder:

- Chest pain(angina)
- Shortness of breath

- Numbness
- Weakness or coldness in your legs or arms
- A fluttering in your chest
- A racing heartbeat
- A slow heartbeat
- Lightheadedness
- Dizziness
- Fainting or near fainting
- Swelling in the hands, ankles or feet
- Easily tiring during exercise or activity
- Fever and sudden severe headaches
- Dry or persistent cough
- Skin rashes or un usual spots
- Loss of vision
- Bluening of the lips
- Nausea
- Confusion, lack of balance or difficulty talking

2.2 Pharmacological Treatment of Cardiovascular Disorders

Vasoprin

Vasoprin (USAN), also known as acetylsalicylic acid is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication.

Vasoprin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstance binds platelet molecular together to repair damaged blood vessels. This is why vasoprin is used in a long-term, low dose to prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots. It has been established that low doses of vasoprin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue. Vasoprin, an anti thrombotic agent is widely used in the prophylaxis of angina and myocardial infarction.

The main undesirable side effect of vasoprin are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in high dose.

Beta-Blocker Drugs

Beta blockers are the most commonly used drugs in medicine. They have been proven useful in treating a host of medical condition such as congestive heart failure, cardiac arrhythmias especially atrial fibrillation, myocardial

infarction (heart attacks) hypertropic cardiomyopathy etc. These drugs block the effect of adrenaline on the cardiovascular system which results in a slowing of the heart rate and a reduction of stress on the heart and the arteries.

Cardio selective beta blocker side effect; the side effect of beta blockers are related mainly to their adrenaline blocking effects .side effect can often be managed by a careful choice of which beta blocker is selected(Herbert, 2004).the effects includes

- Worsening of symptoms in people with asthenia
- Worsening of symptom in people with peripheral artery disease
- Depression
- Fatigue.

2.3 Medicinal Plants

Medicinal plants are plants which have a recognizes medicinal use and also contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs(Abayomi, 2008) .They range from plants which are used in the production of mainstream pharmaceutical products to plant used in herbal medicine preparations(Andrew,2005). Medicinal plants can be growing in numerous setting all over the world.

2.4 Description of *Desmodium Velutinum*

Desmodium velutinum is an erect shrub, usually 100-300cm tall. Branches often dark red, young parts densely hairy, rootstock thickened; leaves 1-foliolate, sometimes mixed with 3-foliolate leaves, stipules narrowly triangular, 2-15mm long, leaflets very variable in size and shape, 4-20cm x 2.5-13cm, chartaceous to coriaceous, upper surface continuously appressed-pubescent, lower surface densely velutinous, lateral veins 8-10, extending to the margin, inflorescence terminal and auxiliary, racemose or paniculate, up to 20cm long, flowers in clusters of 2-5, calyx 4-lobed, densely hairy, corolla pink, purple, blue or reddish-violet, androecium diadelphous; pod (1-) 1.6-2.5cm x 2.2-3.5mm, (3-) 5-7-jointed, articles broadly ablong, densely covered with hairs, seeds very broadly or depressed ovate, 1.3-1.6mm x 1.8-2.5mm. *Desmodium velutinum* belongs to the family Fabaceae. The plant is called Ikeagwani and can be used to control of non-specific diarrhea.



Figure 1: *Desmodium Velutinum*-Typical Appearance.

2.4.1 Taxonomy of the Plant

Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Desmodium*

Species: *Desmodium velutinum*

Common names

Chitkiboota - India

Latkan - India

Local names

Ibo: Ikeagwuani

GEOGRAPHICAL DISTRIBUTION OF THE PLANT

Sub-tropical

Asia(China,Taiwan,India,Indonesia,Laos,Malaysia,Myanmar,Sir

Lanka,Thailand,Vietnam)and tropical Africa.

2.4.2 USES OF *DESMODIUM VELUTNUM*

1. The leaves are used for the control of non-specific diarrhea
- 2 . *Desmodium velutinum* is a source of pharmacological active gent useful in the treatment of aches and pains.
3. The water extract of the leaves is used as an aphrodisiac
4. Its potential use is cut-and –carry or permanently grazed system.

