EVALUATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACTION OF LEAVES OF *RHINACANTHUS NASUTUS (L.)*

A Dissertation submitted to

THE TAMILNADUDr.M.G.R. MEDICAL UNIVERSITY

CHENNAI- 600032.

In partialfulfilmentfor the award of degreeof

MASTEROFPHARMACYIN

PHARMACOLOGY

SubmittedBy

GANESH KUMAR.T

RegNo:261525706

Underthe guidance of

Mr.V.SURESH M.Pharm., (Ph.D).,

Associate Professor

DEPARTMENTOFPHARMACOLOGY

JKKMMRF'S–ANNAIJKKSAMPOORANIAMMAL

COLLEGEOFPHARMACY

B.KOMARAPALAYAM–638183.

TAMILNADU

OCTOBER2017

CONTENTS

1. INTRODUCTION

1.1. Introduction to Herbal medicine

For centuries people have used plants for healing. Plant products as parts of foods or botanical potions and powders have been used with varying success to cure and prevent diseases throughout history. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers (IlyaRaskinand David M. Ribnicky, 2002).

Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent a disease in animals or humans (Weiss RF &Fintelmann V. *et al*, 2000)**.**

About 70-80% of the world populations,particularly in the developing countries, relyonnon-conventional medicine in their primaryhealthcare as reported by the World Health Organization (Akerele O *et al.,* 1993)**.**

In recent years, there has beengrowing interest in alternative therapies and thetherapeutic use of natural products, especially those derived from plants (Vulto AG &Smet PAGM *et al*,1988). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side

effects and other problems, a large percentage of the world, conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless.

About25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO, 1991), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors.

Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeinefrom *Papaver somniferum* (Rates SMK *et al.*,2001).

About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra *et al*., 1956).

The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe and Staden, 1997). Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health.

1.1.1. The role of herbal medicines in traditional healing

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (WHO,1991). Or say, traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine.

The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years.

The classical Indian texts include *Rigveda, Atherveda, Charak Samhita* and *SushrutaSamhita*. The herbal medicines/traditional medicaments have, therefore, beenderivedfrom rich traditions of ancient civilizations and scientific heritage.

1.1.2. Herbal medicine in India

India is one of the 12 mega biodiversity centers having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agroclimatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000–18,000 flowering plants, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million micro-organisms (Drugs and Pharmaceuticals, 1998).

India also has equivalent to 3/4 of its land exclusive economic zone in the ocean harbouring a large variety of flora and fauna, many of them with therapeutic

properties. About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine.

1.1.3. Difference of Herbal and Conventional Drugs

Compared with well-defined synthetic drugs, herbal medicines exhibit some marked differences, namely:

- The active principles are frequently unknown
- Standardization, stability and quality control are feasible but not easy;
- The availability and quality of raw materials are frequently problematic;
- Well-controlled double-blind clinical and toxicological studies to prove their efficacy and safety are rare;
- Empirical use in folk medicine is a very important characteristic;
- They have a wide range of therapeutic use and are suitable for chronic treatments;
- The Occurrence of undesirable side effects seems to be less frequent with herbal
- Medicines, but well-controlled randomized clinical trials have revealed that they also exist;
- They usually cost less than synthetic drugs (Calixto J B *et al.*, 2000).

1.1.4. Relationship between Ayurveda and modern medicine

Ayurveda, one of the major traditional forms of medical practice in India, has produced many useful leads in developing medications for chronic diseases. Almost 25 centuries ago, Hippocrates proclaimed, .Let food be thy medicine and medicine be thy food. (David J. Newman & Gordon M. Cragg, 2003).

Combining the strengths of the knowledge base of traditional systems such as ayurveda with the dramatic power of combinatorial sciences and High Throughput Screening will help in the generation of structure- activity libraries. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity –the three main hurdles in drug development. These records are particularly valuable, since effectively these medicines have been tested for thousands of years on people. Efforts are underway to establish pharmaco epidemiological evidence base regarding safety and practice of ayurvedic medicines.

1.1.5. Herbal medicine standardization

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts.

The extract should then be checked for indicated biological activity in an experimental animal model(s).The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints.

The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies in animals. Determination of the probable mode of action will explain the therapeutic profile.

The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical trial to establish its therapeutic potential would promote clinical use. The herbal medicines developed in this mode should be dispensed as prescription drugs or even OTC products depending upon disease consideration and under no circumstances as health foods or nutraceuticals. (V.P.Kamboj *et al*., 2000).

1.2. INTRODUCTION OF DIABETES MELLITUS

1.2.1. History of diabetes mellitus

The earliest description of diabetes was documented in the writings of Hindu scholars as long as in 1500 BC. They had already described **"***a mysterious diseasecausing thirst, enormous urine output, and wasting away of the body with flies and*

ants attracted to the urine of people.**"**

The term diabetes was probably coined by Apollonius of Memphis around 250 BC, which literally meant **"**to go through**"** or siphon as the disease drained more fluid than a person could consume. Later on, the Latin word **"**mellitus**"** was added because it made the urine sweet. (MacCracken J. 1997).

Sushruta, Arataeus, and Thomas Willis were the early pioneers of the treatment of diabetes. Greek physicians prescribed exercise, preferably on horseback,
to **"**employ moderate friction**"** and alleviate excess urination. Wine, overfeeding to compensate for loss of fluid weight, starvation diet, potato therapy, and oat cure were some of the other curious forms of remedy suggested for the therapy of diabetes in olden days (MacCracken J.1997).

Sir William Osler, in the year 1915, is said to have even recommended opium! Early research linked diabetes to glycogen metabolism, and the islet cells of pancreas were discovered by Paul Langerhans, a young German medical student. In 1916, Sharpey-Shafer of Edinburgh suggested that a single chemical was missing from the pancreas and proposed its name as **"**insulin.**"** The term insulin originates from the word Insel, which is German for an islet or island.

Researchers like E.L. Scott and NikolaePaulesco were successful in extracting insulin from the pancreas of experimental dogs. The key breakthrough, though, came from the Toronto University with the discovery of insulin in 1921. FG Banting and JJR Macleod were awarded the Nobel Prize for Physiology or Medicine in 1923 (Bliss M.1982.).

1.2.2. Definition of Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterised by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The abnormalities in carbohydrate, fat, and protein metabolismthat are found in diabetes are due to deficient action of insulin on target tissues.

If ketones are present in blood or urine, treatment is urgent, because ketoacidosis can evolve rapidly (American diabetes association,2012).

1.2.3. Causes

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or alternatively, when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar, hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

1.2.4. Symptoms of diabetes mellitus

Common symptoms include the following:

- Frequent urination
- Excessive thirst
- Unexplained weight loss
- Extreme hunger
- Sudden vision changes
- Tingling or numbness in the hands or feet
- Feeling very tired much of the time
- Very dry skin
- Sores that are slow to heal
- More infections than usual

Some people may experience only a few symptoms that are listed above. About 50 percent of people with type 2 diabetes don't experience any symptoms and don't know they have the disease. (www.ucsfhealth.org)

1.2.5. Pathophysiology of diabetes mellitus

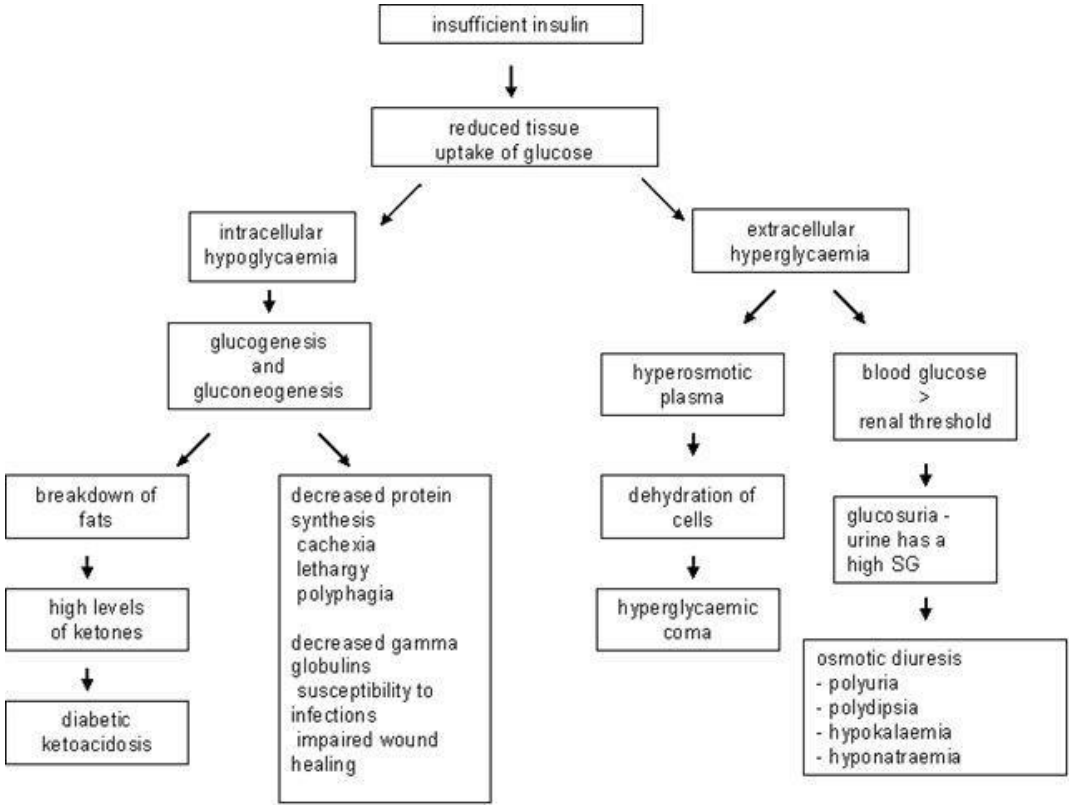


Figure No: 1.Pathophysiology of diabetes mellitus

1.3. CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types ofdiabetes: type 1 diabetes, type 2diabetes, and gestational diabetes.(www.webmd.com)

1.3.1. Type 1 diabetes mellitus: (β-cell destruction, usually leading to absolute insulin deficiency)

Type 1 diabetes represents a heterogenous and polygenic disorder, with a number of non-HLA loci contributing to disease susceptibility (Lernmark A, 1998). Though this form of diabetes accounts for 5 to 10% of all diabetics, there is yet no

identified agent substantially capable of preventing this type of disease (Atkinson MA, 2001). The WHO and the American Diabetics Association (WHO; 1999 and ADA; 2001) have proposed that type 1 diabetes can be divided into autoimmune/ immune-mediated diabetes (Type 1A) and idiopathic diabetes with **β**-cell obstruction (Type 1B). This type of diabetes mellitus requires exogenous insulin to prevent Diabetic ketoacidosis.

1.3.2. Immune mediated diabetes

This form of diabetes, previously encompassed by the terms insulin dependent diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the **β**-cells of the pancreas.(American diabetes association,2012)

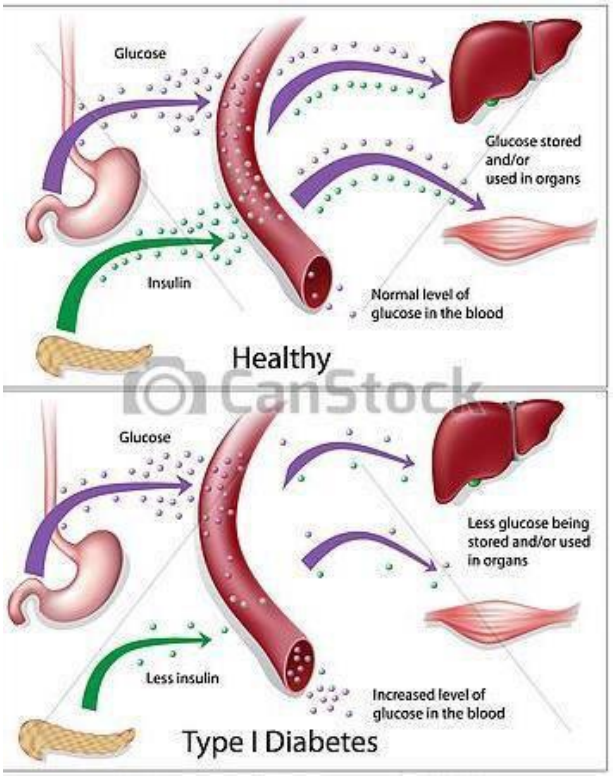


Figure No: 2. Immune mediated diabetes

Type 1 diabetes is an autoimmune condition. It's caused by the body attacking its own pancreas with antibodies. In people with type 1 diabetes, the damaged pancreas doesn't make insulin.

This type of diabetes may be caused by a genetic predisposition. It could also be the result of faulty beta cells in the pancreas that normally produce insulin.

A number of medical risks are associated with type 1 diabetes. Many of them stem from damage to the tiny blood vessels in your eyes (called diabetic retinopathy), nerves (diabetic neuropathy), and kidneys (diabetic nephropathy). Even more serious is the increased risk of heart disease and stroke.(www.webmd.com)

1.3.3. Idiopathic diabetes

Some forms of type1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of Patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry.

Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for **β**-cell autoimmunity (American diabetes association, 2012).

Treatment for type 1 diabetes involves taking insulin, which needs to be injected through the skin into the fatty tissue below. (www.webmd.com)

1.3.4. Type 2 diabetes

Type 2diabetes is far more common and results from a combination of defects in insulin secretion and insulin action, either of which may predominate. People with type 2 diabetes are not dependent on exogenous insulin, but may require it for the control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents. This type of diabetes accounts for 90 to 95% of all diabetic patients (DeFronzo RA, 1997).

All forms of diabetes are characterized by chronic hyperglycemia and the development of diabetes- specific micro vascular pathology in the retina, renal glomerulus, and peripheral nerve. As a consequence of its micro vascular pathology, diabetes is a leading cause of blindness, end-stage renal disease, and a variety of debilitating neuropathies.

When islet b-cell function is impaired, insulin secretion is inadequate, leading to overproduction of glucose by the liver and under-utilization of glucose in peripheral tissue (Bergman RN *et al*, 1989).

Type 2 diabetes is made up of different forms, each of which is characterized by a variable degree of insulin resistance and **β**-cell dysfunction and which together lead to hyperglycemia (American Diabetes Association, 2001).

At each end of this spectrum are single gene disorders that affect the ability of the pancreati**c β** cell to secrete insulin (Fajans SS *et al*, 2001 and Owen K *et al*, 2001) or the ability of muscle, fat, and linear cells to respond to insulin action (Taylor SI *etal*, 1999 and Barroso I *et al*, 1999).

