

**THE EFFECT OF ETHANOL EXTRACT OF *DESMODIUM VELUTINUM* STEM  
ON SOME MARKER ENZYME IN LIVER OF ALBINO WISTAR RATS**

**BY**

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**APPROVAL PAGE**

This is to prove that this research work on, the effect of ethanol extract of *Desmodium Velutinum* stem on liver function tests of albino wistar rats fed with high fat from cow's brain by Ubah Chukwuemeka .O BC/2009/278 has been read and approved as having satisfied the requirement for award of bachelors of science (B.Sc.) Degree in Biochemistry.

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## **DEDICATION**

I dedicated this work to almighty God my beloved parent and my Uncle Dr. Damian Ayichi.

## **ACKNOWLEDGEMENT**

I express immense gratitude to my parents, my uncle Dr. Damian Ayichi and my friends for the relentless supports they gave me throughout my stay in school.

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#### **Abstract**

Present investigations were carried out on the effect of ethanol extract of *Desmodium velutinum* stem on some liver enzymes of albino wistar rats fed with

high fat from cow's brain. Twelve healthy albino wistar rats were divided into four groups. Group I rats were fed with balanced diet (normal feed, that is grower's mash) and tap water. Group II rats were fed with fat diet (cow's brain) (3.6g/day) alone. Group III rats received combined mixture of 70% fat diet (cow's brain) with 30% ethanol extract of *Desmodium elutinum* stem (2.7g/day), Group IV rats were fed with fat diet (cow's brain) and were administered with a drug atorvastatin (5mg/rat/day). The rats were sacrificed at the end of the experimental (two weeks) period. The plant extract was screened for its phytochemical constituents, using standard procedures. Phytochemical screening revealed the presence of tannins, flavonoids, saponins and alkaloids. The liver function tests was assessed by measuring the total bilirubin, total serum protein, direct and indirect bilirubin and liver enzymes such as Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphate (ALP), Gamma-glutamyl transferase (GGT) on the liver of the albino wistar rat. The serum, ALT, ALP, AST and bilirubin concentration of the albino wistar rat increased significantly ( $P>0.05$ ) compared to their total protein concentration. The result obtained indicate that the ethanol extract of *Desmodium Velutinum* stem possess hepatoprotective activity on the fatty liver of albino wistar rat fed with fat from cow's brain. The studied plant showed variable amount of phytochemicals including flavonoid, alkaloid, saponin, tannins, terpenoid which were determined quantitatively using literature methods. The significant effect of the *Desmodium velutinum* stem ethanol extract was compared with standard drug, atorvastatin. The result in the present study suggest that the ethanol extract of *Desmodium velutinum* stem can be used in treating liver diseases.

## CHAPTER ONE

### INTRODUCTION

The liver is the largest and most complex internal organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions. Liver is involved in several vital functions such as metabolism, secretion and storage. Hepatitis or inflammatory disorder involves inflammation and change to the hepatocytes. Hepatitis is one of the most prevalent diseases in the world. Every year 18,000 people had been reported to die due to liver cirrhosis caused by viral hepatitis (Wang *et al.*, 2008). Ectopic fat storage occurs in obesity, particularly in the liver leading to a condition termed non alcoholic fatty liver disease (NAFLD) characterized by varying degree of liver injury that progresses from steatosis to tratrohepatitis, fibrosis and necrosis. Due to its prominent association with insulin resistance/obesity, NAFLD is regarded as the hepatic manifestation of metabolic syndrome. Liver has great capacity to detoxicate toxic substance and synthesizes useful principles. Therefore damage to liver inflicted by hepatotoxic agents is of grave consequences. Experimental studies have reported that animal fed a high fat diet (HFD) for more than two months develop weight, hyperlipidemic, hyperglycemia, oxidative stress and insulin resistance (IR). Besides, consumption of a calorie-rich diet results in lipid accumulation, excess production of inflammatory cytokines and macrophage infiltration that favours the

progression of liver disease. Many medicinal plant/indigenous plant have been mentioned and well established as hepatoprotective agents.

*Desmodium velutinum* is a medicinal plant around the world. The extract of *Desmodium velutinum* showed significant anti-pyretic activity on experimental rats used. *Desmodium velutinum* is very rich in alkaloids and related amino compounds. It is a source of flavonoid, saponins and pharmacological active agent useful in the treatment of aches and pains. It also showed that one of the uses is for erectile enhancing property. *Desmodium velutinum* being one of the important medicinal plant in the world may have effect on the liver. However limited studies are available on the effect of *Desmodium velutinum* treatment on hepatoprotective effect of its extract on high fat diet fed animal. In this regard, the present study was aimed to investigate the effect of ethanol extract of *Desmodium velutinum* stem on liver function tests on albino wistar rats fed with high fat from cow's brain related to different hepatoprotective effect by comparing it to a known drug, atorvastatin treatment which is used to lower the level of cholesterol in blood. Since changes in the concentration and composition of plasma lipid and lipoproteins occur frequently in liver disease, these findings may be useful in following the clinical course of patient with liver diseases of various causes.

Liver disease is associated with characteristic changes in the lipid composition of the surface coat of plasma lipoprotein particles. Cholesterol accumulate as hepatic

secretion of lecithin cholesterol acyltransferase decreases, such abnormal circulating lipoproteins tend to induce corresponding changes in cell membrane lipid composition. Studies in both human and experimental liver disease confirm that this does occur and that it is wide-spread. Low density lipoproteins, which are rich in cholesterol can also be taken up by the liver or extrahepatic tissues by a receptor mediated endocytosis that specially recognizes apolipoproteins. It can be predicted that extra hepatic membrane dysfunction might be a general feature of severe liver disease. It is proposed that many of the cellular disturbances and metabolic abnormalities accompanying hepatic disease result from, or are exacerbated by lipoprotein induced changes in membrane lipid composition and function. Hence *Desmodium velutinum* may be a source of a pharmacological active agent useful in the treatment of high level cholesterol in the blood which has got both liver protecting and nutritional value.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

The use of herbs and medicinal plants as the first medicine is a universal phenomenon. Every culture on earth, through written or oral traditions has relied on the vast variety of natural medicine found in healing plants for their therapeutic properties. All drugs of the past were substances with a particular therapeutic action extracted from plants. This, natural plants have been valuable sources of medicinal agent with proven potential of treating infectious disease and with lesser side effects compared to the synthetic drug agents. Hence potentially useful drugs can often be recognized from their relative importance and use in folk medicine. *Desmodium velutinum* has been reported in traditional medicine to have medicinal properties. Extracts of *Desmodium velutinum* are used traditionally in some disease, hence may be as sources of a pharmacological active agent in the treatment of aches and pains. Ethanolic extract of *Desmodium velutinum* possess antipyretic property

### 2.1 TAXONOMY OF PLANT (*DESMODIUM VELUTINUM*)

#### SCIENTIFIC CLASSIFICATION

Kingdom:            plantae

Division:           magnoliophyta

Class: magnolipsida  
 Order: fabales  
 Family: fabaceae  
 Genus: desmodium  
 Species *Desmodium velutiumm*

### COMMON NAMES

Chit kiboota - India  
 Latka - India

### LOCAL NAMES

IGBO: Ikeagwuani

It is called “Ikeagwuani” because it has erectile enhancing property.