2.5 Lipid Metabolism

Lipid has many roles in biological systems. These roles are a consequence of their chemical and physical properties. Fatty acids and their derivatives (especially triacylglycerols) can act as highly concentrated energy storage molecules. The high energy density (the relatively large amount of energy released per unit of mass) of fat stores is due to three main factors;

- The completely reduced carbon of fatty acids have a higher energy content than the partially oxidized carbons of carbohydrates and proteins.
- The fortuitous fact that the reduced carbons have covalent bonds light atoms (hydrogen rather than to the reduced oxygen) means that the fully reduced hydrocarbon compounds are lighter than the partially oxidized carbohydrate.
- Lipids are hydrophobic molecules and therefore fat stores contain little water, which would add to the weight of the molecules without adding to the energy content.

2.5.1 Lipoprotein Metabolism in the Heart

Lipoproteins are macromolecular aggregates of lipids and proteins that function to transport insoluble lipid molecules through the plasma. Triglycerides, consist of three fatty acids esterified to a glycerol molecule, which are insoluble in water (Small, 1986). Triglyceride are either absorbed from, the diet following a meal or assembled by the liver. Lipoprotein transport triglyceride to muscles, which utilize the fatty acid as a key source of energy. Cholesterol is a critical regulator of membrane structure and function. Its concentration in membrane preserves bilayer fluidity and governs the formation of microdomains. Cholesterol is also the substrate for

bile salt and steroid hormone biosynthesis(Agellon,2002)oxidized cholesterol molecules(oxysterols) serves as ligands for nuclear hormone receptors which regulate cellular lipid metabolism(chawla,2001).

2.5.1 Classification of Lipoprotein

Lipoprotein may be classified as follows, listed from larger and less dense to smaller and denser .they are classified on the basis of electrophoresis and ultracentrifugation. chylomicrons are primarily that triglyceride –bearing lipoproteins produced after a meal during the process of lipid absorption.(livitt,2006)

- Very low density lipoprotein(VLDL) are produced by the liver with a primary function of supplying free fatty acids to tissues and are normally the predominant carriers of circulating triglycerol (Pentikainen,2004).
- Low density lipoprotein (LDL)are by products of very low density lipoprotein metabolism and in the normal state are the primary carriers of plasma cholesterol chylomicrons, very low density lipoprotein and low density lipoprotein all carry apoB, among other apolipoproteins (Hussain,2003).
- High density lipoprotein (HDL) particles are produced by the liver and intestine and then mature and become enriched with other

apolipoproteins and lipids by exchanging chylomicrons and very low density lipoproteins.(Silver,2000).

- Intermediate density lipoprotein (LDL) is intermediate between very low density lipoprotein and low density lipoprotein. They are not usually detectable in the blood.

Functions of lipoprotein

The function of lipoprotein particle is to transport lipids(fat) such as triacylglycerol)around the body in the blood.

Functions of Cholesterol

- It builds and maintains cell membranes(outer layer),it prevents crystallization of hydrocarbons in the membrane.
- It is essential for determining which molecules can pass into the cell and which cannot (cell membrane permeability).
- It is involved in the production of sex hormones(androgen and estrogens).
- It is involved in the production of hormones released by the adrenal glands (cortisol,corticosterone,aldosterone,)
- It is important for the metabolism of fat soluble vitamins, including vitamins A,D,E and K.

2.5.2 Danger of High Cholesterol Levels.

High cholesterol levels can cause

- Atherosclerosis- narrowing of the arteries.
- Higher coronary heart disease risk- an abnormality of the arteries that supply blood and oxygen to the heart.

Heart attack-occurs when the supply of blood and oxygen to an area of heart muscle is

blocked, usually by a clot in a coronary artery. This causes your heart muscle to die

- Angina-chest pain or discomfort that occurs when your heart muscle does not get enough blood.
- Cardiovascular conditions diseases of the heart and blood vessels.
- Stroke and mini-stroke-occurs when a blood clot blocks an artery or vein, interrupting the flow to an area of the brain. Can also occur when a blood vessel breaks, brain cells begin to die.