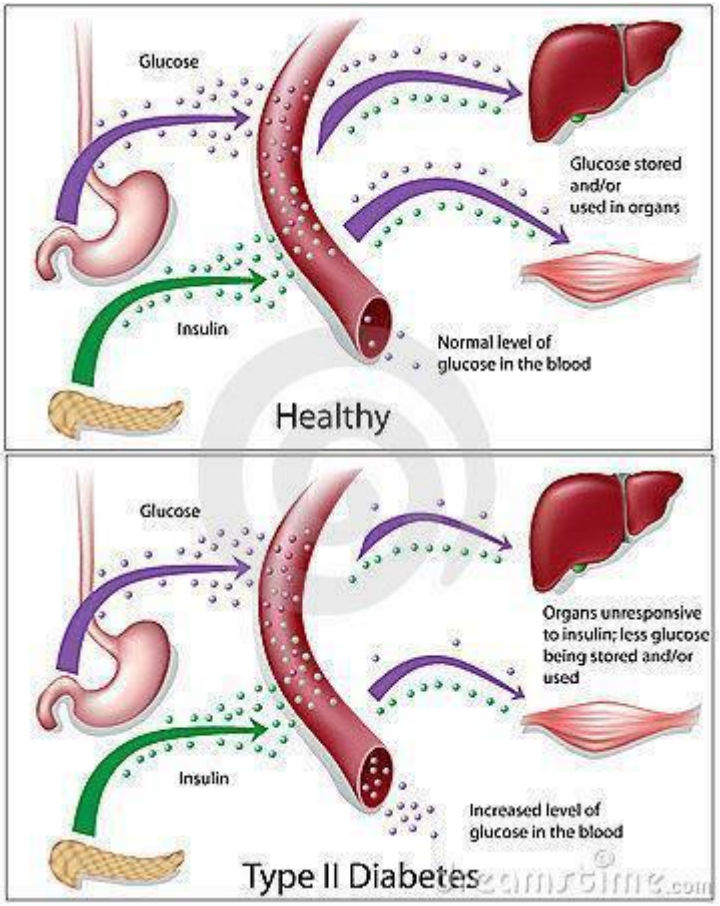


Figure No: 3. Type 2 diabetes

With Type 2 diabetes, the pancreas usually produces some insulin. But either the amount produced is not enough for the body's needs, or the body's cells are resistant to it. Insulin resistance, or lack of sensitivity to insulin, happens primarily in fat, liver, and muscle cells.

People who are obese -- more than 20% over their ideal body weight for their height -- are at particularly high risk of developing type 2 diabetes and its related medical problems. Obese people have insulin resistance. With insulin resistance, the

pancreas has to work overly hard to produce more insulin. But even then, there is not enough insulin to keep sugars normal. (www.webmd.com)

There is no cure for diabetes. Type 2 diabetes can, however, be controlled with weight management, nutrition, and exercise. Unfortunately, type 2 diabetes tends to progress, and diabetes medications are often needed (www.webmd.com).

1.3.5. Gestational Diabetes

Diabetes that's triggered by pregnancy is called gestational diabetes (pregnancy, to some degree, leads to insulin resistance). It is often diagnosed in middle or late pregnancy. Because high blood sugar levels in a mother are circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby's growth and development.

According to the National Institutes of Health, the reported rate of gestational diabetes is between 2% to 10% of pregnancies. Gestational diabetes usually resolves itself after pregnancy. Having gestational diabetes does, however, put mothers at risk for developing type 2 diabetes later in life. Up to 10% of women with gestational diabetes develop type 2 diabetes. It can occur anywhere from a few weeks after delivery to months or years later.

With gestational diabetes, risks to the unborn baby are even greater than risks to the mother. Risks to the baby include abnormal weight gain before birth, breathing problems at birth, and higher obesity and diabetes risk later in life. Risks to the mother include needing a cesarean section due to an overly large baby, as well as damage to heart, kidney, nerves, and eye.Treatment during pregnancy includes,

- Careful meal planning to ensure adequate pregnancy nutrients without excess fat and calories
- Daily exercise
- Controlling pregnancy weight gain
- Taking diabetes insulin to control blood sugar levels if needed(www.webmed.com)

1.3.6. Other specific types of diabetes

a) Genetic defects of the β-cell

Several forms of diabetes are associated with monogenetic defects in **β**-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action.

b) Genetic defects in insulin action

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes.

c) Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in **β**-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage **β**-cells and impair insulin secretion.

d) Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action.Excess amounts of these hormones (e.g.,acromegaly, Cushing**'**s syndromeglucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved.

e) Drug- or chemical-induced diabetes

Many drugs can impair insulin secretion.These drugs may not cause diabetes bythemselves, but they may precipitate diabetes in individuals with insulin resistance.In such cases, the classification is unclear because the sequence or relative importance of **β**-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor(a rat poison) and intravenous pentamidine can permanently destroy pancreatic **β**-cells.

f) Infections

Certain viruses have been associated with **β**-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.(American diabetes association ,2012)

1.4. Evolution of insulin

Insulin is a natural hormone and as essential as air, water, and light. Amongst all the antidiabetic medications, it is the most potent agent that reduces blood glucose levels. Its benefits exceed beyond the realms of glycemic control.

It reduces glucotoxicity and lipotoxicity, reverses insulin resistance, preserves beta cell function, and it improves lipid profile, endothelial dysfunction, anti-inflammatory effects, and anti-platelet effects as well as the quality of life. In spite of all these benefits, there is an inherent clinical inertia before initiating insulin therapy, especially for patients with type 2 diabetes mellitus. (Galloway JA *et al* ;1994.)

1.4.1. Structure of insulin

Insulin was purified and crystallized by Abel within a few years of its discovery. Sanger established the amino acid sequence of insulin in 1960, the protein was synthesized in 1963, and Hodgkin and coworkers elucidated insulin's three-dimensional structure in 1972. Insulin was the hormone for which Yalow and Berson first developed the radioimmunoassay (Kahn and Roth, 2004).

The β cells of pancreatic islets synthesize insulin from a single-chain precursor of 110 amino acids termed *preproinsulin.* After translocation through the membrane of the rough endoplasmic reticulum, the 24-amino-acid N-terminal signal peptide of preproinsulin is cleaved rapidly to form proinsulin. Thereafter, proinsulin folds, and the disulfide bonds form. During conversion of human proinsulin to insulin, four basic amino acids and the remaining connector or C peptide are removed by proteolysis. This gives rise to the A and B peptide chains of the insulin molecule, which contains one intra-subunit and two inter-subunit disulfide bonds.

The A chain usually is composed of 21 amino acid residues, and the B chain has 30; the molecular mass is thus about 5734 daltons. Although the amino acid sequence of insulin has been highly conserved in evolution, there are significant variations that account for differences in both biological potency and immunogenicity (De Meyts, 1994)

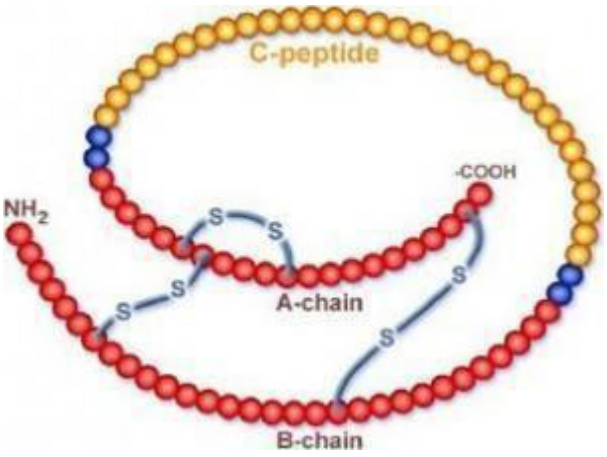


Figure No: 4. Structure of insulin

There is a single insulin gene and a single protein product in most species. However, rats and mice have two genes that encode insulin and synthesize two molecules that differ at two amino acid residues in the B chain.

The crystal structure reveals that the two chains of insulin form a highly ordered structure with a-helical regions in each of the chains. The isolated chains of insulin are inactive.

In solution, insulin can exist as a monomer, dimer, or hexamer. Two molecules of Zn^{2+} are coordinated in the hexamer, and this form of insulin presumably is stored in the granules of the pancreatic **β** cell. It is believed that Zn^{2+} has a functional role in the hexamer formation and that this process facilitates the conversion of proinsulin to insulin and storage of the hormone.

Traditional insulin is hexameric in most of the highly concentrated preparations used for therapy. When the hormone is absorbed and the concentration falls to physiological levels (nanomolar), the hormone dissociates into monomers, and the monomer is most likely the biologically active form of insulin. Monomeric insulin is now available for therapy. (Goodman and Gillman,2006)

Insulin is a member of a family of related peptides termed *insulinlike growthfactors* (IGFs). The two IGFs (IGF-1 and IGF-2) have molecular masses of about7500 daltons and structures that are homologous to that of proinsulin. However, the short equivalents of the C peptide in proinsulin are not removed from the IGFs.

In contrast with insulin, the IGFs are produced in many tissues, and they may serve a more important function in the regulation of growth than in the regulation of metabolism. These peptides, particularly IGF-1, are the presumed mediators of the action of growth hormone, and they originally were called *somatomedins.* The uterine hormone *relaxin* also may be a distant relative of this family of polypeptides, although the relaxin receptor clearly is distinct from those for insulin and IGF-1.

The receptors for insulin and IGF-1 are also closely related (Nakae*et al.*, 2001). Thus, insulin can bind to the receptor for IGF-1 with low affinity and *viceversa.* The growth-promoting actions of insulin appear to be mediated in part throughthe IGF-1 receptor, and there may be discordance between the metabolic potency of an insulin analog and its ability to promote growth.

1.4.2. Synthesis of Insulin

Like other peptide hormones insulin is synthesised as a precursor (preproinsulin) in the rough endoplasmic reticulum. Preproinsulin is transported to the Golgi apparatus, where it undergoes proteolytic cleavage first to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide.[1]

Insulin and C-peptide are stored in granules in B cells, and are normally cosecreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin. The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration.βcells respond both to the absolute glucose concentration and to the rate of change of blood glucose .

Other stimuli to insulin release include amino acids (particularly arginine and leucine), fatty acids, the parasympathetic nervous system, peptide hormones for the gut and drugs that act on *sulfonylurea receptors*.

There is a steady basal release of insulin and also a response to an increase in blood glucose . This response has two phases: an initial rapid phase reflecting release of stored hormone, and a slower, delayed phase reflecting both continued release of stored hormone and new synthesi (Bolli G B *et al* 2000)

1.4.3. Regulation of Insulin Secretion

Insulin secretion is a tightly regulated process designed to provide stable concentrations of glucose in blood during both fasting and feeding. This regulation is achieved by the coordinated interplay of various nutrients, gastrointestinal hormones, pancreatic hormones, and autonomic neurotransmitters. Glucose, amino acids, fatty acids, and ketone bodies promote the secretion of insulin. The islets of Langerhans are richly innervated by both adrenergic and cholinergic nerves. Stimulation of **α**2 adrenergic receptors inhibits insulin secretion, whereas **β2** adrenergic receptor agonists and vagal nerve stimulation enhance release. In general, any condition that activates the sympathetic branch of the autonomic nervous system (such as hypoxia, hypoglycemia, exercise, hypothermia, surgery, or severe burns) suppresses the secretion of insulin by stimulation of **α**2 adrenergic receptors. Predictably, **α**2 adrenergic receptor antagonists increase basal concentrations of insulin in plasma, and **β2** adrenergic receptor antagonists decrease them. (Goodman and Gillman, 2006)

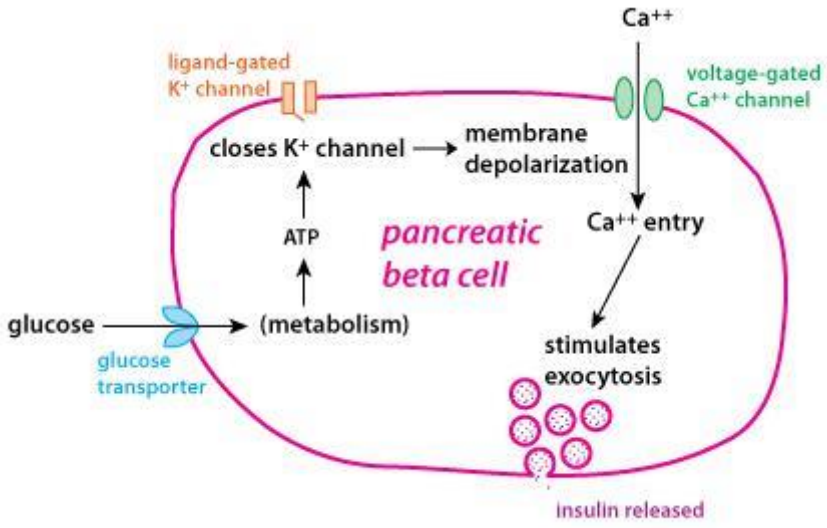


Figure No: 5. Regulation of Insulin Secretion

1.4.4. Distribution and Degradation of Insulin

Insulin circulates in blood as the free monomer, and its volume of distribution approximates the volume of extracellular fluid. Under fasting conditions, the pancreas secretes about 40 mg (1 unit) of insulin per hour into the portal vein to achieve a concentration of insulin in portal blood of 2 to 4 ng/ml (50 to 100 units/ml) and in the peripheral circulation of 0.5 ng/ml (12 units/ml) or about 0.1 nM.

After ingestion of a meal, there is a rapid rise in the concentration of insulin in portal blood, followed by a parallel but smaller rise in the peripheral circulation. A goal of insulin therapy is to mimic this pattern, but this is difficult to achieve with subcutaneous injections.

The half-life of insulin in plasma is about 5 to 6 minutes in normal subjects and patients with uncomplicated diabetes. This value may be increased in diabetics who develop anti-insulin antibodies.

Degradation of insulin occurs primarily in liver, kidney, and muscle (Duckworth, 1988). About 50% of the insulin that reaches the liver *via* the portal vein is destroyed and never reaches the general circulation. Insulin is filtered by the renal glomeruli and is reabsorbed by the tubules, which also degrade it.

Severe impairment of renal function appears to affect the rate of disappearance of circulating insulin to a greater extent than does hepatic disease. Hepatic degradation of insulin operates near its maximal capacity and cannot compensate for diminished renal breakdown of the hormone. Peripheral tissues such as fat also inactivate insulin, but this is of less significance quantitatively (Goodman and Gillman, 2006).

1.4.5. Mechanism of Insulin

Insulin binds to a specific receptor on the surface of its target cells. The receptor is a large transmembrane glycoprotein complex belonging to the kinase-linked type 3 receptor super families and consisting of two **α** and **two β** subunits. Occupied receptors aggregate into clusters, which are subsequently internalised in vesicles, resulting in down-regulation. Internalised insulin is degraded in lysosomes, but the receptors are recycled to the plasma membrane.