Bioni sabota

Orila

### DESCRIPTION OF PLANT

*Desmodium velutinum* is a perennial, erect or semi-erect shrub or sub-shrub, up to 3m high. Branches often dark red, yellow- brown when young, velutinous and short hooked-hairy. Leaves 1- foliolate, rarely 3- foliolate, ovate, ovate- lanceolate, triangular-ovate, or broadly ovate, 4-20 cm long and 2.5-13cm wide, chartaceous

to coriaceous, upper surface continuously oppressed-pubescent, lower surface densely velutinous. Inflorescence often dense, terminal or auxiliary racemose or paniculate, 4-20cm long, with 2-5 flowers at each node, flowers purple to pink. Pods narrowly oblong, 1-2.5 cm long, 2-3 mm wide, with dense yellow straight hairs intermixed with short hooked hairs, 5-7 jointed. Seeds ovate, flat, 1.3-1.6mm x 1.8-2.5mm, yellow when ripe. Depending on the genotype, there are 320,000-830,000 seeds per kg. *Desmodium velutinum* belongs to the botanical family fabacea. It is a perennial, erect or semi-erect shrub up to 3m high. The plant is generally called “Ikeagwuani”, the leaves are used for the control of non-specific diarrhoea. About 30 grams of whole leaves may be boiled in about 150-200ml of water and 20-50ml of the extract taken depending on the severity of the diarrhoea. The dose may be taken once but not more than two doses may be taken in a day. Children may take lower doses. It is also claimed that the water extract of the leaves is used as an aphrodisiac.

## **GEOGRAPHICAL DISTRIBUTION OF THE PLANT**

Sub-tropical Asia (China, Taiwan, India, Indonesia, Laos, Malaysia, Myanmar, Sri-Lanka, Thailand, Vietnam) and tropical Africa.

### **Other Species of Desmodium Include**

*Desmodium lasiocarpum* (P. Beauu). DC

*Desmodium latifolium* (Roxb.ex ker Gawl) DC

*Hedysarum lasiocarpum* (P. Beauv)

*Hedysarum latifolium* Roxb. Ex ker Gawl

*Hedysarum velutinum* wild

*Meibomia lasiocarpa* (P. Beau.) kuntze

## **GROWTH AND DEVELOPMENT**

*Desmodium* appears to be predominantly self-pollinating. However, when flowers are touched, they spring open and release pollen which makes outcrossing possible.

## **ECOLOGY**

*Desmodium* is mainly found humid to sub-humid regions of the tropics and subtropics, on acid soils (pH <6.5). The usual habitats are open wood land and forest clearings. In equatorial regions, *desmodium* species are found from sea-level up to 3000m attitude. The photoperiod sensitivity varies with the specie.

## **PROPAGATION AND PLANTING**

Propagation of *desmodium* is by seed. The degree of scarification necessary for successful germination varies between species.

## DISEASES AND PESTS

*Desmodium* is affected by a range of diseases. Pathogens like the fungi *synchytrium desmodii* and *phanerochaeta salmonicolor*, causing wart and pink disease respectively, the root-knot nematodes *meloïdogyne arenaria*, *meloïdogyne hapla*, *meloïdogyne incognita* and *meloïdogyne javanica* and the stem gall nematode *pterotylendius cecidogenus*.

## USES OF DESMODIUM

*Desmodium* shows a broad range of traditional medicinal uses. In South-East Asia they are considered diuretic. It is used in the treatment of diarrhoea, dysentery and stomach-ache, wound, ulcers and other skin problems, stones in the gall bladder, kidney, headache and toothache. In Philippines, a decoction of *desmodium triflorum* is use as a month wash and as an expectorant and also used internally as a galactagogue. In Taiwan, the whole part is used against fever, rheumatism, jaundice and gonorrhoea. The boiled roots of *desmodium heterocappon* are used in Malaysia to poultice sore breast and a decoction of the plant is regarded as a tonic and a bechic. In Cambodia, the stem are applied to fractures and snaked bites. In India, the whole plant is used to treat stomach ache and abdominal problems. *Desmodium* contains a considerable number of species used as pasture and fodder crops, species used for ground cover and green manure, some of these have

medicinal applications as well. A decoction of the roots is used against rickets in children in Taiwan.

## **PROPERTIES**

*Desmodium* is very rich in alkaloids and related amino compounds, biosynthetically derived from different precursors. Compounds like hyypaphorine-N, N-dimethyl tryptamine; hordenine is known to increase the urinary flow, and to be a remedy for diarrhoea and dysentery. Others are isoflavonoids, desmodin and gangetin which is isolated from the roots of *Desmodium gangeticum* that adversely affect the fertility and reproductive system of male rats. It reduce the vaginal sperm count and enhance pre-implantation losses. It also cause a dose dependent impairment of fertility. Aqueous root extract shows mild diurectic action, relaxant effects on intestine muscles of rats and dogs and anti-bacterial, anti fungal and anti-inflammatory activity. The alkaloid fraction of stems and leaves has shown relaxant (curariform) effects on frog rectal muscles while the methanolic extract of Nigeria *Desmodium gangeticum* plants has in vitro anti leishmanial activity.