2.6 Phytochemical

This refers to a wide variety of compounds made by plants but is mainly used to describe those compounds that may affect human health.

Phytochemicals are found in plant based foods such fruits, vegetables, beans

and grains. Some of the better known phytochemicals includes better carotene and other carotenoids, ascorbic acid (vitamin c) folic acid and vitamin E some phytochemicals have either antioxidant or hormone like actions and they are promoted for the prevention and treatment of many health conditions ,including cancer, heart diseases and high blood pressure.

Several major groups of phytochemicals includes;

- The polyphenols which includes a large sub group of chemicals called Flavonoids and they are plant chemicals found in a broad range of fruit, grains and vegetables. Other polyphenols(including some flavonoids) act as anti-oxidants. These are thought to rid the body of harmful molecules known as free radicals which can damage a cell's DNA .
- Ally Sulfides such are found in garlic and onions these compounds may stimulate enzymes that help the body get rid of harmful chemical they may also help strengthen the immune system.
- Hormonal action; Iso flavones, found in soy , imitate human estrogens and helps to reduce menopausal symptom and osteoporosis.

2.7 Heart Maker Enzymes

Cardiac marker is biomarker measured to evaluated heart function. They are often discussed in the context of myocardial infarction, but other conditions

can lead to an evolution in cardiac level and cardiac marker tests also identify blood chemicals associated with myocardial infarction (MI), commonly known as a heart attack. The myocardium is the middle layer of the heart wall composed of heart muscle. Infarction is tissue death caused by an interruption in the blood supply to an area.

2.7.1 Types of cardiac marker includes

Troponins.

This is a protein complex located on the thin filament of striated muscles consisting of the three subunits namely, Troponin T(TnT), Troponin I(TnI), and Troponin C(TnC) each having different structure and function. Of the three Troponins, Troponin T and Troponin I are being used as the biochemical marker for the diagnosis of myocardial injury(Ikeda, 2002). The troponins found in cardiac tissue (cTn) have a different amino acid sequence than that present in troponin of skeletal muscles. This makes Troponin T and Troponin I more specific for the diagnosis of myocardial injury(Kost,2002). These cardiac troponins(cTns) appear in the blood as early as 3-4 hours of the acute episode and remain elevated for 4-14 days. The pattern of release kinetics is related to the distribution of these proteins within the myocardial cell. About 94-97% of these troponins is bound to myofibril and only 3% of Troponin I and 6% of Troponin T is free in the cytoplasm (Apple, 2003).

When the myocardial damage occurs the cytosolic troponins reach the blood stream quickly resulting in a rapid peak of serum troponin observed during the first few hours which is followed by the release of structurally bound troponin resulting in a second peak lasting for several days. Troponin T and troponin I are now regarded as the most specific biochemical markers of myocardial injury (Mohler, 2008).

Troponin C, I, and T are proteins that form the thin filaments of muscle fibers and regulate the movements of contractile proteins in muscle tissue. Skeletal and cardiac forms are structurally distinct, and antibodies can be produced that react only with the cardiac forms of troponin I and troponin T (Nageh, 2007). Troponin C, I and T are proteins that form the thin filaments of muscle fibers and regulate the movement of contractile proteins in muscle tissue. Skeletal and cardiac forms are structurally distinct, and antibodies can be produced that react only with the cardiac forms of troponin I and T. Cardiac troponin are specific to heart muscle. They have enabled the development of assays (test) that can detect heart muscle injury with great sensitivity and specificity.

Myoglobin

Myoglobin is a protein found in both skeletal and myocardial muscle. It is released rapidly after tissue injury and may be elevated as early as one hour

after myocardial injury, through it may also be elevated due to skeletal muscle trauma(Hetland,2004) .However, if myoglobin values do not rise within three to four hours after a person show acute symptoms, it is highly unlikely that he or she had ML. There are several measurement methods available.