- The signal transduction mechanisms that link receptor binding to the biological effects of insulin are complex. Receptor autophosphorylation -the first step in signal transduction-is a consequence of dimerisation, allowing each receptor to phosphorylate the other.

- Insulin receptor substrate (IRS) proteins undergo rapid tyrosine phosphorylation specifically in response to insulin and insulin-like growth factor-1 but not to other growth factors. The best characterised substrate is IRS-1, which contains 22 tyrosine residues that are potential phosphorylation sites.

- It interacts with proteins that contain a so-called SH2 domain, thereby passing on the insulin signal. Knockout mice lacking IRS-1 are hyporesponsive to insulin (insulin-resistant) but do not become diabetic because of robust B-cell compensation with increased insulin secretion. By contrast, mice lacking IRS-2 fail to compensate and develop overt diabetes, implicating the IRS-2 gene as a candidate for human type 2 diabetes.

- Activation of phosphatidylinositol 3-kinase by interaction of its SH2 domain with phosphorylated IRS has several important effects, including recruitment of insulin-sensitive glucose transporters (Glut-4) from the Golgi apparatus to the plasma membrane in muscle and fat cells.

- The longer-term actions of insulin entail effects on DNA and RNA, mediated partly at least by the Rassignalling complex. Ras is a protein that regulates cell growth and cycles between an active GTP-bound form and an inactive GDP-bound form.

- Insulin shifts the equilibrium in favour of the active form, and initiates a phosphorylation cascade that results in activation of mitogen-activated protein kinase, which in turn activates several nuclear transcription factors, leading to the expression of genes that are involved both with cell growth and with intermediary metabolism. Regulation of the rate of mRNA transcription by insulin provides an important means of modulating enzyme activity.

1.4.6. Cellular action of Insulin

Insulin is the main hormone controlling intermediary metabolism, having actions on liver, muscle and fat. It is an anabolic hormone: its overall effect is to conserve fuel by facilitating the uptake and storage of glucose , amino acids and fats after a meal. Acutely, it reduces blood sugar. Consequently, a *fall* in plasma insulin increases blood glucose . The biochemical pathways through which insulin exerts its effects and molecular aspects of its mechanism are discussed below.

1.4.7. Effect of insulin on Carbohydrate metabolism

Insulin influences glucose metabolism in most tissues, especially the liver, where it inhibits glycogenolysis (glycogen breakdown) and gluconeogenesis (synthesis of glucosefrom non-carbohydrate sources) while stimulating glycogen synthesis. It also increases glucoseutilisation (glycolysis), but the overall effect is to increase hepatic glycogen stores.

In muscle, unlike liver, uptake of glucose is slow and is the rate-limiting step in carbohydrate metabolism. The main effects of insulin are to increase facilitated transport of glucose via a transporter called Glut-4, and to stimulate glycogen synthesis and glycolysis.

Insulin increases glucose uptake by Glut-4 in adipose tissue as well as in muscle, enhancing glucoseol, which is esterifies with fatty acids to form triglycerides, thereby affecting fat metabolism metabolism. One of the main end products of glucose metabolism in adipose tissue is glycerol, which is esterifies with fatty acids to form triglycerides, thereby affecting fat metabolism.

1.4.8. Effect of insulin on fat metabolism

Insulin increases synthesis of fatty acid and triglyceride in adipose tissue and in liver. It inhibits lipolysis, partly via dephosphorylation (and hence inactivation) of lipases. It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagon by opposing their actions on adenylatecyclase.

1.4.9. Effect of insulin on protein metabolism

Insulin stimulates uptake of amino acids into muscle and increases protein synthesis. It also decreases protein catabolism and inhibits oxidation of amino acids in the liver.

1.4.10. Other metabolic effect of insulin

Other metabolic effects of insulin include transport into cells of K^{+},[3] Ca^{2+}, nucleosides and inorganic phosphate.

1.4.11. Insulin administration

Because insulin is a polypeptide, it is degraded in the gastrointestinal tract if taken orally. It therefore is generally administered by subcutaneous injection. [Note: In a hyperglycemic emergency, regular insulin is injected intravenously.] Continuous subcutaneous insulin infusion has become popular, because it does not require multiple daily injections.

Insulin preparations vary primarily in their times of onset of activity and in their durations of activity. This is due to differences in the amino acid sequences of the polypeptides. Dose, site of injection, blood supply, temperature, and physical activity can affect the duration of action of the various preparations. Insulin is inactivated by insulin-degrading enzyme (also called insulin protease), which is found mainly in the liver and kidney. (lippincot)

1.4.12. Long term effect of insulin

In addition to its rapid effects on metabolism, exerted via altered activity of enzymes and transport proteins, insulin has long-term actions via altered enzyme synthesis. It is an important anabolic hormone during fetal development. It stimulates cell proliferation and is implicated in somatic and visceral growth and development. (Rang and Dale's)

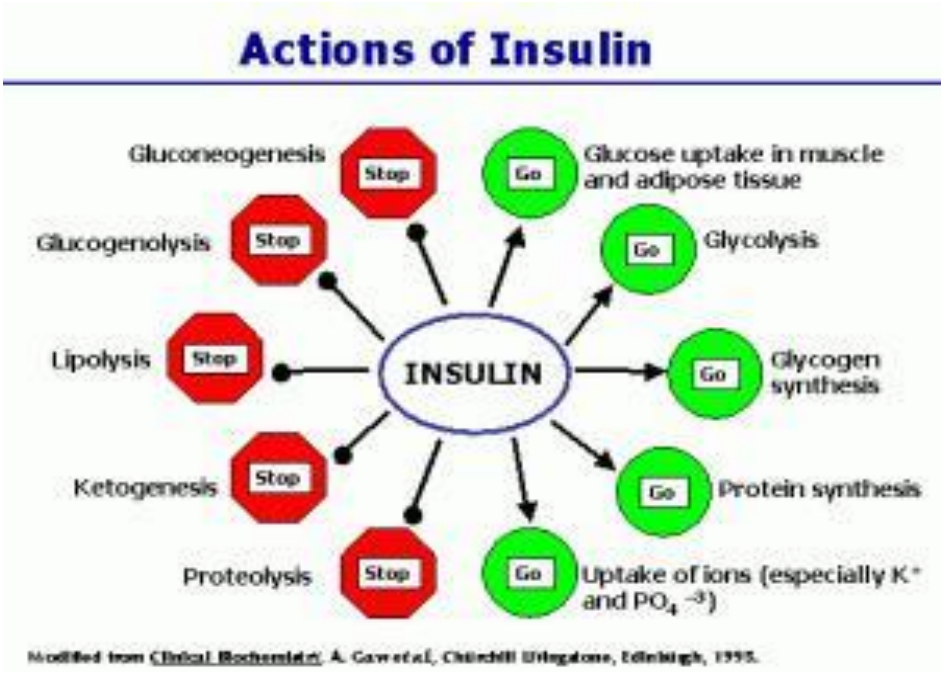


Figure No: 6. Long term effect of insulin

1.4.13. Adverse reactions to insulin

The symptoms of hypoglycemia are the most serious and common adverse reactions to an overdose of insulin. Long-term diabetics often do not produce adequate amounts of the counter-regulatory hormones (glucagon, epinephrine, cortisol, and growth hormone), which normally provide an effective defense against hypoglycemia. Other adverse reactions include weight gain, lipodystrophy (less

common with human insulin), allergic reactions, and local injection site reactions. Diabetics with renal insufficiency may require adjustment of the insulin dose.(lippincot)

1.5. Consequences of diabetes mellitus

People living with diabetes may have to deal with short-term or long-term complications as a result of their condition.

- Short-term complications include hypoglycaemia diabetic ketoacidosis (DKA), and hyperosmolar hyperglycaemic state (HHS).

- Long-term complications include how diabetes affects your eyes (retinopathy), heart (cardiovascular disease), kidneys (nephropathy), and nerves and feet (neuropathy).

1.5.1. Acute complications

These include diabetic ketoacidoses (DKA) and non-ketotic hyper-osmolar state (NKHS). While the first is seen primarily in individuals with type 1 DM, the latter is prevalent in individuals with type 2 DM. Both disorders are associated with absolute or relative insulin deficiency, volume depletion, and altered mental state.

1.5.2. Chronic complications

The chronic complications of diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications (coronary artery disease, peripheral

vascular disease, and cerebrovascular disease). Nonvascular complications include problems such as gastroporesis, sexual dysfunction, and skin changes.

- **Diabetic retinopathy**

Diabetic retinopathy occurs in 3/4 of all persons having diabetes for more than 15 years and is the most common cause of blindness. There is appearance of retinal vascular lesions of increasing severity, culminating in the growth of new vessels.

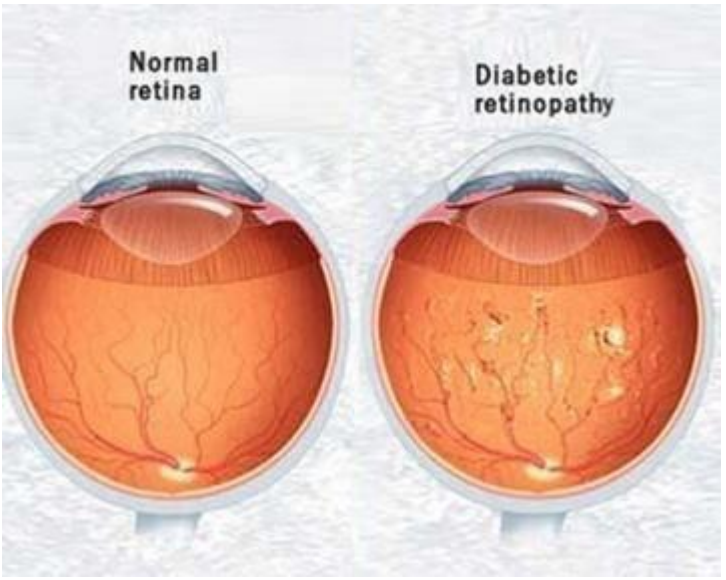


Figure No: 7. Diabetic retinopathy

Diabetic retinopathy is classified into two stages: non proliferative and proliferative.

The non-proliferative stages is marked by retinal vascular microneurisms, blot hemorrhages, and cotton-wool spots and includes loss of retinal pericytes, increased retinal vascular permeability, alterations in regional blood flow, and abnormal retinal microvasculature, all of which lead to retinal ischemia.

In proliferative retinopathy there is the appearance of neovascularization in response to retinal hypoxia. The newly formed vessels may appear at the optic nerve and/or macula and rupture easily, leading to vitreous hemorrhage, fibrosis, and ultimately retinal detachment (Aiello LP *et al*, 1998).

- **Neuropathy**

About half of all people with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy and/or autonomic neuropathy.

In polyneuropathy there is loss of peripheral sensation which, when coupled with impaired microvascular and macrovascular junction in the periphery,can contribute to non-healing ulcers, the leading cause of non-traumatic amputation. There is thickening of axons, decrease in microfi laments, and capillary narrowing involving small myelinated or non-myelinated C-fi bers.

It can occur both from direct hyperglycemia-induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of microvessels, such as endothelial cell activation, pericyte degeneration, basement membrane thickening, and monocyte adhesion.

Mono-neuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems, including cardiovascular, gastrointestinal, genitourinary, sudomotor, and metabolic systems (Chen YD *et al*, 1997).

- **Nephropathy**

This is a major cause of end-stage renal disease. There are glomerular hemodynamic abnormalities resulting in glomerular hyper-filtration, leading to glomerular damage as evidenced by microalbuminurea. There is overt proteinuria, decreased glomerular filtration rate, and end-stage renal failure.

Dysfunction of the glomerular filtration apparatus is manifested by microalbuminurea and is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening.

Another possible mechanism to explain the increase in permeability of the glomerulus is the increase in renal VEGF levels, since VEGF is both an angiogenic and a permeability factor (Ritz E *et al*, 1999).

- **Cardiovascular morbidity and mortality**

In diabetes mellitus there is marked increase in several cardiovascular diseases, including peripheral vascular disease, congestive heart failure, coronary artery disease, and myocardial infarction, and a one- to fivefold increase in sudden death. The absence of chest pain (silent ischemia) is common in individuals with diabetes, and a thorough cardiac evaluation is indicated in individuals undergoing major surgical procedures.

Despite proof that improved glycemic control reduces microvascular complications in diabetes mellitus, it is possible that macrovascular complications may be unaffected or even worsened by such therapies.

An improvement in the lipid profiles of individuals in the intensive group (lower total and low-density lipoprotein cholesterol, lower triglycerides) suggested that intensive therapy may reduce the risk of cardiac vascular mortality.

In addition to coronary artery disease, cerebrovascular disease is increased in individuals with diabetes mellitus (threefold increase in stroke).

Individuals with DM have increased incidence of congestive heart failure (diabetic cardiomyopathy). The etiology of this abnormality is probably multifactorial and includes factors such as myocardiac ischemia from atherosclerosis, hypertension, and myocardial cell dysfunction secondary to chronic hyperglycemia.

Though DM itself does not increase levels of LDL, LDL particles found in type 2 DM are more atherogenic and are more easily glycated and susceptible to oxidation (Grundy SM *et al*, 1999).

- **Hypertension**

Hypertension can accelerate other complications of diabetes mellitus, particularly cardiovascular disease and nephropathy. (Tripathi B.K *et al*)

1.6. ANIMALS USED FOR THE SCREENING OF ANTIDIABETIC DRUG

1. Obese mouse
2. Diabetic mouse
3. Sand mouse (Psammomysobesus)
4. Spiny mouse (Acomyscahirinus)
5. BB rats

7. KK mouse
8. Yellow mouse
9. Yellow KK mouse
10. New Zealand obese mouse
11. Tuco-tuco (clenomystalarum)- these are burrowing rodents from Argentina.
12. Chinese hamster (Cricetulusgriseus)
13. NOD mouse
14. Japanese wistar rat (Goto rat) etc. (www.Pharma info.net)

1.6.1. CHEMICALS USED TO INDUCE DIABETES MELLITUS

A. Irreversible beta cytotoxic agents

1. *Alloxan*
2. *Streptozotocin*
3. *Diphenylthiocarbazine*
4. Oxine-9-hydroxyquinolone
5. Vacor

B. Reversible beta cytotoxic agents

1. 6-amino nicotinamide
2. l-aspartase
3. Azide
4. Cyanide

5. *Cyproheptadine*
6. *Phenytoin* (www.Pharmainfo.net).