## 2.2 LIVER

The liver disease is associated with characteristics changes in the lipid composition. Liver which is the largest organ of the body is located between the portal and the general circulation, between the organs of the gastrointestinal tract and the heart. The main function of the liver is to take up nutrients, to store them, and to provide nutrients to the other organs. The liver is not only an important power and sewage treatment plant of the body but the best example for a cheap recycling system. The function of the liver as clearance organ, however, harbours the danger that the substances that should be degraded and/or eliminated lead to tissue damage. Thus, effective defense mechanisms are necessary. During the process of elimination there is chance of accumulation different kinds of toxic materials inside the hepatocytes and there is chance of liver infection, and hepatic disorders such as hepatitis. So an urgent need of developing a herbal medicine which has got both liver protecting and nutritional value is require hence at attempt has been made to screen the hepato protective activity of extract of *Desmodium velutinum* stem.

## 2.3 WHAT THE LIVER DOES

The liver is in the upper right part of the abdomen. The functions of the liver include storing glycogen (fuel for the body) which is made from sugars, helping to

process fats and proteins from digested food, making proteins that are essential for blood to clot (clotting factors); processing many medicines which you may take; helping to remove poisons and toxins from the body. The liver also makes bile. This is a greenish-yellow fluid that contains bile acids, bile pigments and waste products such as bilirubin; liver cells pass bile into bile ducts into larger and large ducts, eventually leading to the common bile duct. The gallbladder is like a ‘cul-de-sac’ reservoir of bile which comes off the common bile duct. After you eat, the gallbladder squeezes bile back into the common bile duct and down into the duodenum ( the first part of the gut after the stomach). Bile in the gut helps to digest fats.

#### **2.4 LIVER FUNCTION TESTS/MARKER ENZYMES**

The term “liver function test” is commonly used, that is applied to a variety of blood tests that assess the general state of the liver and biliary system. Routine blood test can be divided into those test that are simply makers of liver or biliary tract disease, such as the various liver enzymes. Liver function test measure various chemicals in the blood made by the liver. An abnormal results indicates a problem with the liver and may help to identify the cause. In addition, further test may be needed to clarify the cause of the liver problems to the usual liver test obtained on routine automated chemistry panels, physicians may order more

specific liver test such as viral serological test or auto-immune test that if positive, can determine the specific cause of a liver disease.

There are two general categories of “liver enzymes”. The first group includes the alanine amino transferase (ALT) and the aspartate amino transferase (AST), formerly referred to as the SGPT and SGOT. These are enzymes that are indicators of liver cell change. The other frequently used liver enzymes are the alkaline phosphatase and gamma-glutamyl transpeptidase (GGT) that indicate obstruction of the biliary system, either within the liver or in the larger bile channels outside the liver. The ALT and AST are enzymes that are located in liver cells and leak out and make their way into the general circulation when liver cells are injured. The ALT is thought to be a more specific indicator of liver inflammation, since the AST may be elevated in disease of other organs such as the heart or muscle. In acute liver injury such as acute viral hepatitis the ALT and AST may be elevated to the high 100s or over 1000 U/L. In chronic hepatitis or cirrhosis, the elevation of these enzymes may be minimal (less than 2-3 times normal) or moderate ( 100 300U/L). Mild or moderate elevations of ALT of AST are non specific and may be caused by a wide range of liver disease. ALT and AST are often used to monitor the course of chronic hepatitis and the response to treatments such as prednisone and interferon.

The alkaline phosphatase and the GGT are elevated in a large number of disorders that affect the drainage of bile such as a gallstone or tumor blocking the common bile duct or alcoholic liver disease or drug-induced hepatitis, blocking the flow of bile in smaller bile channels within the liver. The alkaline phosphatase is also found in other organs, such as bone, placenta, and intestine. For this reason, the GGT is utilized as a supplementary test to be sure that the elevation of alkaline phosphatase is indeed coming from the liver or the biliary tract. In contrast to the alkaline phosphatase, the GGT is not elevated in diseases of bone, placenta, or intestine. Mild or moderate elevation of GGT in the presence of a normal alkaline phosphatase is difficult to interpret and is often caused by changes in the liver cell enzymes induced by alcohol or medications, but without causing injury to the liver.

## **2.5 LIVER MARKERS ENZYMES**

### **THE LIVER FUNCTION TESTS (AST, ALT, ALP, GGT, LDH)**

As the liver performs its various functions, it makes chemicals that pass into the bloodstream and bile. Various liver disorders alter the blood level of these chemicals. Some of these chemicals can be measured in a blood sample. Some tests that are commonly done on a blood sample are called liver function tests. These usually measure the following:

**ALANINE TRANSAMINASE (ALT)**

This is an enzyme that helps to process proteins. They are marker of injury to liver cells. Large amounts of ALT occur in liver cells when the liver is injured or inflamed (as in hepatitis), the blood level of ALT usually rises.

**ASPARATATE AMINO TRANSFERASE (AST)**

This is another enzyme usually found inside liver cells. when a blood test detects high level of this enzyme in the blood it usually means the liver is injured in some way. However AST can also be released if heart or skeletal muscle is damaged. For this reason ALT is usually considered to be more specifically related to liver problems.

**ALKALINE PHOSPHATASE (ALP)**

This enzyme occurs mainly in liver cells next to bile ducts, and in bone. The blood level is raised in some types of liver and bone disease. Its level in plasma will rise with large bile duct obstruction intrahepatic cholestasis or infiltrative disease of the liver. It is higher in growing children (as their bone are being remodelled) and elderly patients with Paget's disease. ALP is a marker of injury to the system of bile ducts.

**ALBUMIN**

This is the main protein made by the liver and it circulates in the blood stream. The ability to make albumin (and other proteins) is affected in some types of liver disorder. A low level of blood albumin occurs in some liver disorders. It is a protein made specifically by the liver and can be measured cheaply and easily. It is the main constituent of total protein (the remaining from globulins). Albumin levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephrotic syndrome, where it is lost through the urine. The consequence of low albumin can be edema since the intra-vascular oncotic pressure is higher than the extravascular space.

**GAMMA GLUTAMYL TRANSFERASE (GGT)**

This is another enzyme that occurs in liver cells. A high level of this enzyme is particularly associated with heavy alcohol drinking (the liver breaks down and clears alcohol from the body and this enzyme is involved in the process). It can also be helpful in identifying the cause of an isolated elevation in ALP. GGT may be elevated with even minor, sub-clinical levels of liver dysfunction.

**TOTAL PROTEIN**

This measures albumin and all other proteins in blood.