Myoglobin, a 18KD cytosolic protein, appears in the blood earlier after myocardial injury than any other marker available so fast(Chan,2004) .The detectable levels of myoglobin in the blood are found as early as 2 to 3 hours after the onset. Its peak values is obtained at 6-12 hours after the onset of symptoms and then it normalizes over the next 24hours.However,it is not cardiac specific as its release from the skeletal muscle cannot be distinguished from that released due to cardiac injury (Christenson,2002).Several studies have compared the diagnostic utility of serum myoglobin with other marker like CKMB,CKMB mass, CKMB isofoms and cardiac Troponins but the results have been controversial (Pentilla,2002). The high negative predictive value of serum myoglobin for excluding early infarction has encouraged its use along with more specific markers such as CKMB and cardiac troponin and this two- marker approach has improved the diagnosis of MI(Pantighini,2003).

Creatin Kinase (CK-MB);

Creatin Kinase resides in the cytosol and facilitates movement of high energy phosphates into and out of mitochondria. It is distributed in a large number of tissue even in the skeletal muscle .Since it has a short duration, it cannot be used for late diagnosis of acute MI but can be used to suggest infarct extension if levels rise again and is usually back to normal within 2-3 days(Hetland,2004). It is relatively specific when skeletal muscle damage is not present.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase catalyses the conversion of pyruvate to lactate .Lactate dehydrogenase 1 (LDH-1) isozyme is normally found in the heart muscle and lactate dehydrogenase -2(LDH-2) is found predominately in blood serum.A high LDH-1 level to LDH-2 suggest MI.LDH levels are also high in tissue breakdown or hemolysis.it can mean caner,meningitis,encephalitis or HIV this is usually back to normal 10-14 days.LDH is not as specific as troponin.

Aspartate transaminase

This was the first used (Nissen, 1964). It is not specific for heart damage, and is also one of the liver function tests.

Glycogen phosphorylase isoenzyme BB (GPBB)

This is an isoenzyme of glycogen phosphorylase. Glycogen phosphorylases exist in three isoforms. One of these isoforms is GP-BB. This isoform exists in the heart and the brain tissue because of the blood-brain barrier GP-BB can be seen as heart muscle specific. During the process of ischemia, GP-BB is converted into a soluble form and is released into the blood. This isoform of the enzyme exists in cardiac (heart) and brain tissue. GP-BB is one of the new cardiac markers which are discussed to improve early diagnosis in acute coronary syndrome. A rapid rise in blood levels can be seen in myocardial infarction and unstable angina. GP-BB elevated 1-3 hours after process of ischemia.

Pro-brain natriuretic peptide

This is increased in patients with heart failure. It has been approved as a marker for acute congestive heart failure. Pt with <80 have a much higher rate of symptom free survival within a year. Generally, pt with CHF will have >100 .

CHAPTER 3

MATERIALS AND METHOD

3.0 Materials and chemicals:

The chemicals used during extraction, phytochemical analysis and experimental animals model are

Chloroform (biolab)

Ethanol

Lead Acetate

Methanol

Petroleum ether

Ethyl acetate

Ammonia sloke

Phospomolybdic acid reagent

Hydrochloric acid (sigma alorich Supplies)

Distilled water

Ammonia slake

Alkaline copper reagent

Potassium Ferricyanide

Picric acid

Vasoprine

Color reagent

Alkaline picrate solution

Equipments

The materials used during identification and extraction, phytochemical analysis and experimental animals model are

Soxhlet apparatus (Hesol)

Water bath (Griffen)

Nose marks (Jinxiany)

Weighing balance (camry, China)

Cage (Locally made)

Hand gloves (Jinxiang)

Centrifuge (Haracus Christ)

Electric grinder (Moulinex, 2000 France)

Spectrophotometer (Pee, Medical USA)

Refrigerator (Thermocool)

The Glass Wares includes:

Beaker (Pyrex)

Pipette (Pyrex)

Conical Flask (Pyrex)

Measuring Cylinder (Pyrex)

Test tube (Pyrex)

3.1 Identification and Extraction of Plant Material

Healthy fresh stems of *Desmodium velutinum* were harvested from Prof. J.C. Okafor's garden at Independence Layout, Enugu State in the month of February, 2013. The plants were also authenticated by him.