Commonly used diabetes inducing agents are,

1. *Alloxan monohydrate*
2. *Streptozotocin (STZ)*

Alloxan Monohydrate

The name *alloxan* is derived from Allantoin, a product of uric acid excreted by the fecus into the allantois and oxaluric acid derived from oxalic acid and urea, found in urine.

Biological effects

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas **(β** cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (*Alloxan* Diabetes) in these animals, with characteristics similar to type I diabetes in humans.

Mechanism of action

Alloxan is selectively toxic to insulin-producing pancreatic beta cells becauseit preferentially accumulates in beta cells through uptakes via the GLUT2 glucose transporter. *Alloxan,* in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid.

The beta cell toxic action of *alloxan* is initiated by free radicals formed in this redox reaction. One study suggests that *alloxan* does not cause diabetes in humans. Other

show some correlation between *alloxan* plasma level and diabetes Type I in children. (www.wikipedia.com)

Strepetozotocin

Streptozocin or *Izostazin* or *Zanosar* (STZ) is a synthetic antineoplastic agentthat is classifically an anti-tumor antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy. *Streptozotocin* sterile powders are provided and prepared a chemotherapy agent.

Each vial of sterilized *Streptozotocin* powder contains 1 gr. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[[(methylnitrosoamino) carbonyl] amino]-D-glucopyranose and 200 mg. citric acid. *Streptozotocin* was supplied by Pharmacia Company. *Streptozotocin* is available for intravenous use as a dry-frozen, pale yellow, sterilized product. Pure *Streptozotocin* has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial will be 3.5-4.5 because of the presence of citric acid. This material is prepared in 1-gr vials and kept in cold store and refrigerator temperature (2-8 °C) away from light. (A. Akbarzadeh *et al*, 2007)

Glibenclamide

Glibenclamide is a popular antidiabetic drug, belonging to class ofsulfonylureas.The drug is widely used for treating type II diabetes.(www.neisslabs.com)

Mechanism of action

The drug works by inhibiting ATP-sensitive potassium channels in pancreatic β cells. This inhibition causes cell membrane depolarization, opening of voltage

dependent calcium channels, thus triggering an increase in intracellular calcium into the beta cell which stimulates insulin release. (www.neisslabs.com)

Dosage

Dosage should be adapted to each individual patient and is determined by results of medical examinations. In general the initial dose is 2, 5 mg daily (half a *glibenclamide* tablet). The daily dose can then be raised gradually in steps of halftablets, but only after repeating medical examination. Raising the dose beyond three tablets daily dose not produce any increased response. When changing over from another oral antidiabetic preparation, with a similar mode of action, the dosage of *Glibenclamide* is determined by the amount of the previously administered dose and themedical examination. (www.neisslabs.com)

LITERATURE REVIEW

Literature review is the first and most important step for the proper selection of plants and it also forms basis for the planning of any scientific work that has to be performed. Due to this reason, there view of literature regarding has been done under various divisions like Pharmacognostical, Phytochemical, Pharmacological, Ethno medical and also miscellaneous reviews www.indianmedicine.eidoc. ub.rug.nl/).

1. Babu RK, Vinay K, Sameena SK, Prasad SV, Swapna S, Appa Rao ARC. Antihyperglycemic and antioxidant effects of Talinum portulacifolium leaf extracts in streptozotocin diabetic rats: a dose-dependent study. *Pharmacognosy Magazine*. 2009;5(19):1–10.

2. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocin induced diabetic rats. *Food and Chemical Toxicology*.2008;46(7):2376–2383.

3. Cho WCS, Chung WS, Lee SKW, Leung AWN, Cheng CHK, Yue KKM. Ginsenoside Re of Panax ginseng possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats.*European Journal of Pharmacology*. 2006;550 (1–3):173–179.

4. Abdul Nabi S, Kasetti RB, Sirasanagandla S, et al. Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. *BMCComplementary and Alternative Medicine*. 2013;13, article 37

5. Pari L, Srinivasan S. Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. *Biomedicineand Pharmacotherapy*. 2010;64(7):477–481.

6. Kirtikar KR, Basu BD. *Indian Medicinal Plants with Illustration*. 2nd edition. Vol. 3. Dehra Dun, India: International Book Distributors; 2005.

7. James MB, Tewin T. *Rhinacanthus nasutus* protects cultured neuronal cells against hypoxia induced cell death. *Molecules*. 2011;16(8):6322–6338.

8. Kupradinun P, Siripong P, Chanpai R, Piyaviriyagul S, Rungsipipat A, Wangnaitham S. Effects of *Rhinacanthus nasutus* Kurz on colon carcinogenesis in mice. *Asian Pacific Journalof Cancer Prevention*.2009;10(1):103–106.

9. Rojanapo W, Tepsuwan A, Siripong P. Mutagenicity and antimutagenicity of Thai medicinal plants. *Basic life sciences*. 1990;52:447–452.

10. Visweswara Rao P, Madhavi K, Dhananjaya Naidu M. Hypolipideic properties of *Rhinacanthus nasutus* in streptozotocin-induced diabetic rats. *Journal of Pharmacologyand Toxicology*. 2011;6:589–595.

11. Visweswara Rao P, Sujana P, Vijayakanth T, Dhananjaya Naidu M. *Rhinacanthusnasutus*—its protective role in oxidative stress and antioxidant status in streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Disease*. 2012;2(4):327–330.

12. Kasetti RB, Rajasekhar MD, Kondeti VK, et al. Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food and ChemicalToxicology*. 2010;48(4):1078–1084.

13. Rao PV, Naidu MD. Anti diabetic effect of *Rhinacanthus nasutus* leaf extract in streptozotocin induced diabetic rats. *Libyan Agriculture Research Center JournalInternational*. 2010;1(5):310–312.

14. Harborne JB. *Phytochemical Methods a Guide to Modern Techniques of PlantAnalysis*. 3rd edition. New Delhi, India: Springer; 2005.

15. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 4th edition. Pune, India: NiraliPrakashan; 1996.

16. Peach K, Tracey MV. *Modern Method of Plant Analysis*. New Delhi, India: Narosa Publishing House; 1959.

17. Trease GE, Evans WC. *Pharmacognosy*. 11th edition. London, UK: Bailliere Tindall; 1978.

18. Prameelamma Y, Swami KS. Glutamate dehydrogenase activity in the normal and denervated gastrocnemius muscle of frog. Rana hexadactyla. *Current Science*. 1975;44:739–740.

19. Lohr GD, Waller HD. Glucose 6-phosphate dehydrogenase. In: Bergmayer HU, editor. *Methods in Enzymatic Analysis*. Vol. 2. London, UK: Academic Press; 1974. pp. 636–643.

20. Nachlas MM, Margulies SI, Goldberg JD, Seligman AM. The determination of lactic dehydrogenase with a tetrazolium salt. *Analytical Biochemistry*. 1960;1:317–326.

21. Lee YP, Lardy HA. Influence of thyroid hormones on L-***α***-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat. *The Journal ofBiological Chemistry*. 1965;240:1427–1436.

22. Farswan M, Mazumder PM, Percha V. Protective effect of Cassia glauca Linn. on the serum glucose and hepatic enzymes level in streptozotocin induced NIDDM in rats. *IndianJournal of Pharmacology*.2009;41(1):19–22.

23.Patel SS, Goyal RK. Prevention of diabetes-induced myocardial dysfunction in rats using the juice of the Emblica officinalis fruit. *Experimental & ClinicalCardiology*. 2011;16:87–91.

24. Prasenjit M, Parames CS. Impaired redox signaling and mitochondrial uncoupling contributes vascular inflammation and cardiac dysfunction in type 1 diabetes: protective role of arjunolic acid. Biochimie.2012;94:786–797.

25. Singh SN, Vats P, Suri S, et al. Effect of an antidiabetic extract of Catharanthusroseuson enzymic activities in streptozotocin induced diabetic rats. Journal ofEthnopharmacology. 2001;76(3):269–277.

26. Ainscow EK, Zhao C, Rutter GA. Acute overexpression of lactate dehydrogenase-A perturbs**β**-cell mitochondrial metasbolism and insulin secretion. Diabetes. 2000;49(7):1149–1155.

27. Narendhirakannan RT, Subramanian S, Kandaswamy M. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. Clinical and Experimental Pharmacology and Physiology. 2006;33(12):1150–1157.

28. Luzi L, Pozza G. Glibenclamide: an old drug with a novel mechanism of action. Acta Diabetol. 1997;34:239–244.

29. Satav JG, Katyare SS. Effect of streptozotocin-induced diabetes on oxidative energy metabolism in rat liver mitochondria—a comparative study of early and late effects. *IndianJournal of Clinical Biochemistry*.2004;19(2):23–31.

30. Dudley GA, Staron RS, Murray TF. Muscle fiber composition and blood ammonia levels after intense exercise in humans. *Journal of Applied Physiology RespiratoryEnvironmental and Exercise Physiology*.1983;54(2):582–586.

31. Rasineni K, Bellamkonda R, Singareddy SR, Desireddy S. Antihyperglycemic activity of *Catharanthus roseus* leaf powder in streptozotocin-induced diabetic rats. *PharmacognosyResearch*. 2010;2(3):195–201.

32. Sellamuthu PS, Muniappan BP, Perumal SM, Kandasamy M. Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rats. *Journal of Health Science*. 2009;55(2):206–214.

33. Aseervatham J, Palanivelu S, Panchanadham S. *Semecarpus anacardium* (Bhallataka) alters the glucose metabolism and energy production in diabetic rats. *Evidence-based Complementary and Alternative Medicine*.2011;2011:9 pages.142978

34. Díaz-Flores M, Ibáñez-Hernández MA, Galván RE, et al. Glucose-6-phosphate dehydrogenase activity and NADPH/$NADP^+$ ratio in liver and pancreas are dependent on the severity of hyperglycemia in rat. *Life Sciences*.2006;78(22):2601–2607.

35. Jiang H, Xie Z, Koo HJ, McLaughlin SP, Timmermann BN, Gang DR. Metabolic profiling and phylogenetic analysis of medicinal Zingiber species: tools for authentication of ginger (Zingiber officinale Rosc.) *Phytochemistry*. 2006;67(15):1673–1685.

36. Shankar PK, Kumar V, Rao N. Evaluation of antidiabetic activity of ginkgo biloba in streptozotocin-induced diabetic rats. *Iranian Journal of Pharmacology and Therapeutics*. 2005;4:16–19.

37. Gandhi GR, Ignacimuthu S, Paulraj MG. Hypoglycemic and b-cells regenerative effects of Aegle marmelos (L.) Corr. Bark extract in streptozotocin-induced diabetic rats. *Food andChemical Toxicology*. 2012;50:1667–1674.

38. Newsholme P, Haber EP, Hirabara SM, et al. Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *TheJournal of Physiology*. 2012;583:9–24.

3. AIM AND PLAN OF WORK

3.1 AIM OF PRESENT STUDY

In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications.

The selection of this plant, *Rhinacanthusnasutus* was made on the basis of its

- High therapeutic value
- Easy availability
- Degree of research work which is not done

Very less pharmacological studies have been carried out on the leaves of Hence, I have decided to choose the *Rhinacanthus nasutus* project on Which *Rhinacanthus nasutus* includes detailed studies on Preliminary Phytochemical and Pharmacological activities of Oral glucose tolerance, and In-vivo Anti- diabetic studies.

3.2 THE PLAN OF WORK

The plan of work for the study of *Rhinacanthus nasutus* was carried out as follows

Collection and authentifiction of raw material

1. **Pharmacognostical studies**
    a. Ash value
        - Total ash
        - Water soluble ash
        - Acid insoluble ash
    b. Extractive value
    c. Loss on drying
2. **Preliminary phytochemical studies**
    a. Preparation of extract
    b. Qualitative phytochemical studies
3. **Pharmacological studies**
    a. Acute oral toxicity study
    b. Oral glucose tolerance test
    c. Screening of Anti- diabetic activity
        - *In-vivo* study of anti-diabetic activity (*Alloxan* induced diabeticRat)

4. PLANT PROFILE

Plant Classification	**: Rhincanthus nasutus**
Kingdom	**: Plantae**
Order	**: Lamiales**
Family	**: Acanthaceae**

Native to India, this useful plant is a slender, erect, branched, somewhat hairy shrub 1-2 m in height. The leaves are oblong, 4-10 cm in length, and narrowed and pointed at both ends. The inflorescence is a spreading, leafy, hairy panicle with the flowers usually in clusters. The calyx is green, hairy, and about 5 mm long. The corolla-tube is greenish, slender, cylindric, and about 2 cm long. The flowers is 2-lipped; the upper lip is white, erect, oblong or lance like, 2-toothed at the apex, and about 3 mm in both length and width; and the lower lip is broadly obovate, 1.1-1.3 cm in both measurements, 3-lobed, and white, with a few, minute, brownish dots near the base. The fruit (capsule) is club-shaped and contains 4 seeds. *Rhinacanthusnasutus*is a herb used in traditional system of medicine. It is native to Sri Lanka, India, Malaysia, Philippine Islands and Tropical Africa. In Sri Lanka, it is a very common road-side plant at the edges of the jungle in dry region of Tissamaharama, Polonnaruva. Appearance and shape of the flower is very similar to a white water crane1.

The aim of the present study is to collect and record therapeutic usages of *R. nasutus* phytochemicals and scientifically proven bio activities of this plant. R. nasutus is used in treatment of common disorders including cancer, fungal infections, eczema, pulmonary tuberculosis and herpes virus infections. R. *nasutus* can be used for health promotion due to its immunomodulating activity. Through various researches, it has been reported that the plant has no toxicity. *R. nasutus* contains several chemical compounds such as rhinacanthin A, B, C, and D which are active against human cytomegalovirus, microbials, diabetes mellitus, cancers and hypertension. Preparations of *R. nasutus* are very effectively used by traditional practitioners in Sri Lanka in treatment of skin diseases.

Part Used:

Leaf:

The leaves are oblong, 4-10 cm in length, and narrowed and pointed at both ends. The inflorescence is a spreading, leafy, hairy panicle with the flowers usually in clusters. The calyx is green, hairy, and about 5 mm long. The corolla-tube is greenish, slender, cylindric, and about 2 cm long.

RhinacanthusNasutus – Flowers

RhinacanthusNasutus – Plant

5. MATERIALS AND METHODS

5.1 COLLECTION AND IDENTIFICATION

5.1.1 Collection of specimen:

The species for the proposed study that is leaves of Rhinacanthus Nasutus collected carefully from the Kollimalai hills, Namakkal Dt, Tamilnadu.

5.1.2 Taxonomical identification:

5.1.3 Shade drying:

After collection, the leaves of we *Rhicanthus nasutus* rewashed thoroughly with water to remove the dirt particles and ant other foreign material adheres to leaves. Then after, the leaves were wiped off with cotton cloth and transferred to newspaper and evenly spreader on to paper.