## **BILIRUBIN**

This chemical gives bile its yellow/green colour. A high level of bilirubin in your blood will make you jaundiced (yellow). Bilirubin is made from haemoglobin. Haemoglobin is a chemical in red blood cells that is released when the red blood cells breakdown. Liver cells take in bilirubin and attach sugar molecules to it. This is then called 'conjugated' bilirubin which is passed into the bile ducts.

A raised blood level of 'conjugated' bilirubin occurs in various liver and bile duct conditions. It is particularly high if the flow of bile is blocked. For example, by a gallstone stuck in the common bile duct or by a tumor in the pancreas. It can also be raised with hepatitis liver injury or long-term alcohol abuse.

A raised level of 'unconjugated' bilirubin occurs when there is excessive breakdown of red blood cells. For example in haemolytic anaemia.

## **LACTATE DEHYDROGENASE (LDH)**

This is an enzyme found in many body tissues, including the liver. Elevated levels of LDH may indicate liver damage. LDH isotype-3 (or cardiac) is used for estimating damage to cardiac tissue.

## **BLOOD CLOTTING TEST**

This is another liver function tests of the liver. The liver makes many of the proteins needed to make blood clot. In certain liver disorders the liver cannot make enough of these proteins and so blood does not clot so well. Therefore, blood clotting test may be used as a marker of the severity of certain liver disorders.

### **2.6 USES OF LIVER FUNCTION TESTS**

- To help diagnose liver disorders if you have symptoms which may be due to liver disease (such as jaundice). The pattern of the blood result may help to say which disorder is causing the problem. For example depending on which enzyme is highest, it may point to a particular disorder.
- To monitor the activity and severity of liver disorders.
- As a routine precaution after starting certain medicines to check that they are not causing liver damage as a side effect.
- To screen for any potential liver disease (example in alcoholics or people who have been exposed to a hepatitis virus).

The normal range of liver tests can often vary between different laboratories, so it is not always possible to compare results directly if they have been taken at different places.

## 2.7 ATORVASTATIN

Atorvastatin belongs to the group of medications known as HMG CoA reductase inhibitors (“statins”). It is used to treat people who have high cholesterol levels, including those people who have certain inherited cholesterol disorders. Atorvastatin works by blocking an enzyme that is used to make cholesterol in the liver. When that enzyme is blocked, less cholesterol is produced and the amount of cholesterol in the blood decreases. Atorvastatin lowers the level of total cholesterol and low density lipoprotein (LDL, or “bad” cholesterol), and raises high density lipoprotein (HDL, or “good” cholesterol) levels. Reduction of cholesterol levels in the blood has been shown to reduce the risks associated with heart diseases, such as heart attack. It is also used to reduce the risk of heart attack in people experiencing symptoms associated with heart diseases and people with high blood pressure who have at least three additional risk factors for heart disease but not experiencing symptoms associated with heart diseases. The risk factors of heart disease for these individuals include;

- Being male
- Being 55 years old or older
- Enlarged heart ventricles
- Family history of heart disease
- Smoking

- Specific abnormalities on an ECG
- The total cholesterol to HDL cholesterol ratio that is greater than or equal to 6
- Type II diabetes
- Specific types of protein in the urine

Atorvastatin is also used to reduce the risk of heart attack and stroke in people with high blood pressure and type II diabetes who have other risk factors for heart disease but not experiencing symptoms associated with heart disease. The risk factors of heart disease for these individuals include.

- A specific type of protein in the urine
- Being 55 years old or older
- Disease of the retina
- Smoking

Atorvastatin may be used in addition to diet to reduce cholesterol for adolescents between 10 and 17 years of age with increased cholesterol levels and family history of early heart disease or two or more risk factors for cardiovascular disease. The medication usually takes about two to four weeks to have a significant effect on the cholesterol level in your blood. After which you (a patient) can go for blood test to check changes in your cholesterol levels.

A person taking atorvastatin should be on a cholesterol lowering diet. Thus, when using this medication, one needs to make lifestyle changes, including switching to a diet low in fat and cholesterol, quitting smoking and increasing the amount of exercise you do.

The recommended starting dose of atorvastatin for adults is 10mg or 20mg daily, preferably in the evening, with or without food. The maximum recommended dose for adult is 80mg taken once daily.

For children 10 to 17 years age who are taking this medication to treat inherited cholesterol disorder, the recommended dose ranges from 10mg to 20mg daily. For best results in lowering cholesterol it is very important to closely follow the diet suggested by a doctor, because many things can affect the dose of medication that a person needs such as body weight, other medical conditions and other medications. It is also very important that atorvastatin be taken regularly and exactly as prescribed by a doctor.

## **2.8 PHYTOCHEMICALS**

Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. They are non-nutritive plant chemicals that have protective or disease preventive properties. They are non essential nutrients meaning that they are not required by the human body for sustaining life.

It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. Phytochemicals are many and work differently. They are conferred from diets high in fruits, vegetables, beans, cereals and plant-based beverages such as tea and wine. They have some possible actions like;

- **ANTIOXIDANTS:-** It helps to protect our cells against oxidative damage and to reduce the risk of developing certain type of cancer. This antioxidant can be found in onion, garlic, fruit, vegetables, carrots, tea and grapes.
- **HORMONAL ACTION:-** It helps to reduce menopause system and osteoporosis. It is found in soy-isoflavones
- **STIMULATION OF ENZYMES:-** It helps to stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. It is found in cabbages and citrus fruits.
- **INTERFERENCE WITH DNA REPLICATION:-** Saponins in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells.

## 2.9 TYPES OF PHYTOCHEMICALS

Alkaloids, flavonoids, saponins, tannins.

**FLAVONOIDS:-** They are the most diverse group of phytochemicals. They have capacity to neutralize the free radicals and to prevent the pernicious effects these

have on the health of our body. It inhibit the growth of cancer cells. It strengthen the heart muscles and improve circulation. It lowers cholesterol. It reduce triglycerides and has protective power against liver diseases. Flavonoid together with apigenion and quercetin are very useful to eliminate certain digestive ailment related to liver. It is found in fruits, onion, berries, tea, soy, grape fruit and coffee.

**SAPONINS:-** They are glucosides with foaming characteristics. It consists of a polycyclic aglycones attached to one or more sugar side chain. It is a phytochemical which can be found in most vegetable, beans and herbs. The best known sources of saponins are peas, soybeans and some herbs. It has bitter-taste. The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. It has beneficial effect on blood cholesterol levels, cancer, bone health and stimulation of immune system. It binds with bile salt and cholesterol in the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponin cause a reduction of blood cholesterol by preventing its re-absorption. It also an antioxidant, that is its non-sugar part, which may results in reduction of cancer risk and heart diseases.