The stem was dried at room temperature for twenty-one days. The dried stems were ground into fine powder using a clean dry electric grinder (moulinex, optilend 2000, made in France). A 150g portion of the ground stem was soaked in 150ml of distilled water for 12 hours filtered and then extracted with double-distilled by hot continuous percolation method on a soxhlet apparatus.

The solid dry extract was weighed using Weighing balance and the weight was 15.0g, the extract was placed in a sterile container, labeled and stored in a refrigerator greater at 40⁰C. The 15.0g was later divided into two containers. The first container containing 4.0g was used for experimental animal model while the remaining 11.0g was used for phytochemical analysis

3.2 Hot Continuous Percolation Method:

Hot continuous percolation method is a method uses when active constituent of herbs are not freely soluble in a solvent, then it becomes necessary to

extract the crude by the action of hot menstruum for a considerable length of time. The fixed oils from seed and alkaloids from the herb are extracted continuous hot percolation process using benzene, chloroform and petroleum ether and is also one of the extraction method used to determine the fat content by continuous extraction of a food with the non polar organic solvent such as petroleum ether for about one (1) hour or more in a soxhlet apparatus.

3.3 Phytochemical Analysis

Evans(1996).The phytochemical tests done and their procedures are:

Determination of Terenoid

About one (1)gram of the sample was weighed, macerated with 50mls of ethanol and filtered. About 2.5 mls of the filtrate was pipette, and added in 2.5mls of 5% aqueous phosphomolybdic acid solution and 2.5mls concentrated H_2SO_4 gradually mixed and allowed to stand for 30munites.Made-up to12.5mls with ethanol and absorbance measured at 700nM

Determination of Glycoside

About one (1) gram of the sample was weighed and 2.5ml of 15% lead acetate was added and filtered. Then 2.5mls of chloroform was added and Shaked vigorously the lower layer and evaporate to dryness. After that,3mls

of glacial acetic acid, 0.1ml of 5% ferric chloride and 0.25ml concentrated H_2SO_4 was added and Shaked. Put it in the dark for 2hours and absorbance measured at 530nM

Determination of Steroid

About one (1) gram of the sample was weighed, macerated with 20ml of ethanol and filtered. About 2mls of the filtrate was pipette and 2mls of colour reagent was added in the filtrate and stand for 30minutes. Absorbance was measured at 550nM

Determination of Saponin

About one (1) gram of the sample was weighed, macerated with 10mls of petroleum ether and decanted into a beaker. Another 10mls of petroleum ether was added and decants into a beaker. The filtrates were combined and evaporated to dryness. Then, 6mls of ethanol was added in the filtrate together with 2mls of colour reagent pipette. Another 2mls of colour reagent was added and stand for 30minutes. Absorbance was measured at 550nM.

Determination of Flavonid

About one (1) gram of the sample weighed, macerated with 20mls of ethyl acetate and filtered.

About 5mls of the filtrate was pipette and 5mls of dilute ammonia slake was added in it. The upper layer was collected and absorbance measured at 490nm

Determination of Reducing Sugar

About one(I) gram of the sample was weighed, macerated with 20mls of distilled water and filtered. About 1ml of the filtrate was pipette and added in 1ml of alkaline copper reagent,boiled for 5minutes.Another 1ml of phosphomolybdic acid reagent and 7mls of distilled water was added and absorbance was at 420nm.

Determination of Alkaloid

About one (I) gram of the sample was weighed, macerated with 20mls of 20% H_2SO_4 in ethanol (1:1) and filtered. About 1ml of the filtrate was pipette and added in 5mls 60% H_2SO_4 and 5mls of 0.5% formaldehyde in 60% H_2SO_4 , mixed and allowed to stand for 3hours.Absorbance was measured at 567nm.