The *Rhicanthus nasutus* leaves were subjected to shade drying to treat fungus until complete dryness of leaves. Then the dried leaves were powdered by mixer grinder until to get coarse powder, which was used for further detailed studies, extraction with solvent and phytochemical studies.

5.2 PHARMACOGNOSTICAL STUDIES

5.2.1 ANALYTICAL PARAMETERS

5.2.1.1 Physico-chemical Parameters:

A. Ash value: (Kokate*et al.*,1985)

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form.

Principle:

The ash content of a crude drug is generally taken to be the residue remaining after incineration. Ash standards have been established for a number of official drugs. Usually these standards get a maximum limit on the total ash or on the acid insoluble ash permitted.

The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.

The ash or residue yielded by an organic chemical compound is as a rule, a measure of the amount of inorganic matters present as impurity. In most cases, the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drugs in powder form. Procedures given in Indian pharmacopoeia were used to determine the different ash values such as total ash and acid insoluble ash.

1) Determination of total Ash value:

Weighed accurately about 3 gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon cooled and weighted and then calculated the percentage of total ash with reference to the air dried drug.

2) Determination of acid insoluble ash value:

The ash obtained as directed under total ash above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

3) Determination of Water soluble ash value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding **450˚C.** The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

B. LOSS ON DRYING:

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Desiccators or

hot air oven). If the sample is in the form of large crystals, then reduce the size by quick crushing to a powder.

Procedure:

About 1.5 gm of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105˚**C** in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

C. EXTRACTIVE VALUES:

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, value indicates the nature of the constituents present in a crude drug.

1) Determination of alcohol soluble extractive value:

5 gm of the air-dried coarse powder of the Leaves bodies of *Rhinacanthus Nautus* was macerated with 100ml of 90% ethanol in a closed flask for24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug. The results are recorded in the table.

2) Determination of water soluble extractive value:

Weigh accurately 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in closed flak for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

5.2.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of *Rhinacanthus Nasutus* **:**

Ethanol extract:

About 250gm of air dried powdered material was taken in 3000ml soxhlet apparatus and extracted with petroleum ether for 7 days. At the end of 7^{th} day the powder was taken out and dried. After drying it was again packed and extracted by using ethanol (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. The temperature was maintained at 55ºC-65ºC. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of ethanolic extract were noted.

Table: 5.1 Nature of extract of *Rhinacanthus Nautus*

S.No.	Name of extract	Colour	Consistency	Yield% W/W
1.	Ethanolic extract	Greenish	Sticky mass	6%

5.3 CHEMICAL TESTS:

A) Test for carbohydrates:

1. **Molisch's Test:** It consists of treating the compounds ofα-naphthol andconcentrated sulphuric acid along the sides of the test tube.

   Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C.K *et al.*,, 2000)

2. **Fehling's Test:** Equal quantity of Fehling's solution A and B is added. Heatgently, brick red precipitate is obtained.

3. **Benedict's test:** To the 5ml of Benedict's reagent, add 8 drops of solution underexamination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.

4. **Barfoed's test:** To the 5ml of the Barfoed's solution add 0.5ml of solution underexamination, heat to boiling, formation of red precipitate of copper oxide is obtained.

B) Test for Alkaloids:

1. **Dragendroff's Test:** To the extract, add 1ml of Dragendroff's reagent Orange redprecipitate is produced.

2. **Wagner's test:** To the extract add Wagner reagent. Reddish brown precipitate isproduced.

3. **Mayer's Test:** To the extract add 1ml or 2ml of Mayer's reagent. Dull whiteprecipitate is produced.

4. **Hager's Test:** To the extract add 3ml of Hager's reagent yellow Precipitate isproduced.

C) Test for Steroids and Sterols:

1. **Liebermann Burchard test:** Dissolve the test sample in 2ml of chloroform in adry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. **Salkowski test:** Dissolve the sample of test solution in chloroform and add equalvolume of conc. sulphuric acid. Bluish red cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides:

1. **Legal's test:** Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.

2. **Baljet test:** To the drug sample, sodium picrate solution is added. Yellow too range colour is produced.

3. **Borntrager test:** Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. **Killer Killani test:** Sample is dissolved in acetic acid containing trace of ferricchloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins:

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids:

Shinoda test: Red colour is produced when the sample, magnesium turnings and then concentrated hydrochloric acid is added.

Ferric chloride test – Test solution when treated with few drops of Ferric chloridesolution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution,shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test – Test solution when treated with few drops of leadacetate (10%) solution would result in the formation of yellow precipitate.(Sathees kumar bhandary et al., 2012)

G) Test for Tri-Terpenoids:

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Tests for Tannins and Phenolic Compounds:

The Phenol content in the raw material of caesalpiniasappan extract was estimated by spectroscopically method.

To 2-3 ml of extract, add few drops of following reagents:

a) 5% $FeCl_3$ solution: deep blue-black color.

b) Lead acetate solution: white precipitate.

c) Gelatin solution: white precipitate

d) Bromine water: decolouration of bromine water.

e) Acetic acid solution: red color solution

f) Dilute iodine solution: transient red color.

g) Dilute HNO_3: reddish to yellow color.

I) Test for Fixed Oils and Fatty acids

a). Spot test:

Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

b). Saponification test:

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

J) Test for Gums and Mucilage:

Ruthenium red test:

Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage.

K) Test for Proteins and Amino acids

a) Biuret test:

Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

b) Ninhydrin test:

Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

c) Xanthoprotein test:

To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

5.4 TOXICOLOGICAL EVALUATION

Determination of LD_{50}value of ethanolic extract of Rhinacanthus Nasutus
Acute Oral Toxicity Study:

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the

animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which cause acute toxicity.

Animals:

Female albino mice weighing 20-25g were used in the present study. All rats were kept at room temperature of 22-25ºC in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions.

Procedure:

Twelve animals Albino mice, (25-30gm) were selected for studies.

The starting dose of ethanolic extracts of RhinacanthusNasutus\300mg/kg, b.w, p.o, was administered.

Most of the crude extracts possess LD_{50}, value more than 2000mg/kg of the body weight of the animal used. Dose volume was administered 0.1ml/100gm body weight to the animal by oral route.

After giving the dose toxic signs were observed within 3-4 hours. Body weight of the animals before and after administration, onset of toxicity and signs of toxicity

like changes in the skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, sign of tremors, convulsion, salivation, diarrhea, lethargy and sleep and coma was also to be noted, if any, was observed. The animal toxic or death was observed up to 14 days.

Observation

Acute toxicity studies and evaluation of dates are studied as per the guideline of OECD (423).

No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD_{50} of the ethanolic extract of leaves of Rhinacanthus Nasutus was greater than 2000mg/kg (LD_{50}>2000mg/kg).

Hence the biological dose was fixed at three levels, 200 and 400mg/kg body weight for the extract.

5.5 PHARMACOLOGICAL EVALUATION:

5.5.1 ORAL GLUCOSE TOLERANCE TEST (OGTT):

The overnight fasted (18hr) normal rats were taken and divided into four groups consists of six animals. They were provided with drinking water only. Normal saline solution was administered to group I animals. Group II animals were received *Glibenclamide* (3mg/kg,b.w) as a standardRhinacanthusNasutusethanol extract(200 and 400 mg/kg) was administered by oral route to group III and IV Glucose (2mg/kg) load was fed 30 minutes after the administration of extracts. Blood was withdrawn from tail vein under mild ether anesthesia initial, 30,60 and 90

minutes after glucose administration (V.Babu *et al.,* 2003) and glucose level were estimated using glucose strips and a glucometer (Standard diagnostics Ltd). Blood glucose levels were noted and reported.

5.5.2 EVALUATION OF ANTI-DIABETIC ACTIVITY:

Animals:

Female albino- Wistar rats weighing 150-250g were used in the present study. All rats were kept at room temperature of 22-25ºc in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions in accordance with the recommendations for the proper care and use of laboratory animals.

Chemicals:

Alloxan monohydrate (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study.

Glibenclamide is an oral antidiabetic preparation with an efficient hypoglycemic action. Diaonil *(Glibenclamide)* (S.K.Prasad *et.al*, 2009) manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature.

Induction of Experimental Diabetes:

The adult albino- wistar rats (150-250gm) were overnight fasted and determine the fasting blood glucose level. The sequence blood glucose level of animals were selected and except group I animals used to induce diabetes by single i.p injection of 120 mg/kg of *Alloxan monohydrate* was dissolved in normal saline (pH-4.5). Animals were fed with 5% glucose solution in order to prevent hypoglycemic shock for 18 hrs (Prince PSM *et al.,* 1989). Hyperglycemia is to be confirmed the elevated blood glucose levels, determined at 72 hrs and then on day 0 after injection. The threshold value of fasting blood glucose level >200mg/dl was taken as diabetic animal and rats found with permanent diabetes were used for the antidiabetic study.

Experimental Design:

Experimental rats were divided into 5 groups of six animals each all the group of animals were induced diabetic except control and treated for 21days as follows.

- Group I: Normal control rats fed with vehicles only. (Normal saline with 1%CMC)
- Group II: Diabetic controls rats (*Alloxan monohydrate* 120mg/kg body weight of rats, once i.p injection).
- Group III: Diabetic rats treated with standard drug, *Glibenclamide* 3mg/kg per oral body weight.
- Group IV: Diabetic rats treated with ethanolic extract of RhinacanthusNasutus200mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).

- Group V: Diabetic rats treated with ethanolic extract of RhinacanthusNasutus400mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).

Sample collection:

Fasting blood glucose (FBG) of all rats was determined before the start of the experiment. Blood sample was collected at weekly intervals from tail vein puncture till the end of study. In the continuous 21 days of drug treatment, a blood glucose level of all animals was determined at the 0, 7, 14, 21 day by using one touch glucometer (SD Check) method.

On day 21, overnight fasted animals were under mild ether anaesthesia, the blood was collected by direct cardiac puncture. Blood was collected in tubes containing EDTA as anticoagulant for estimation of fasting plasma glucose and HbA1c.

Plasma was separated by centrifugation at 3000 rpm for 10 minutes, the clear supernatant colorless liquid was used for the analysis of various biochemical parameters. The pancreas tissues were excised and rinsed in ice-cold saline and kept in formalin solution for further histopathological studies.

5.6 EVALUATION OF PARAMETERS:

1. Estimation of changes in body weight of the animals:

Body weight of all rats was measured on starting day (0 day) of the experiment and 21stday of the experiment. Both initial and final body weights were noted and reported.

2. Estimation of blood glucose level:

Glucose level in plasma was estimated by glucose oxidase/ peroxidase method using a commercial kit from Med source Ozone Biomedicals Pvt Ltd followed by Trinder, p.(1969)Annals.Clin. Bio chem.6, 24.

Reagents:

1. Enzyme reagent
2. Buffer solution
3. Glucose standard (100 mg%)

Procedure:

10 µl of plasma was added to 1.0 ml of working enzyme reagent, mixed well and incubated at 37°C for 15 min. The colour developed was read at 505 nm against blank containing distilled water instead of the sample. A standard was also processed similarly.

The level of glucose is expressed as mg/dl.

3. Estimation of total cholesterol:

Total cholesterol was estimated by the following method

Reagents:

1. Ferric chloride-acetic acid reagent: 0.05% ferric chloride in acetic acid.
2. Concentrated sulfuric acid.
3. Cholesterol stock standard: 1 mg/ ml in acetic acid.
4. Cholesterol working standard: 40µg in ferric chloride-acetic acid reagent.

Procedure:

0.1ml of plasma was evaporated to dryness and 5.0 ml ferric chloride – acetic acid reagent was added, mixed and centrifuged. To the supernatant 3.0 ml of concentrated sulfuric acid was added and the absorbance was read after 20min at 560nm against a reagent blank. A standard in the concentration range of 40-200µg was treated similarly.

Values were expressed as mg/dl Plasma.

4. Determination of triglycerides:

Triglycerides were determined by the following method.

Triglycerides are extracted by isopropanol, which upon saponification with potassium hydroxide yield glycerol and soap. The glycerol liberated is treated with meta per iodate, which releases formaldehyde, formic acid and iodide. The formaldehyde released reacts with acetyl acetone and ammonia forming yellow coloured compound, the intensity of which is measured at 420nm.

Reagents:

1. Isopropanol

2. Activated aluminium oxide (neutral)

3. Saponification reagent- 5 g of potassium hydroxide was dissolved in 60ml of distilled water and 40ml of isopropanol was added to it

4. Sodium meta per iodate reagent -77 g of anhydrous ammonium acetate was dissolved in about 700ml of distilled water, 60ml glacial acetic acid was added to

it followed by 650mg of sodium meta periodate. The mixture was diluted to 1litre with distilled water

5. Acetyl acetone reagent – 0.75 ml of acetyl acetone was dissolved in 60 ml of distilled water and 40 ml of isopropanol was added to it

6. Standard triolein solution – 1 g of triolein was dissolved in 100ml isopropanol. 1 ml of stock standard was diluted to 100 ml to prepare a working standard 100 µg of triolein/ml.

Procedure:

An aliquot of plasma lipid extract was evaporated to dryness. 0.1 ml of methanol was added followed by 4 ml isopropanol, 0.4 g of alumina was added to all the tubes and shaken well for 15 min. Centrifuged and then 2 ml of the supernatant was transferred to labeled tubes. The tubes were placed in a water bath at 65ºC for 15 min for saponification after adding 0.6 ml of the saponification reagent followed by 0.5 ml of acetyl acetone reagent.

After mixing, the tubes were kept in a water bath at 65ºC for 1 hr, the contents were cooled and absorbance was read at 420nm. A series of standards of concentrations 8-40µg triolein were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 420nm.

The triglyceride content was expressed as mg/dL.

5. Estimation of HDL –Cholesterol:

The HDL – Cholesterol was estimated by the reagent precipitation method using phosphotungstic acid in presence of Mg^{2+}.

Reagent:

a) HDL – Cholesterol precipitating reagent

b) Phosphotungstic acid

c) Magnesium chloride

Procedure:

To a test tube add 0.5ml sample and 0.5ml HDL – precipitating reagent. Mixed thoroughly and centrifuged at 4000rpm for 10 minutes. Then a supernatant was obtained. Take three clean, dey test tubes and labeled as test, standard and blank. Take 0.05ml supernatant in test–test tube and 0.058ml standard in standard test tub. Then add 1ml working solution to all these three tubes and incubate 37°C for 10 min. measure the absorbance of all three.(www.ncbi.nlm.nih.gov)

Calculation:

$$\text{HDL – Cholesterol (mg \%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Normal value: 30 – 70 mg%

6. Estimation of LDL – Cholesterol and VLDL – Cholesterol

The amounts of LDL - cholesterol and VLDL - cholesterol were calculated by using Friedwald follows.