**TANNINS:** They are astringent compounds of bitter taste that appear in plants such as grapes, apples or strawberries and in medicinal plants like green tea. They

are important in healing wounds treating diarrhea or in preventing the onset of cholesterol. They are very important antioxidant.

**ANTIOXIDANTS:** They are substance that help fight the harmful effect of unstable molecules in our body called free radicals.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### MATERIALS

- Albino wistar rats (twelve)
- *Desmodium velutinum* stem
- Hand gloves

#### REAGENTS

- Ethanol                      BDH
- Colour                      BDH
- Peteroleum ether    HOPKINS AND WILLIAMS
- Ethyl acetate              BDH
- Ammonia                    BDH
- Distilled water            MAG AND BAKER
- H<sub>2</sub>SO<sub>4</sub>                      SGMALORICH
- Alkaline picrate solution
- Picric acid
- Alkaline copper
- Phosphomolybdic acid
- Formaldehyde

- Sulphanillic acid
- Sodium nitrate
- Diazo reagent
- Caffeine reagent
- Alkaline tartrate reagent
- Ascorbic acid solution
- Ferric chloride
- Sodium methylate                      ARONDAL
- Sulphuric acid                              MAG AND BAKER
- Acetyl acetone                              BDH

## **EQUIPMENTS**

- Beaker    APPROX
- Test tube    PYREX
- Spatula
- Weighing balance                              OHAUS
- Filter paper    WHATMANN
- Barrel (syringe)
- UV Spectrophotometer
- Electric grinder                                      MOULINEX



The solid extract weighing 18.3g was placed in a sterile container labeled and stored at 40<sup>0</sup>C in a refrigerator. The 18.3g was later divided into two containers (6.3g and 12g). the first container of 6.3g was used for experimental animal model while the other of 12g was used for phytochemical analysis. The hot continuous percolation method is used to determine the fat content by continuous extraction of a food with non-organic solvent such as petroleum ether for about one (1) hour or more in a soxhlet apparatus.

### **3.2 PHYOCHEMICAL ANALYSIS**

The phytochemical analysis on the solid extract was carried out based on procedures outlined by Harbourne (1973) and later modified by Trease and Evans (1996).

The phytochemical analysis done and their procedures are:

#### **STEROID DETERMINATION**

One gram (1g) of the sample was weighed into a clean dried beaker using an electric weighing balance and was macerated using 20mls of ethanol. It was then filtered using whatmann filter paper. 2mls of the filtrate was pipette into a beaker and 2mls of colour reagent was added to it and then allowed to stand for 30mins. The absorbance was measured at 550nm using UV spectrophotometer.

### **FLAVONOID DETERMINATION**

One gram (1g) of the sample was weighed into a beaker and I macerated it with 20mls of ethyl acetate. I filtered using whatmann filter paper. 5mls of the filtrate was pipette into a beaker with 5mls of dilute ammonia slake added to it. The upper layer was collected and absorbance measured at 490nm using UV spectrophotometer.

### **SAPONIN DETERMINATION**

One gram (1g) of the sample was weighed into a beaker using an electric weighing balance and was macerated using 10mls of petroleum ether. I decant into a beaker and added another 10mls of petroleum ether, and also decant into the beaker. The filtrate was combined, then evaporated to dryness. 6mls of ethanol was added and 2mls was pipette into a test tube. I added 2mls of colour reagent which was allow to stand for 30mins. The absorbance was measured at 550nm using UV spectrophotometer.

### **REDUCING SUGAR DETERMINATION**

One gram (1g) of the sample was weighed into a beaker and was macerated with 20mls of distilled water. 1ml of the filtrate was pipette and 1ml of alkaline copper reagent added to it. It was boiled for 5mins and allow to cool. 1ml of

phosphomolybdic acid reagent was added and 7mls of distilled water also added. The absorbance was measured at 420nm.

### **ALKALOID DETERMINATION**

I weighed one gram (1g) of the sample into a beaker and macerate with 20mls of 20%  $H_2SO_4$  in ethanol (1:1). I filtered using whatmann filter paper. Then pipette 1ml of the filtration into a beaker and added 5mls of 60%  $H_2SO_4$  and 5mls of 0.5% formaldehyde in 60%  $H_2SO_4$ . I mix properly and allow to stand for 3hours. The absorbance was measured at 565nm using UV spectrophotometer.

### **TERPENOID DETERMINATION**

One gram (1g) of the sample was weighed into a clean dried beaker and was macerated with 50mls of ethanol. It was then filtered and 2.5 mls of the filtrate was pipette into a beaker. 2.5 mls of 50% aqueous phosphomolybdic acid solution was added and 2.5mls concentrated  $H_2SO_4$  was also gradually added. It was mix, then allow to stand for 30mins. I make up to 12.5mls with ethanol. The absorbance was then measured at 700nm.

### **GLYCOSIDE DETERMINATION**

One gram (1g) of the sample was weighed into a beaker using electric weighing balance. 2.5mls of 15% lead acetate was added and filtered. 2.5mls of chloroform was added and shaken vigorously. I collected the lower layer and evaporate to dryness. 3mls of glacial acetic acid was added and 0.1ml of 5% ferric chloride and 0.25ml concentrated  $H_2SO_4$  was added. I shaked properly, then put in the dark for 2 hours. The absorbance was measured at 530nm.

### **TANNIN DETERMINATION**

One gram (1g) of the sample was weighed into a beaker and macerate with 50ml of methanol. I filtered and 5mls of the filtrate was pipette into a beaker. 0.3mls of 0.1M ferric chloride in 0.1M HCL was added and another 0.3mls of 0.0005M potassium ferricyanide was added. The absorbance was measured at 720nm.

### **CYANIDE DETERMINATION**

One gram (1g) of the sample was weighed into a beaker and 50mls of distilled water was macerated. It was allow to stand for 24hrs. I filtered and 1ml of the filtrate was pipette into a beaker 4mls of alkaline picrate solution was added and then boiled for 5 mins. It is the then allow to cool. The absorbance was measured at 490nm.