Determination of Cyanide

About one (1) gram of the sample was weighed, macerated with 50mls of distilled water and allowed to stand for 24hours and filtered. About 1ml of the filtrate was pipette and added in 4mls of alkaline picrate solution, boiled for 5minutes and allowed to cool. Absorbance was measured at 49nm.

3.4 Experimental Animal model

Twelve healthy male albino wister rats were obtained from University of Nigeria Nsukka, Enugu State and the rat were weighed using weighing balance. The rats were distributed into four (4) groups 1-1V consisting of 3 rats each. The rats were housed separately and fed with water and grower's mash (guinea feed Nigeria) and allow to acclimatize for 3 days.

A high lipoprotein food (cow's brain) was gotten from the local market and 300g was weighed out using weighing balance. The 300g was dissolved in 500ml of distilled water forming a semi-solid mixture, a known cardiovascular drug; with generic name aspirin 25mg and brand name vasoprin was prepared by dissolving 12.5mg (half of one tablet) in 2ml of distilled water. The N-hexane extract of the *Desmodium velutinum* leaves weighing 4.0g was dissolved in 8ml of distilled water forming a liquid drug extract.

Rats in group i, iii, iv were fed with the aid of a syringe without needle with 6ml of the lipoprotein food mixture containing 3.6g of the cow's brain twice a day for seven days.

Rats in group ii was fed with only grower's mash and water for seven days

Rats in group iii was later administered orally 2ml of dissolved vasoprin drug once in a day for 3 days.

Rats in group iv were also administered orally 0.5ml of the liquid drug extract of demodium velutinum stem once a day for 3 days. During these three (3) days the rats were given grower's mash and water

3.5 Collection of Blood Sample

The blood samples were collected by dissecting the rats, followed by a cardiac puncture after a mild anesthesia with chloroform. About 6-9mls of blood samples were collected in EDTA tube from each group using a medical syringe. Serum was separated from the blood after clotting by centrifugation and used for heart marker enzyme test.

Blood samples were collected from the rats in group i and ii on the following day after the 7th day of orally feeding the rats with lipoprotein food mixture and normal feed (Grower's mash and water) respectively.

Blood samples were collected from the rats in group iii and iv on the following day after the 3rd day of orally administering a known drug vasoprin and the liquid drug extract respectively

3.6 Enzyme profile Analysis

In the enzyme profile analysis, the following test was conducted: Creatin Kinase(CK-MB),Troponin and Myoglobin.

Test for Troponin

Materials/Reagents involved

Test Kit that contains 30 test devices,

Plastic dropper,

Positive control,

Negative control

Clock or Timer

Assay procedure

Specimen and test components were brought to room temperature, if refrigerated or frozen and mixed thoroughly the test device was placed on a clean flat surface and the plastic dropper filled with the specimen. On holding the dropper vertically, 2-3 drops (about 60-90 μ l) of specimen was added into the sample well making sure that there are no air bubbles. The timer was set and results read in 10 minutes. Open the pouch at the notch and remove device, place the test device on a clean flat surface.

Test for Creatine Kinase (CK-MB)

Materials and Reagents involved

Antibody- coated microtiter plate with 96 wells,

Liquid creatine Kinase (CK-MB) standards.

Enzyme conjugate reagent,

TMB reagent

Stop solution

Precision pipette

Vortex mixer

Absorbent paper

Graph paper

Micro fiter plate reader

Procedure

Desired number of coated wells was secured in the holder and 20mls of standard, specimens was dispensed and controls into appropriate wells. Another 200mls of enzyme conjugate reagent was dispensed to each well and mixed thoroughly for 30 seconds. Incubate at room temperature (18-25⁰C) for 60munites. The incubation mixture by emptying plate into a waste container rinsed and emptied the microfiter wells 5 times with distilled water. The wells were strike onto sharply onto absorbent paper or paper towels to remove all residual water droplets. 100ul of TMB reagent was added to each well mixed gently for 5 seconds and incubate at room temperature for 20 minutes. Then, 100ul of stop solution was added to each well, mixed, gently for 30 seconds for the reaction to stop. Optical density was read at 450nM with a microfiter plate reader.