VLDL-Cholesterol =Triglycerides/5

LDL-Cholesterol = Total cholesterol-(HDL +VLDL)

The levels of HDL, LDL and VLDL-cholesterol are expressed as mg/dL.

7. Estimation of Alkaline Phosphatase

The activity of alkaline phosphatase was estimated by pNPP-AMP method. The colour formed was measured at 450nm and is proportional to ALP activity in the sample.

Reagents:

a) AMP-(2-amino-2methyl-1-propanol) buffer

b) p-NPP substrate

Procedure:

1000µl of the working ALP reagent and 20µl of serum were taken in a test tube. Mixed well and read the absorbance at 405nm.

Calculation:

ALP activity (IU/L) = $^{\Delta}$A/min × kinetic factor (2712)

Where, $^{\Delta}$A/min - Change in absorbance per minute.

8. Estimation of total protein:

The amount of total protein present in the given serum sample was estimated by Biuret method.

Reagents:

a) Biuret reagent

b) Total protein standard

Procedure:

Take 3 clean test tubes and pipette out 1 ml biuret reagent into it.Added 0.01ml protein standard in protein test tube and 0.01ml serum sample in test. Incubated at 37°C for 5min. Measure the absorbance of test and standard against blank at 546nm.

Calculation:

$$\text{Total protein (gm \%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Normal value: 6.3 - 8.4 gm%

9. Estimation of Albumin:

The amount of albumin present in the sample was estimated by BCG dye method. Albumin binds with Bromocresol green (BCG) at pH4.2 causing a shift in the absorbance of the yellow BCG dye. The blue green colour formed in proportional to the concentration of albumin present, when measured photometrically between 580-630nm with maximal absorbance at 625nm.

Reagents:

a) Albumin reagent

b) Albumin standard

Procedure:

Take 3 clean test tubes. Pipette out 1000µl of albumin reagent in all tubes. Add 10µl of albumin standard and 10µl sample serum in standard and test-test tubes respectively. Mix well and incubate all room temperature for 1min. measure the absorbance of test and standard against blank at 630nm.

Calculation :

$$\text{Albumin} = \frac{\text{Albumin of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Normal value: 3.2 - 5.0 g/dl

10. Estimation of uric acid:

The amount of uric acid present in a given serum sample is estimated by modified Trinder peroxide method using TBHB.

Reagents:

a) Uric acid reagent

b) Uric acid standard

Procedure:

Take 3 clean dry test tubes and pipette out 1000µl of uric acid reagent into it. Add 20µl of uric acid standard in standard test tube and 20µl of serum sample in test-test tube. Mix well and incubate for 5 min at 37°C. Read the absorbance of standard and test against blank at 505nm.

Calculation:

$$\text{Uric acid (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Normal value:

Male: 3.5-7.2 mg/dl

Female: 2.5-6.2 mg/dl

11. Estimation if creatinine:

The amount creatinine present in the given serum sample is estimated by initial rate method.

Reagent:

a) Picrate reagent

b) Diluent reagent

Procedure:

1ml of working reagent and 0.05ml of serum reagent was taken in a test tube. Mix well and read the absorbance at 492nm.

Calculation:

$$\text{Creatinine (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 2$$

Normal value:

Male: 0.7 - 1.2mg%

Female: 0.5 - 1.0mg%

The levels of HDL, LDL and VLDL-cholesterol are expressed as mg/dL.

5.7 THE HISTOLOGICAL STUDY

After blood sampling for the biochemical analysis, the animals were sacrificed, quickly dissected and small slices of pancreas were taken and fixed in 10% formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6µm in thickness were prepared and stained with Haematoxylin and Eosin then examined under microscopy (Pearse AG. 3rd edn, vol.I)

5.8 STATISTICAL ANALYSIS

All the values of body weight, fasting blood glucose level, and biochemical parameter estimations were expressed as mean ± standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multible comparison test, using InStat v.2.02 software (GraphPad Software Inc.). Differences between groups (p Value) were considered significant at $P<0.05$ level.

All data were graphically represented by using Prism Software V 2.02.

6. RESULTS AND DISCUSSION

Based on literature review the leaves of RhinacanthusNasutuswere selected and project work was carried out in RhinacanthusNasutusbelonging to the family *Acanthaceae*was collected and authenticated. The result of the present studyshow that the ethanol extract exerts anti-diabetic RhinacanthusNasutus effect against alloxan induced diabetes.

6.1 PHARMACOGNOSTICAL STUDIES

6.1.1 ANALYTICAL PARAMETERS

The analytical parameters were investigated and reported as, total Ash value (9.2%w/w), acid insoluble ash value (2.9%w/w), water soluble ash value (3.2%w/w), water soluble extractive (11.2), alcohol soluble extractive(3.3) and loss on drying (4%w/w). The above studies were enabled to identify the plant material for further investigation and from an important aspect of drug studies. The results were given in Table No.: 6.1, 6.2.

Ash valuess

Table No.6.1: Data for ash values for powdered leaves of *Rhicanthus nasutus*

Ashes	Ash values(%w/w)
Total ash	9.2
Acid insoluble ash	2.9
Water soluble ash	3.2

2. Extractive values and Loss on drying

Table No.6.2: Data for extractive values and loss on drying for powered leaves of RhinacanthusNasutus

Analytical parameters	Percentage(%w/w)
Water soluble extractive	11.2
Alcohol soluble extractive	3.3
Loss on drying	4

6.2 PRELIMINARY PHYTOCHEMICAL STUDIES

Table No.6.3: Weight of extract of RhinacanthusNasutus

Name of extract	Yield(%w/w)
Ethanol	6

The extract obtained were subjected to qualitative Phytochemical test to find out the active constituents.

Table No.6.4: Qualitative Phytochemical analysis of heart wood parts extract

TEST FOR PHYTOCONSTITUENTS	RESULT
Saponins	–
Alkaloids	+
Glycosides	–
Tannins and phenolic compounds	+
Carbohydrates	+
Fixed oils	+
Flavanoids	+
Steroids	+

(+) - Present (-) - Absent

DISCUSSION:

The preliminary Phytochemical studies were done in the ethanolic extract of RhinacanthusNasutusleaves result suggest that presence of**Alkaloids,**

Carbohydrate,Fixed oils, flavonoids, Steroids, phenolic compounds and tannins.

6.3 PHARMACOLOGICAL STUDIES

6.3.1 ACUTE ORAL TOXICITY STUDIES

The acute oral toxicity of the ethanolic extract of *Rhicanthus nasutus* was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that LD_{50}>2000mg/kg for the extract. Hence, the biological dose was fixed at EERN 200mg and 400mg of body weight for the extract.

6.3.2 EFFECT ON GLUCOSE TOLERANCE

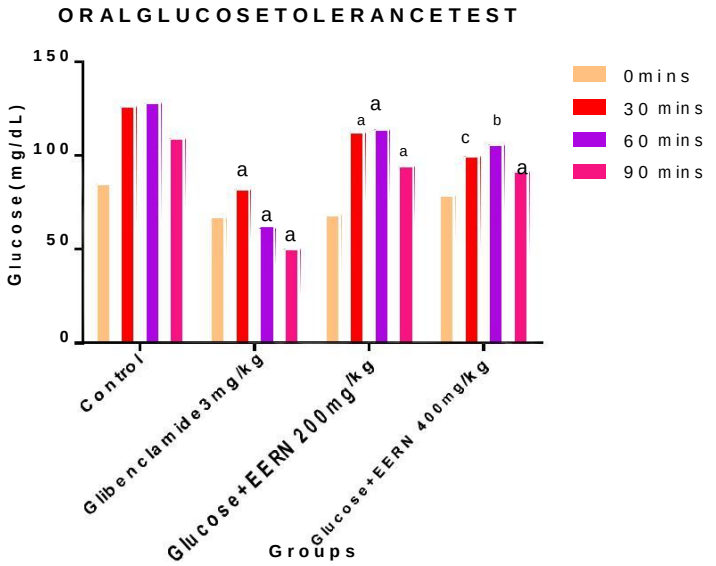
In OGTT, the doses of EERN 200 mg/kg and 400 mg/kg increased the tolerance for glucose suggesting increased peripheral utilization of glucose. The reduction in blood glucose level was dose dependent.

The results were given in Table No.: 6.6 and Figure No. : 6.2

Table No.: 6.6 Effect of ethanolic extract of *RhinacanthusNasutus* and *Glibenclamide* on glucose tolerance of diabetic rats.

Groups	Treatment	Change in blood glucose levels(mg/dL)			
		Fasting	After 30 Minutes	After 60 minutes	After 90 minutes
I.	Glucose 2mg/kg	85.35±3.80	126±3.01	128.32±2.20	109.4±3.66
II.	*Glibenclamide* 3mg/kg	67.65±4.33	82.32±2.58 [a]	61.84±2.47 [a]	50.6±4.40 [a]
III.	EERN 200mg/kg	66.83±1.52	112.67±6.28 [a]	114.33±12.00 [a]	94.67±6.89 [a]
IV.	EERN 400mg/kg	79±3.18	100±2.41 [c]	106.15±8.91 [b]	91.65±3.95 [a]

Figure No.6.1: Effect of ethanolic extract of *Rhinacanthus Nasutus* and *Glibenclamide* on glucose tolerance of diabetic rats.



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05.EERN. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests).

Extract treated group III, IV were compared with group I (Normal) and Group II (Standard).

6.3.4 EVALUATION OF PARAMETERS

1. Changes in body weight:

Vehicles control animals were found to be stable in their body weight but significant reduction in diabetic control group during 21 days (Table. 6.7). Alloxan caused body weight reduction, which is slightly reversed by ethanolic extract of RhinacanthusNasutustreated (200mg/kg and 400mg/kg) groups after 21 days.

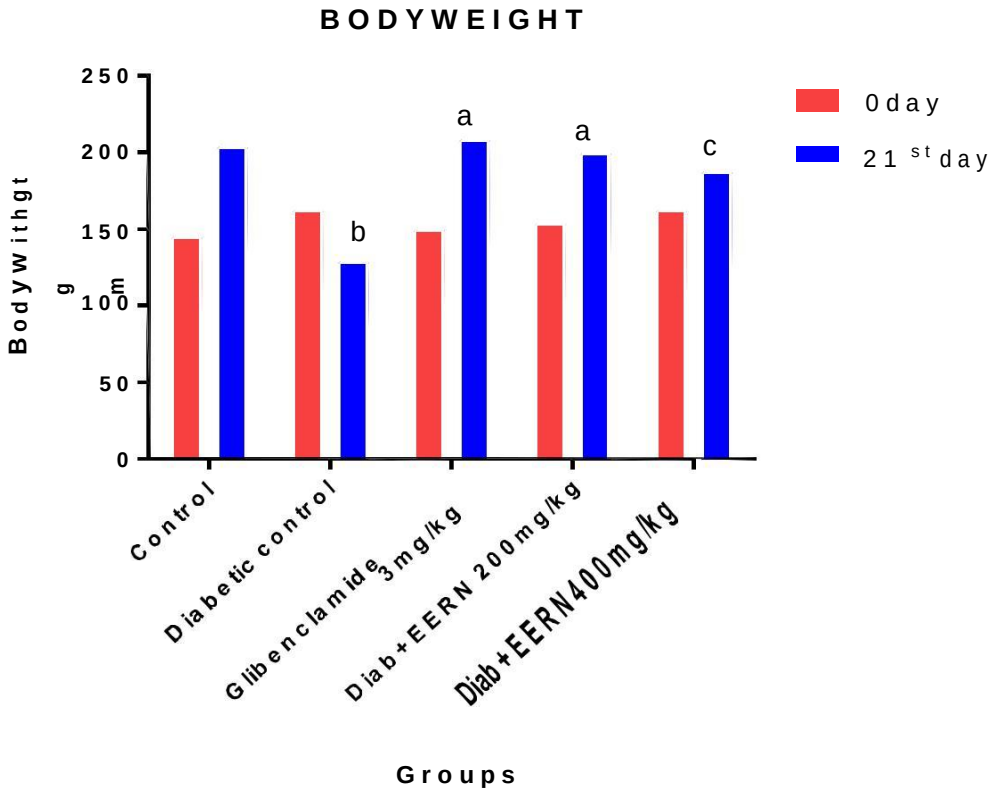
While, significant ($p<0.01$,$p<0.001$) increase in body weight was observed in rats treated with ethanolic extract of RhinacanthusNasutusThe EERN treated diabetic rats (400mg/kg) were slightly increased the body weight level and showed in Table No: 6.7, Fig No: 6.2.

Table No: 6.7 Body weight changes in ethanolic extract of *Rhinacanthus Nasutus* and *Glibenclamide* on control and experimental groups of rats

Group	Treatment	Body weight changes (g)	
		Day 0	Day 21
I	Normal control rats (vehicles only)	145±7.67	204.15± 11.94
II	Diabetic control rats	162.5±8.54 [b]	129.18± 7.67 [b]
III	Diabetic group + Glibenclamide 3mg/kg	150±6.44 [a]	208.37± 12.37 [a]
IV	Diabetic group + EERN (200/kg)	154.17±7.67 [b]	200± 6.46 [b]
V	Diabetic group + EERN (400mg/kg)	162.6 ±14.05 [c]	187.6± 17.98 [c]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.001$; b=** = $p<0.01$; c=*=$p<0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

Figure: 6.2 Body weight changes in ethanolic extract of and *Rhinacanthus Nasutus Glibenclamide* on control and experimental groups of rats



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b= ** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

2. Changes in blood glucose:

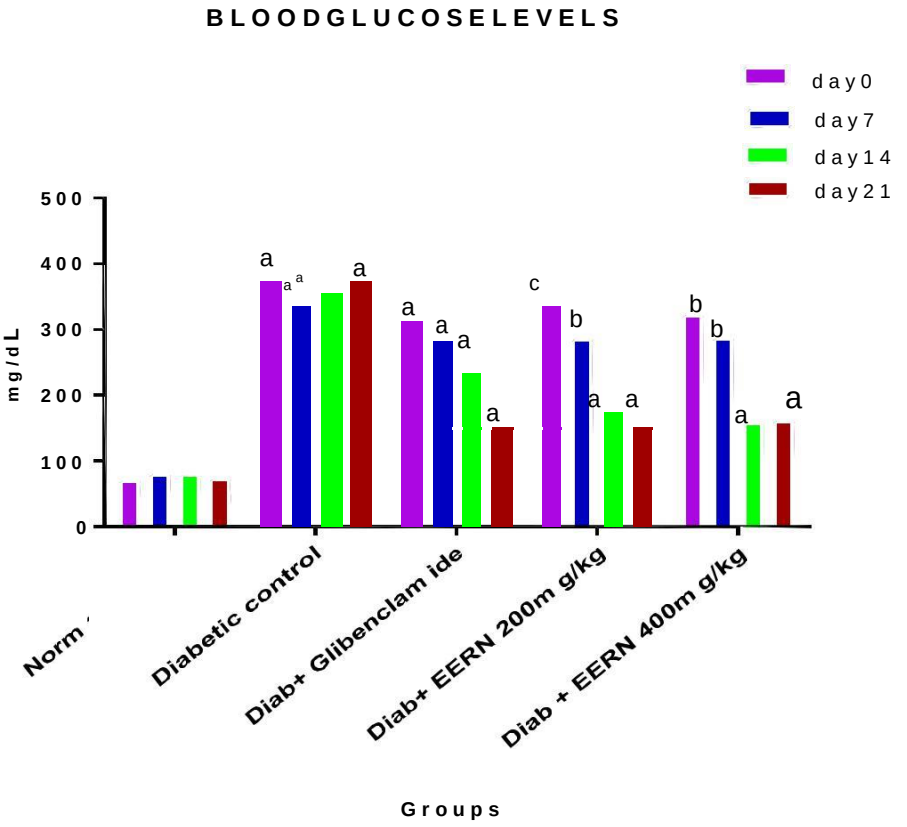
A significant increase in the level of blood glucose, was observed in diabetic control rats when compared to control rats. Administration of EEMK and *Glibenclamide* to diabetic rats significantly decreased the elevated level of blood glucose, near tocontrol level. Showed Table No: 6.8, Figure No: 6.4.