## **SOLUBLE CARBOHYDRATE DETERMINATION**

One gram (1g) of the sample was weighed into a beaker and macerated with 50mls of distilled water. I filtered and 1ml of the filtrate was pipette into a beaker. 2mls of saturated picric acid was added. The absorbance was measured at 530nm using UV spectrophotometer.

### **3.3 EXPERIMENTAL ANIMAL MODEL**

Twelve (12) healthy albino wistar rats with mean weight of  $1.50 \pm 0.60$ kg were obtained locally from Nsukka, Enugu State. The rats were randomly distributed into four (4) groups (I-V) of the (3) rats each. They were housed separately and fed with water and grower's mash (Guinea feed Nigeria) and allowed for 3days to acclimatize.

A high fat from cow's brain was prepared by dissolving 300g of fresh cow's brain in 500ml of distilled water forming a semi-solid mixture. A known drug; Atovarstatin (brand name –Lipitor, 10mg) was prepared by dissolving 5 mg (half of one tablet) in 2ml of distilled water. Also *Desmodium Velutinum* stem ethanol extract weighing 2.7g was dissolved in 7ml of distilled water forming a liquid drug extract.

Group I rats were fed orally with 6ml; of the high fat food mixture which contains 3.6g of the cow's brain twice a day (morning and evening) for seven (7) days.

Group II rats was fed orally with only grower's mash and water for also seven (7) days.

Rats in groups III were also fed orally with 6ml of the high fat food mixture from cow's brain for seven (7) days (morning and evening) and later were administered orally with the 2ml dissolved atorvastatin drug for the following three (3) days (once each day) during which grower's mash and water was their food.

Rats in group IV were also fed orally with 6ml of the high fat food mixture from cow's brain for seven (7) days (morning and evening) and later were administered orally with 0.5ml of the liquid drug extract(*Desmodium Velutinum* stem ethanol extract mixture) for the following three (3) days (once a day) during which grower's mash and water was their food.

NB- Both the high fat food mixture from cow's brain and the extract doses of *Desmodium Velutinum* stem were orally administered by the use of a syringe (needle part removed).

### **3.4 COLLECTION OF BLOOD SAMPLE**

The collection of blood samples from the rats was simply done by dissecting of the rats, followed by cardiac puncture after a mild anesthesia with chloroform. About 5-9mls of blood sample were collected in an EDTA tube from each group using a

medical syringe. Serum was separated from the blood after clotting by centrifugation and then used for lipid analysis.

Blood sample were collected from the rats in group I rats and II rats on the following day of after the 7<sup>th</sup> day of orally feeding the rats with high fat from cow's brain and normal feed (Grower's mash and water ) respectively.

Blood sample were collected from the rats in group III and IV on the following day of after the 3<sup>rd</sup> day of orally administering a known drug (atorvastatin; Lipitor) and the liquid drug extract (Ethanol extract of *Desmodium velutinum* stem mixture) respectively.

### **3.5 PROCEDURES FOR CARRYING OUT LIVER FUNCTION TESTS**

#### **REAGENT PREPARATION**

##### **SULPHANILIC ACID REAGENT SUCH AS**

Distilled water up to 1 litre is prepared with concentrated hydrochloric acid of 15ml and sulphanilic acid up to 50g.

##### **SODIUM NITRATE SOLUTION**

Distilled water up to 100ml is prepared kept at 4<sup>0</sup>C for two weeks.

## **DIAZO REAGENT**

Diazo reagent is divided into two reagent which is labeled reagent I and II. The reagent I is prepared up to 10.0ml while reagent II is prepared up to 0.25ml. (The reagent should be stable for about three hours)

## **CAFFEINE REAGENT SUCH AS**

Sodium benzoate up to 75g, Caffeine up to 50g, sodium acetate anhydrous up to 75g, ethylene diamine tetracetic acid up to 1g and distilled water up to 1 litre are used for caffeine reagent.

## **ALKALINE TARTRATE REAGENT SUCH AS**

NaOH up to 100g with sodium potassium tartarate up to 350g are used for the alkaline tartrate reagent.

## **ASCORBIC ACID SOLUTION**

Ascorbic acid solution up to 0.20g with distilled water up to 5ml are used for ascorbic acid solution.

**FIRST STAGE****MAKE AN ARRANGEMENT OF FOUR TEST TUBES**

Total	blank	STD	STD		
Bilirubin				blank	
Caffeine reagent	Absent	Absent	0.2ml	Absent	
Diazo reagent	-	-	-	-	-
Distilled water	-	-	-	-	-
Standard up to 200 $\mu$ mol/l	-	-	-	-	-

Total	blank	STD	STD		
Bilirubin				blank	
Serum/plasma	0.2ml	-	-	-	-
Caffeine reagent	2.0ml	-	2.0ml	-	-
Diazo reagent	0.5ml	-	0.5ml	-	-
Distilled water	0.8ml	0.8ml	0.8ml	0.8ml	0.8ml
Standard up to 200 $\mu$ mol/l	-	-	0.2ml	-	-

Allow the rack containing the first test tube to stand at room temperature for about 10 minutes.

Still on the tubes containing the reagents as in first stage;

Add the following reagents

Total	blank	STD	STD		
Bilirubin				blank	
Diazo reagent	-	5.0ml	-	0.5ml	
Alkaline tartarate	1.5ml	1.5ml	1.5ml	1.5ml	
Caffeine reagent	-	2.0ml	-	2.0ml	
Serum/plasma	-	0.2ml	-	-	
Standard up to 200 $\mu$ mol/l	-	-	-	-	

Calculation:

$$\mu\text{mol/l of total bilirubin} = \frac{\text{Absorbance total bilirubin} - \text{Absorbance blank}}{\text{Absorbance STD} - \text{Absorbance STD blank}} \times 200.$$

Conjugated/Direct bilirubin: the test tubes are arranged as below

Serum	0.2ml
Alkaline tartarate	1.5ml
Caffeine reagent	2.0ml
Ascorbic acid	2.0ml
Distilled water	0.8ml

Note: the absorbance of all the test tubes should be read at 600nm within 30 minutes

Calculation:

$$\frac{\text{Absorbance (B)} - \text{Absorbance (B)}}{\text{Absorbance STD} - \text{Absorbance STD blank}} \times 200$$

Absorbance STD – Absorbance STD blank

Then to calculate unconjugated (indirect) bilirubin the formula is simply;