Test for Myoglobin

Material/Reagents involved

Test cards

Plastic dropper

Package insert

Timer/clock

Pipette

Procedure

The test device, whole blood, serum or plasma was allowed to equilibrate to room temperature (15-30⁰C) prior to testing. The test devices on a clean and level surface and the plastic dropper filled with the specimen. On holding the dropper vertically, two (2) drops of specimen was transferred to the specimen well in the test device, and waited for 1-5 minutes until the plasma appears in the test window. The result was read within 10minutes.

CHAPTER FOUR

4.0 RESULTS

The statistical analysis was done using the statistical package for social science

4.1 Quantitative Analysis. Table 1: phytochemical Composition of sample

Sample code	Soluble CHO	Cyanide	Reducing sugar	Saponin	Tannin	Flavonoid	Alkaloid	Steroid	Terpenoids
E	1.49±0.003 ^c	0.58±0.020 ^b	373.682±0.006 ^e	1.43±0.004 ^e	3.31±0.007 ^e	3.35±0.004 ^a	3.25±0.003 ^e	0.55±0.007 ^d	0.39±0.003 ^e

(Mg/100g)

(SPSS Data are means of triplicate determination ± standard deviation (SD) Data in the same column bearing different superscript differed significantly (P≤0.05)

Key

E=N-hexane extract of stem

4.2 Table 2: Qualitative Phytochemical

Sample code	E
Tanin	+++
Alkaliod	+++
Carbohydrate	+
Saponin	+
Steroid	+
Hydrogen cyanide	+
Flavonoid	++
Reducing sugar	++
Terpenoid	+

TABLE 3 HEART MAKER EMZYMES OF RATS FED WITH VARIOUS SAMPLES

SAMPLE CODE	Tropo ph in T	Troponin I	Ck-Total	Ck-MB	Myoglobin	Myosin	LD-1	AST
A1 COW'SBRAIN	70.00±2.83 ^a	90.00±1.41 ^a	75.00±1.41 ^a	93.00±1.41 ^a	70.00±1.41 ^a	60.00±2.83 ^a	85.00±1.41 ^a	75.00±2.83 ^a
A2 NORMAL FEED	60.00±0.00 ^b	75.00±1.41 ^b	70.00±2.83 ^b	85.00±0.00 ^b	60.00±1.41 ^b	55.00±1.41 ^b	70.00±0.00 ^b	65.00±0.00 ^b
B1 VASOPRIN	40.00±1.41 ^c	35.00±0.00 ^c	30.00±0.00 ^c	50.00±0.00 ^c	30.00±2.83 ^c	20.00±0.00 ^c	40.00±0.00 ^c	35.00±16.01 ^c
B2 EXTRACT	45.00±1.41 ^d	45.00±0.00 ^d	30.00±0.00 ^c	40.00±1.41 ^d	25.00±0.00 ^c	30.00±2.83 ^d	45.00±1.41 ^d	40.00±0.00 ^d

Data are means of triplicate determination ± standard deviation (SD)

Data in the same column bearing different superscript differed significantly (P≤0.05)

Data in the same column bearing different superscript differed significantly (P≥0.05)

Keys

A1- Rat fed with cow's brain(group 1 rats)

A2- Rats fed with Normal feed (group 2 rats)

B1- Rats fed with cow's brain + vasoprin (group 3 rats)

B2-Rats fed with cow's brain+ N-hexane extract of *Desmodium Velutinum* stem (group 4 rats)

CHAPTER FIVE

5.0 DISCUSSION

From table 2, the qualitative analysis showed the high presence of tannin, alkaloid, carbohydrate, flavonoid and reducing sugar. Also there were presence of saponin, steroid, hydrogen cyanide and terpernoid. The quantitative analysis of phytochemical in *Desmodium velutinum* stem showed that N-hexane extract of the stem contained (1.49±0.003) of carbohydrate and carbohydrate are one of the main type of nutrient and also the most important source of energy for the body.