Table No. 6.8. Effect of Rhinacanthus Nasutus **ethanolic extract of and *Glibenclamide* on blood glucose level**

Group	Treatment	Blood glucose level (mg/dL)			
		Day 0	**Day 7**	**Day 14**	**Day 21**
I	Normal control rats (vehicles only)	70.65 ± 1.42	80±2.34	78.83 ± 2.36	72.33 ±1.82
II	Diabetic control rats	380.6±13.57 [a]	336.84±7.18 [a]	354.84±10.81 [a]	369.32±12.91 [a]
III	Diabetic group + Glibenclamide 3mg/kg	313.6±9.09 [a]	281.34±9.56 [a]	233.65±5.42 [a]	147.67±8.05 [a]
VI	Diabetic group + EERN (200mg/kg)	334.66±8.90 c	285±13.26 [a]	174.82±8.91 a	161±10.81 [a]
V	Diabetic group + EERN (400mg/kg)	321.84±12.16 [b]	286±5.08 [b]	157.82±7.30 [a]	160.5±7.74 [a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.05$; b=** = $p<0.01$; c=* =$p<0.001$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups IV, V and Standard III compared with Diabetic control group II.

Figure No: 6.4 Effect of ethanolic extract of RhinacanthusNasutus and *Glibenclamide* on blood glucose level.



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c= * =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

4. Changes in lipid profile:

4.1 Changes in total cholesterol and triglycerides:

The serum lipid values of TC, TG of those were treated with EERNextract returned to values near to control group. The level of cholesterol and triglyceride increased in diabetic animals when compared to control animals.

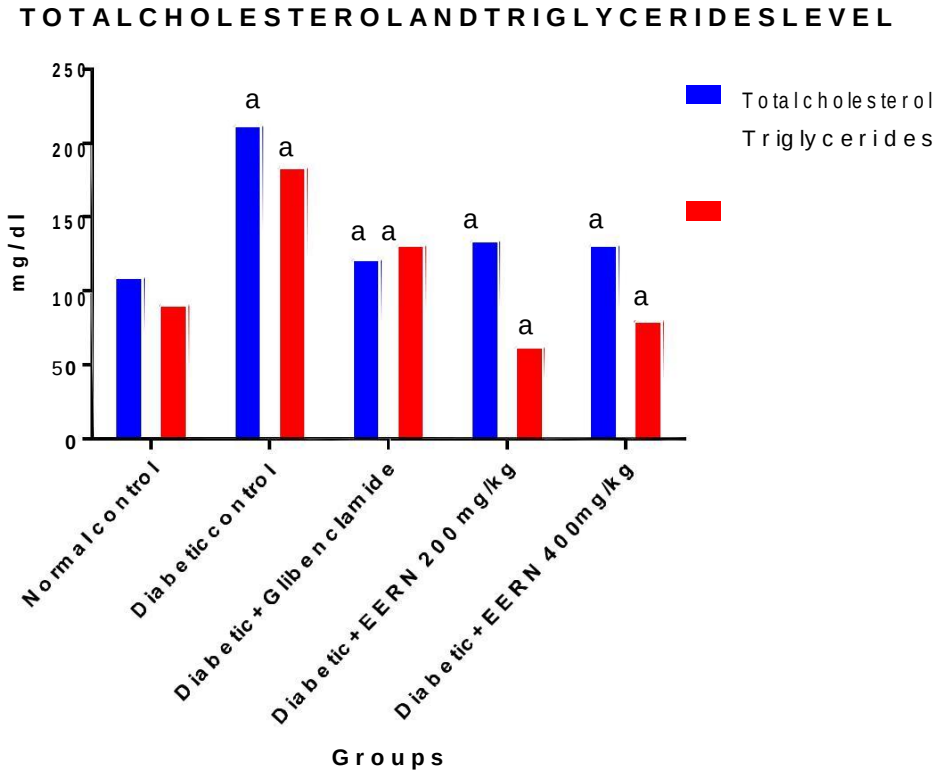
The showed that treatment with EERN significantly (200mg/kg and 400mg/kg) $p<0.001$ improved the lipid profile in Alloxan induced diabetic rats. Showed in Table No: 6.10 Figure No: 6.6.

Table No: 6.10. Effect of ethanolic extract of *Rhinacanthusnasutus* and *Glibenclamide* in Total cholesterol, Triglycerides levels of control and experimental groups of rats

Group	Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)
I	Normal control group (vehicles only)	109.82±0.47	91.16±1.71
II	Diabetic control rats	212.82±1.84 [a]	184±2.63 [a]
III	Diabetic group + *glibenclamide* (3mg/kg)	122.17±1.94 [a]	132±2.63 [a]
IV	Diabetic group + EERN (200mg/kg)	134.6±2.11 [a]	62.83±1.45 [a]
V	Diabetic group + EERN(400mg/kg)	131.67±2.94 [b]	80.83±2.11 [a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.001$; b=** = $p<0.01$; c=* =$p<0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

Figure No. 6.6: Effects of ethanolic extract of *RhinacanthusNasutus* and *glibenclamide*on total cholesterol, triglycerides levels of control andexperimental groups of rats.



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

4.2 Changes in HDL, LDL and VLDL:

The plasma lipid values of HDL, LDL and VLDL of those were treated with EERN extract returned to values near to control group. The level of HDL in plasma of diabetic animals was decreased. These lower levels of HDL- cholesterol were restored significantly near to normal in EERN treated diabetic groups.

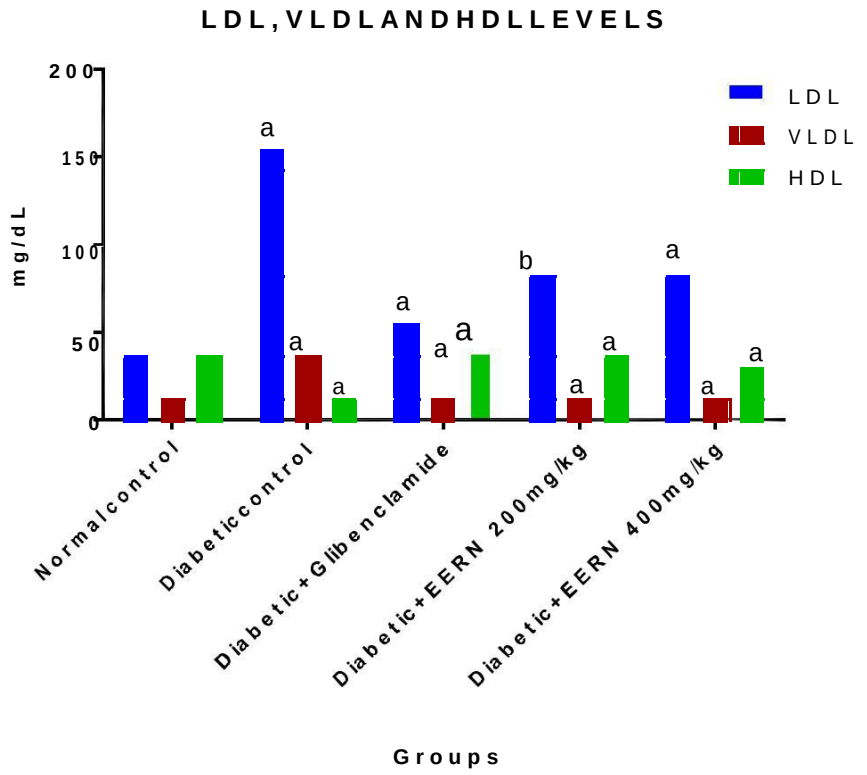
The level of LDL and VLDL increased in diabetic animals when compared to control animals. After EERN treatment, the higher level of both LDL, VLDL were increased to near control. The showed that treatment with EERN significantly (200mg/kg and 400mg/kg) $p<0.001$ improved the lipid profile in Alloxan induced diabetic rats. Showed in Table No. 6.11 Figure No. 6.7

Table No: 6.11: Effect of ethanolic extract *Rhinacanthus Nasutus* of and *Glibenclamide* on LDL, VLDL, HDL of control and experimental group of rats

Group	Treatment	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)
I	Normal control group (vehicles only)	49.92±1.19	18.24±0.34	41.65±0.87
II	Diabetic control rats	155.4±2.15 [a]	36.7±0.52 [a]	21±1.06 [a]
III	Diabetic group + glibenclamide (3mg/kg)	56.74±1.67 [a]	26.52±0.52 [a]	38.84±1.07 [a]
IV	Diabetic group + EERN (200mg/kg)	83.1±2.80 [b]	12.55±0.27 [a]	38.82±1.07 [a]
V	Diabetic group + EERN (400mg/kg)	85.32±1.85 [a]	16.17±0.44 [a]	31.84±0.82 [a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

Figure No: 6.7 Effects of ethanolic extract of *Rhinacanthus Nasutus* and *Glibenclamide* on LDL cholesterol, VLDL cholesterol, HDL cholesterol of control and experimental groups



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a= *** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

4.3 Changes in Total protein and Albumin:

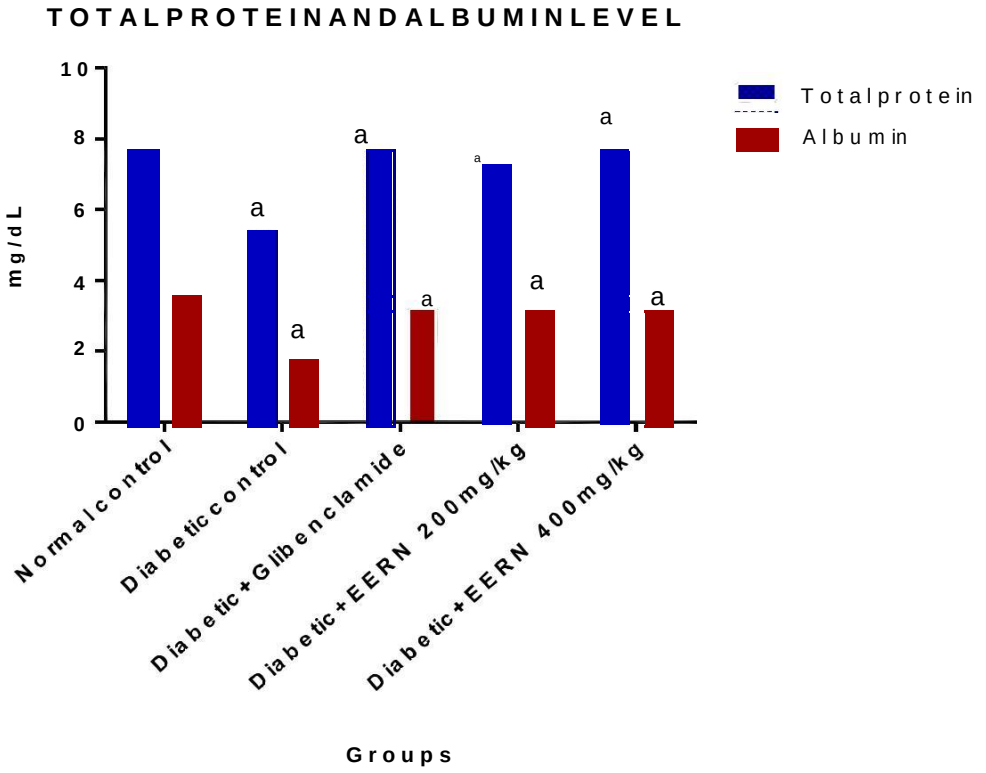
The plasma lipid values of Total protein and Albumin of those were treated with EEMK extract returned to values near to control group. The level of Total protein and Albumin in plasma of diabetic animals was increased. Total protein and Albumin where restored significantly near to normal in EERN treated diabetic groups. Showed in Table No: 6.12 Figure No: 6.8

Table No. 6.12 Effect of ethanolic extract of *Rhinacanthus nasutus* and *Glibenclamide* in Total protein, Albumin of control and experimental groups of rats

Group	Treatment	Total protein (mg/dl)	Albumin (mg/dl)
I	Normal control group (vehicles only)	8±0.13	3.867±0.10
II	Diabetic control rats	5.44±0.19 [a]	1.77±0.11 [a]
III	Diabetic group + *glibenclamide* (3mg/kg)	7.67±0.14 [a]	3.22±0.12 [a]
IV	Diabetic group + EERN (200mg/kg)	7.3±0.13 [a]	3.08±0.09 [a]
V	Diabetic group + EERN (400mg/kg)	8±0.18 [a]	3.26±0.08 [a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.001$; b=** = $p<0.01$; c=* =$p<0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

Figure No. 6.8: Effect of ethanol extract of *Rhinacanthus Nasutus* and *Glibenclamide* on Total protein and Albumin levels of control and experimental groups of rats.