Total bilirubin- conjugated bilirubin

To convert  $\mu\text{mol/l}$  to  $\text{mg/dl}$  the formula is;

$$\text{Bilirubin } \mu\text{mol/l} \times 17.1$$

## CHAPTER FOUR

### 4.0 RESULTS

#### QUANTITATIVE ANALYSIS OF THE ETHANOL EXTRACT OF *DESMODIUM VELUTINUM* STEM SHOWING THE PHYTOCHEMICAL COMPOSITION OF SAMPLES

Quantitative analysis table I phytochemical composition of samples (mg/100g)

PHYTOCHEMICAL SAMPLES	QUANTITATIVE COMPOSITION (MG/100G)
Soluble carbohydrate	1.92±0.003
Cyanide	0.52±0.003
Reducing sugar	334.743±0.003
Saponin	1.34±0.004
Tannin	2.14±0.003
Flavonoid	2.94±0.003
Alkaloid	3.45±0.006
Steroid	0.64±0.004
Terpenoids	0.37±0.002

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

Data in the same column bearing different superscript differed significantly ( $P \leq 0.05$ )

#### 4.1 QUALITATIVE ANALYSIS OF ETHANOL EXTRACT OF *DESMODIUM VELUTINUM* STEM SHOWING THE QUALITATIVE PHYTOCHEMISTRY OF SAMPLES

Qualitative analysis table II: qualitative phytochemistry

PHYTOCHEMICAL SAMPLES	QUALITATIVE ANALYSIS OF THE SAMPLES
Tannin	+++
Alkaloid	+++
Carbohydrate	++

Saponin	+
Steroid	+
Hydrogen cyanide	+
Flavonoid	++
Reducing sugar	++
Terpenoid	+

**4.2 TABLE III: LIVER FUNCTION TESTS OF RATS FEED WITH VARIOUS SAMPLES.**

Parameters	Group 1 Rats feed with cow's brain	Group 2 Rats feed with normal feed (grower's mash)	Group 3 Rats feed with atorvastatin	Group 4 Rats feed with Ethanol extract of the stem
Total bilirubin (mg/dl)	0.91±0.10 <sup>e</sup>	0.71±0.01 <sup>d</sup>	0.40±0.00 <sup>f</sup>	0.81±0.01 <sup>a</sup>
Direct bilirubin (mg/dl)	0.81±0.00 <sup>d</sup>	0.10±0.00 <sup>d</sup>	0.20±0.00 <sup>e</sup>	0.10±0.00 <sup>a</sup>
Indirect bilirubin (mg/dl)	0.70±0.00 <sup>a</sup>	0.60±0.00 <sup>d</sup>	0.50±0.21 <sup>e</sup>	0.70±0.00 <sup>a</sup>
Total serum/plasma protein( g/dl)	3.35±0.07 <sup>b</sup>	4.85±0.07 <sup>d</sup>	1.55±0.07 <sup>e</sup>	5.05±0.07 <sup>a</sup>
Albumin (mg/dl)	3.71±0.01 <sup>e</sup>	2.51±0.01 <sup>a</sup>	0.70±1.14 <sup>f</sup>	4.00±0.00 <sup>a</sup>
Globulin (g/dl)	1.85±0.07 <sup>e</sup>	2.40±0.00 <sup>e</sup>	0.81±0.01 <sup>f</sup>	1.00±0.00 <sup>a</sup>
Serum plasma Alkaline phosphatase (ALP) (KA units/100ml)	5.01±0.01 <sup>e</sup>	3.50±0.00 <sup>d</sup>	1.51±0.01 <sup>f</sup>	3.00±0.00 <sup>a</sup>
Serum plasma phosphate acid phosphate (units/ml)	1.20±0.00 <sup>e</sup>	0.51±0.01 <sup>d</sup>	1.20±0.00 <sup>e</sup>	0.31±0.00 <sup>a</sup>
Creatine phosphate (units/ml)	35.00 ±0.00 <sup>e</sup>	30.00±0.00 <sup>d</sup>	0.41±0.01 <sup>f</sup>	10.43±0.07 <sup>a</sup>
Aspartate amino transferase (AST) (u/l)	13.00±0.00 <sup>e</sup>	11.00±0.00 <sup>d</sup>	8.75±0.07 <sup>f</sup>	8.01±0.00 <sup>a</sup>
Alanine amino transferase (ALT) (u/l)	15.00±0.00 <sup>d</sup>	13.91±0.01 <sup>c</sup>		11.00±0.01 <sup>a</sup>
Gamma-glutamyl transferase (GGT) (u/l)	8.01±0.01 <sup>e</sup>	6.91±0.01 <sup>d</sup>	3.71±0.01 <sup>f</sup>	5.01±0.01 <sup>a</sup>
Lactate dehydrogenase (LDH)(u/l)	3.34±0.01 <sup>e</sup>	2.50±0.00 <sup>a</sup>	1.41±0.01 <sup>f</sup>	1.06±0.01 <sup>a</sup>

Data are means of duplicate determinations ± SD. Data in the same row bearing different superscript are significantly different (P<0.05)

## CHAPTER FIVE

### 5.0 DISCUSSION

Consumption of a calorie-rich diet results in lipid accumulation excess production of inflammatory cytokines and macrophage infiltration that favour the progression of liver disease showed that high fat diet could induce the hyperlipidemia in rats and hyperlipidemia could alter the related marker enzyme profiles in serum and liver tissue and progress to liver cirrhosis have revealed that high fat diet promote hyperglycemia(Ishii *et al.*, 2010) and its effects on muscle and liver physiology as well as endothelial functions.

Liver is the key organ in the metabolism detoxification and secretory function in the body and its disorders are numerous with no effective remedies however, the search for new medicines still going(Jamshidzadeh *et al.*, 2005). Many folk remedies from plant origin have been long used for treatment of liver diseases. Management of liver diseases is still challenge to the modern medicine. In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders(Prveen *et al.*,1992). From the above statement it is generally accepted that high fat diets can be used to generate a valid rat model for the analysis of pathophysiology of hyperlipidemia. Therefore, in this study high fat diet fed to rat model for the induced alterations on marker enzymes in serum and liver tissue to examine the comparative effect of ethanol extract of

*Desmodium Velutinum* stem and atorvastatin combination with dietary (high fat from cow's brain) administration for their hepatoprotective activity.

Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP which are enzymes originally present in higher concentration in cytoplasm, when there is hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage. Indication of hepatocellular integrity most commonly measured in clinical toxicology studies are the enzymes AST, ALT and bilirubin levels. ALT is frequently included in biochemical profile for the purpose of assessing hepatic injury.

In the present investigation, the increased levels of AST ALT and ALP have been observed in both liver and serum of high fat fed rat compared to control groups (table 3) indicating the hepatotoxic role of high fat diet. An elevation in the levels of serum marker enzymes is generally regarded as the most sensitive index of the hepatic damage. ALP reduces the liver mainly from bone and its elevation in serum occurs through biliary excretion during hepatobiliary diseases. The elevation of ALP in liver tissue of high-fat fed rat indicates the disturbed excretory function of liver. As we know, the enzymes ALT and AST are present in the hepatic and biliary cells. Observed elevated level of these enzymes in serum and liver tissue of high fat from cow's brain fed to the albino wistar rats indicates that these elevation might be due to hepatocellular damage caused by high fat diet toxicity. These

elevated enzymes in hepatocytes usually released into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary tract cells. In table three(3) which is the liver function test of the albino wistar rats fed with various samples, the AST, ALP, ALT levels increased significantly( $P>0.05$ ) in the pretreatment of the ethanol extract of *Desmodium Velutinum* stem when compared to their total protein concentration. This mean that the pretreatment with plant extract for seven(7) days showed significant( $P<0.05$ ) protective effect against the fatty liver of albino wistar rats.

In present study, serum as well as liver protein level were decreased in high fat fed rats. The depletion in the protein levels might be due to liberate energy of their metabolism during the toxicity of high-fat fed rats. The reduction of protein in high-fat fed rat is possible due to localized damage in the endoplasmic reticulum(Sureshkumar and Mishra, 2006).

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary diseases and severe disturbance of hepatocellular function (Martin and Friedman,1992). In the present investigation, the rats fed with high fat from cow's brain showed significantly increased levels of bilirubin as compared to control rats(Group II). This trend of result coincide with the statement of induced hepatitis is characterized by increased levels of bilirubin in serum (Lenaerts

*et al*, 2005). In table 2, which is the qualitative analysis of the phytochemistry, tannin, alkaloid was found to be highly present, while flavonoids is moderately present. Saponin and terpenoid was found to be present. These phytochemicals are antioxidants which fight against unstable molecules (Free radicals) in the body system. Saponin reduce blood cholesterol by preventing its re-absorption and also flavonoids which has protective power against liver diseases reduces cholesterol in the body system.

Findings on the rat fed with atorvastatin and ethanol extract of *Desmodium velutinuem* stem in separately mixing with high fat from cow's brain showed the appreciable normal level of enzyme profile (ALT, AST and ALP) and total protein in serum and liver tissue as well as bilirubin in serum of albino wistar rat. The results on atorvastatin group indicted that atorvatation was effective in the treatment of high fat fed rats possible through the mechanism of regulating the enzymes metabolism. Research on atorvastatin for the treatment of liver fibrosis induced in rats by either high fat diet administration or bile duct ligation indicated that atorvastatin inhibit both hepatic inflammation, collagen synthesis and hepatic stellate cell activation, thereby ameliorating early-phase fibrogenesis in the liver. In this study the atorvastatin treated group (in table 3) with great prevention on the alteration of biochemical changes which indicates the atorvatatin act as a hepato-protector from high-fat toxicity in rat, research recently points to an unfavourable

effect of atorvastatin to cause insulin resistance and increases ambient glycemia in hypercholesterolemic patients (Koh *et al.*, 2010). Further, the atorvastatin therapy is associated with a decrease in cardiac outcomes, including recurrent heart attack.

The extract of *Desmodium Velutinum* stem with high fat from cow's brain fed to rat showed the perfect tendency of biochemical component (enzymatic level) in both serum and liver tissue compared with control groups, indicating the antihepatotoxic role of *Desmodium Velutinum* stem. The *Desmodium Velutinum* treatment exerted beneficial effect on ameliorating the enzyme resistance in high fat fed rat. Administration of atorvastatin and *Desmodium Velutinum* extract with high fat fed to rats showed decreased bilirubin level when compared to high fat from cow's brain alone fed rats. The atorvastatin and ethanol extract of *Desmodium Velutinum* stem mediated reduction of the increased bilirubin level mixing with high fat suggests the possibility of the extract being able to stabilize biliary dysfunction.

The present investigation showed that the *Desmodium Velutinum* ethanol extract of the stem act as an important mediator of enzyme resistance in high fat diet induced obesity, through its ability to decrease the elevated activity of ALT, ALP and AST at the cellular level. The results on ethanol extract of *Desmodium Velutinum* stem treatment indicated that *Desmodium Velutinum* extract maybe more effective for the treatment of high fat diet toxicity than the treatment of

atorvastatin. High amount of hordenine and desmodin have been identified in *Desmodium Velutinum* which are know to increase the urinary flow and remedy to dysentery and diarrhoea and also affect the fertility and reproductive system of male rats. Also, its aqueous roots extract shows mild diurectic action, relaxant effects on intestine muscles of rats and dogs and anti-inflammatory activity of the alkaloid fraction of the stem. The extracts of *Desmodium Velutinum* successive ethanol extract of the stem, showed significant hepato protective effect. From the above experimental study, it showed that the extract of *Desmodium velutinum* produces adequate hepato protective effect on the liver of albino wistar rats fed with high fat from cow's brain.

## 5.1 CONCLUSION

From this work it is concluded that the usage of ethanol extract of *Desmodium Velutinum* stem as a hepatoprotective drug has been validated, it is useful in treating different liver infections and diseases. The study conclusively stated that *Desmodium Velutinum* has antihepatotoxic effects on serum, hepatic marker enzyme activities and blood bilirubin level as well as improving protein level from high-fat diet induced hepatotoxicity. The presence of phytochemical constituents like flavonoids, saponins, alkaloids in *Desmodium Velutinum* possess antioxidant and hepatoprotective properties. *Desmodium Velutinum* treatment was effective in

hepatoprotective role by improving biochemical components in high fat diet fed rat towards normal tendency without any side effect after two(2) weeks experimental period in comparison to atorvastatin treatment. It showed potent hepaprotective activity, if the pretreatment of *Desmodium Velutinum* stem of ethanol extract dose is increased for a duration of time or the experiment is prolong, it may be even more effective than the drug atorvastatin.

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