N-hexane extract of the stem contained (0.58±0.20) of cyanide. Cyanide is dangerous to human system as it ceases aerobic respiration. However, its content in the N-hexane extract of *Desmodium Velutinum* stem is very small and poses no risk no human health.

Desmodium Velutinum stem contained (1.43±0.004) of saponin. Saponin is a good secondary metabolite that reduces Low Density Lipoprotein in the blood which thereby reduces artherosclerosis effect (Cormwell *et al*, 2004). The reduction in artherosclerosis aids cardio protective effect.

Tannin content was (3.31±0.007) in N-hexane extract of the stem. Tannins have been considered to have anti-nutritional activities but it is now known that their anti-nutritional properties depend upon their chemical structure

and dosage. They also accelerate blood clotting, reduce blood pressure and decrease the serum lipid level (Chung, 1998).

Flavonoid content was (3.35 ± 0.004) in N-hexane extract of the stem. Flavonoids are polyphenolic compounds which have an anti-oxidant activity that protects the body against damage in blood vessels, thus it decreases the risk of cardiovascular disorders. It also lowers blood pressure and cholesterol which reduces the risk of heart diseases (Maria, 2009).

N-hexane extract of *Desmodium Velutinum* stem contained (3.43 ± 0.006) of Alkaloid. Alkaloids are any class of naturally occurring nitrogen-containing bases (Andreas, 2009). It has so many medicinal values and when compared to the effect of it on the heart, it is an anti-hypertensive agent and also an anti-arrhythmia agent.

Terpenoids content was (0.39 ± 0.003) in N-hexane extract of the stem. Terpenoids are antioxidants, it is very friendly to the heart and can thereby be used as a cardiovascular drug. Terpenoids are a large and diverse class of naturally occurring organic chemicals (Mann, 2002).

From table 5.3 which is the heart marker enzyme of rats fed with various samples.

Rat fed with cow's brain (A1 rats) showed high lipoprotein content in biomarker. TroponinT (70.00 ± 2.83) , TroponinI (90.00 ± 1.41) , CK-Total

(75.00±1.41), CK-MB (93.00±1.41), Myoglobin (70.00±1.41), Myosin (60.00±2.83), LD-I (85.00±1.41) and AST (75.00±2.83), against those fed with normal feed, because cow's brain has no protective activity but it increases cardiovascular disease.

This leads to the proper investigation into the cardio protective effect of the extract and the known drug (Vasoprin).

Rat fed with normal feed (A2 rats) showed high lipoprotein in biomarker like TroponinT(60.00±0.00), TroponinI (75.00±1.41), CK-Total (70.00±2.82), CK-MB (85.00±0.00), Myoglobin (60.00±1.41), Myosin (55.00±1.41), LD-I (70.00±0.00), AST (65.00±0.00). Grower mash has no protective activity but increases the lipoprotein levels which causes cardiovascular diseases. Rats fed with Vasoprin (B1 rats) showed a decrease in lipoprotein of biomarker TroponinT(40.00±1.41), TroponinI(35.00±0,00), CK-Total (30.00±0.00), CK-MB (50.00±0.00), Myoglobin (30.00±2.83), Myosin (20.00±0.00), LD-I (40.00±0.00), AST (35.00±16.01). This showed that vasoprin has an effect in the treatment of cardiovascular diseases. Though, vasoprine cardioprotective activity was higher than that of the extract of N-hexane of *Desmodium velutinum* stem, the extract has also shown that it has a good cardio protective potential. However, if the dose and the duration of administration was increased

according to body weight, there is possibility of the extract having more cardioprotective potential might be seen.

5.0.1 CONCLUSION

This study in qualitative, quantitative and heart marker enzyme test showed that N-hexane extract of *Desmodim Velutinum* Stem extract has a potential cardio protective drug .

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