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

4.5 Changes in Alkaline Phospahtase and Uric acid:

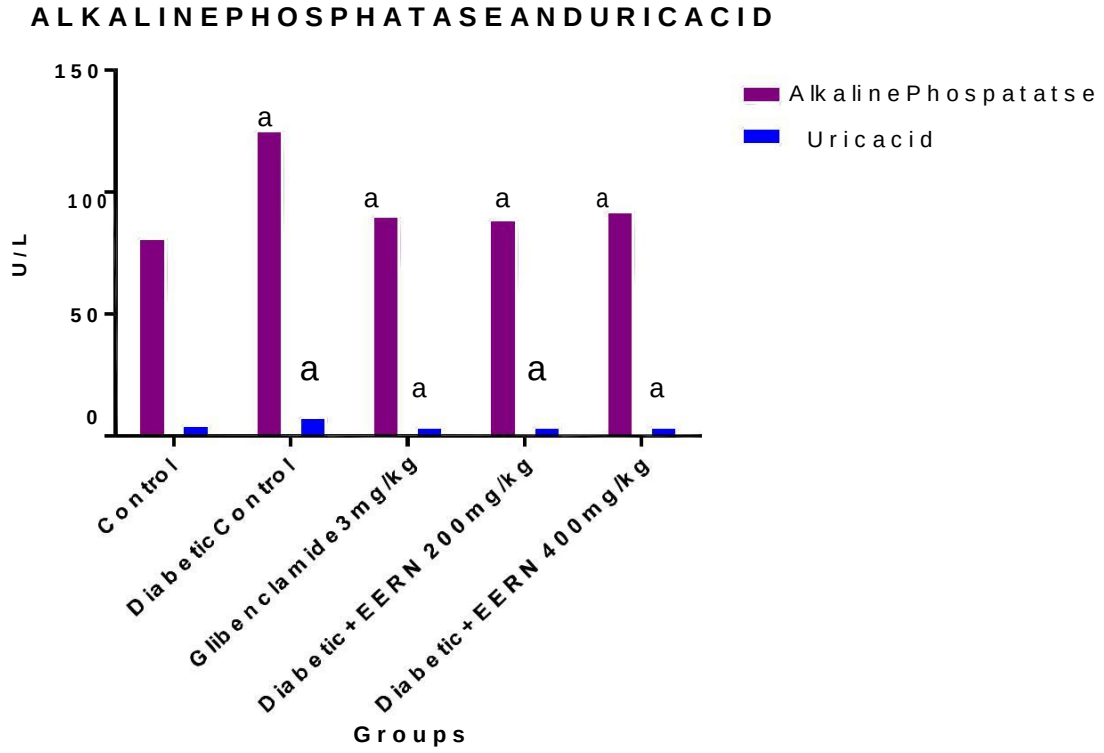
The plasma lipid values of Alkaline Phosphatase of those were treated with EERN extract returned to values near to control group. The level of Alkaline Phosphatase in serum of diabetic animals was increased. Total protein and Albumin were restored significantly near to normal in EERN treated diabetic groups. Showed in Table No: 6.12, Figure No: 6.8.

Table No. 6.12 Effect of ethanolic extract *Rhinacanthus nasutus* and *Glibenclamide* in Alkaline Phosphatase of control and experimental groups of rats

Group	Treatment	Alkaline Phospahtase	Uric acid
I	Normal control group (vehicles only)	81.6±1.05	4.71±0.12
II	Diabetic control rats	125.5±1.95 [a]	8.14±0.14 [a]
III	Diabetic group + *glibemclamide* (3mg/kg)	90.4±0.99 [a]	4.05±0.10 [a]
IV	Diabetic group + EERN (200mg/kg)	89±1.28 [a]	4.06±0.08 [a]
V	Diabetic group + EERN(400mg/kg)	92.34±0.85 [a]	4.24±0.11 [a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.001$; b=** = $p<0.01$; c=* =$p<0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

Figure No. 6.8: Effect of ethanol extract of *Rhinacanthus Nasutus* and *Glibenclamide* on Alkaline Phosphatase and Uric acid levels of control and experimental groups of rats.



Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Diabetic control group II was compared with Normal control group I and extract treated groups III, IV and V compared with Diabetic control group II.

6.5 HISTOPATHOLOGY OBSERVATION

Examination of Pancreatic tissue of diabetic rats treated with ***Rhinacanthus Nasutus***indicated that pancreatic section appeared more (or) less like control.

Group I: Normal control (Figure No: 6.9)

MICROSCOPY: Multiple section studied shows cells of pancreas with normal architecture and proportion. The acinar cells which stained strongly are arranged in lobules. The islet cells are embedded within acinar cells surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

IMPRESSION: Normal histology-Pancreas biopsy.

Group II: Diabetic Control (Figure No: 6.10)

Multiple section studied shows pancreatic tissue with hyalinization of islets of langerhans cells with focal mild degenerative changes. Interstitium showing mild fibrosis, dilated, thick walled and congested blood vessels along with focal chronic inflammatory cell infiltrate.

IMPRESSION: Features shows strong pancreatic inflammation-Pancreas biopsy

Group III: Diabetes group with Glibenclamide (Figure No: 6.11)

MICROSCOPY: Multiple section studied shows cells of pancreas withmarked atrophy of pancreatic isle cells and minimal degenerative changes in acinar population. The within shows admixed eosinophilic material, pericapsular fibrosis, congested blood vessels and scattered mononuclear inflammatory cell infilteration

surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

IMPRESSION: Features are that of insulinitis-Pancreas biopsy

Group IV: Diabetes group with 200 mg/kg EERN (Figure No: 6.12)

MICROSCOPY: Multiple section studied shows cells of pancreas with mildatrophy of pancreatic isle cells and minimal degenerative changes in acinar population. The acinarcells shows dark staining and are arranged in lobules. The islet cells are embedded within acinarcells which shows minimal pericapsular fibrosis, congested blood vessels and scattered mononuclear inflammatory cell infilteration surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

IMPRESSION: Features show mild decrease in pancreatic inflammation-Pancreas biopsy

Group V: Diabetes group with 400 mg/kg EERN (Figure No: 6.13)

MICROSCOPY: Multiple section studied shows cells of pancreas with mildatrophy of pancreatic isle cells and normal acinar population. The acinarcells shows dark staining and are arranged in lobules. The islet cells are embedded within acinar cells which shows minimal pericapsular fibrosis, edema, congested blood vessels and very few scattered mononuclear inflammatory cell infilteration surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

IMPRESSION: Features show marked decrease in pancreatic inflammation-

Pancreas biopsy.

IMPRESSION: Features show marked decrease in pancreatic inflammation-Pancreas biopsy.

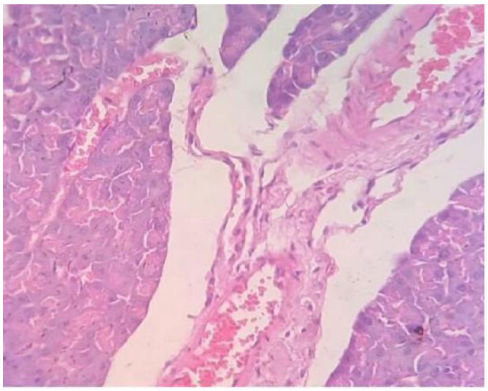


Figure No: 6.9 (Normal control)

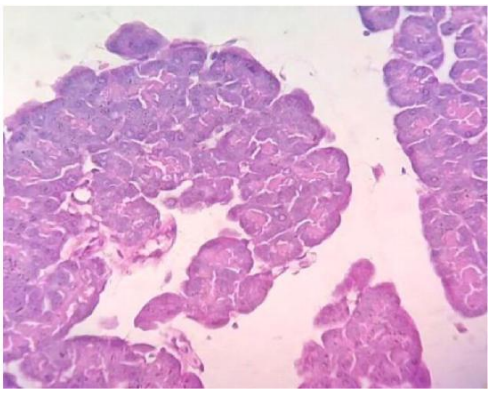


Figure No: 6.10 (Diabetic control)

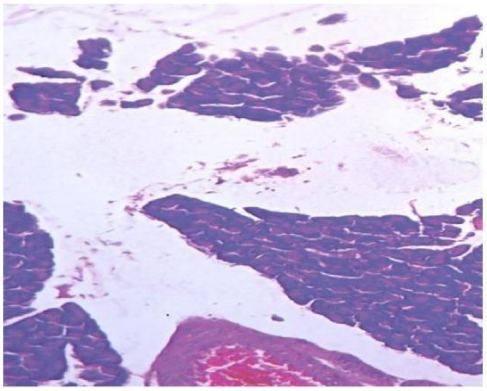


Figure No: 6.11 (Diabetic group with glibenclamide

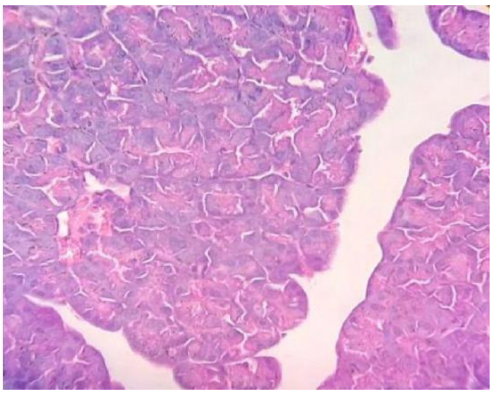


Figure No: 6.12 (Diabetic group with 200 mg EERN

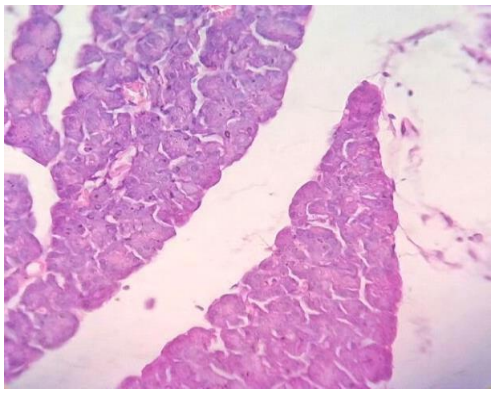


Figure No: 6.13 (Diabetic group with 400 mg EERN)

7. SUMMARY AND CONCLUSION

The leaves of ***Rhinacanthus Nautus*** belonging to family ***Rhinacanthus Nasutus*** has been examined to gain an insight of its Phytochemical and pharmacological behaviors.

The Pharmacognostical studies made on powdered ***Rhinacanthus Nasutus*** like ash value, loss on drying gave valuable information. Preliminary phytochemical investigation of showed the presence of **Carbohydrate, Alkaloids, Phytosteroids, Flavonoids, Phenolic compounds and Tannins.**

The pharmacological and acute toxicity studies of ethanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight. The Biological dose of extract ***Rhinacanthus Nasutus*** dose was selected 200mg/kg and 400mg/kg in this dose possessed significant antidiabetic activity.

In conclusion, in the present study on the ethanolic extract of ***Rhinacanthus Nasutus*** leaves having antidiabetic activity more over nearest activity of*Glibenclamide*. This study shows that flavanoids present in this extract may bepossibly responsible for the antidiabetic activities.

Histopathological studies on isolated pancreas revealed that ethanolic extract of ***Rhinacanthus Nasutus*** reversed the changes which produced due to diabetes caused by Alloxan. The normal pattern of histology of pancreas was observed.

Further pharmacological and biochemical investigation are to be done to find out the active constituent responsible for the antidiabetic activity. However, the future study may also include cataloging, standardizing, for quality control and above all developing new drugs/ pharmaceuticals keeping the disease and cost factor in view.

BIBLIOGRAPHY

- Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon. With taxonomic updating by Senaratna LK. The national science foundation Sri Lanka, Colombo; part 1, 2006 ; 21.

- Shinto Arthur W. Rhinacanthus nasutus Herbal Remedies: 2013; (cited 2015 Jnne 20), Available from http:/www. *Rhinacanthus nasutus Herbal Remedies* . *Pharma Reseach Library/ mht* .

- *Rhinacantus nasutus* (Species). Bold systems :Taxonomy Browser: 2014;[Cited 2015 June 20], Available from http://www.boldsystems.org/index.php/IDS OpenI dEnne Taxbrowser.

- Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon. With taxonomic updating by Senaratna LK. The national science foundation Sri Lanka, Colombo; part 1, 2006; 20-21.

  Anonymous, Compendium of Medicinal Plants A Sri Lankan Study. Department of Ayurveda, Sri Lanka, volume 1, 2001; 19.

- *Rhinacantus nasutus* (Species). Bold systems Taxonomy Browser: 2014;[Cited 2015 June 20]; Available from http://www.boldsystems.org/index.php/IDS OpenI dEnne Taxbrowser.

- *Rhinacantus nasutus.* Available from http:/www. Wikipedia, / Rhinacanthusnasutus the free encyclopedia.mht.

- Compendium of Medicinal Plants A Sri Lankan Study. Department of Ayurveda, Sri Lanka, volume 1, 2001; 1

- Compendium of Medicinal Plants A Sri Lankan Study. Department of Ayurveda, Sri Lanka, volume 1, 2001; 19

- Anna Sendl, *et al*., Two New Naphthoquinones With Antiviral Activity from Rhinacanthus nasutus. Article in Journal of Natural Products 1996; Available from http:/www./researchgate.net/ publication.

- Pasupuleti visweswara Rao et al. *Rhinacanthus nasutus* Ameliorates Cytosolic and Mitochondrial Enzyme Levels in Streptozotocin Induced Diabetic Rats. 2013 Feb. thirteenth (cited 2015 june 22); Available from http://www.hindawi.com/journals/ecam/2013/486047.

- Gotoh A. et al., Anti proliferative activity of *Rhinacanthus nasutus* extracts and the activite moiety. Rhinacanthin 2004 July twenty seventh (cited 2015 june 20); Available from *http:/www./ Rhinacanthus nasutus /PubMed/Antiproliferative.mht.* Visweswara Rao.et.al., Efficacy of AntimicrobialActivity of *Rhinacanthus nasutus* (L) Leaves in Different Extractions. International Journal of Pharma and Bio Sciences. 2010 Feb (cited 2015 june 20); Available from *http:/www./antimicrobial Pdf- Adobe Reader*.

- Brahma Srinivasa Rao Desu and CH. Saileela. Antihyperlipidemic activity of methanolic extract of *rhinacanthus nasutus*. International journal of research in pharmacy and chemistry, 2013 March third (cited 2015 june 21); Available from www.ijrpc.com.

- Linda Suift. The Vascular Effect of Nifedipine and the plant Extract. *Rhinacanthus nasutus* in Hypertensive Rats. 2003; (cited 2015 june 21)Journal of Biological Research Vol 4, Available from *http:/www./hartwick.edu.*

- Sharma PV. Dravyaguva Vijnana. Chaukhambha Bharati Academy Varanasi India, Vol. ii ,1974; 188,. Jayasingha DM. Charma Roga Nidana Chikithsa Sangrahaya. Department of Ayurveda, Sri Lanka, 1990: 93.

- Abesekara Harald. Osuthuru visithuru. Department of Ayurveda, Sri Lanka, 1994; 1: 127

- Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon. With taxonomic updating by Senaratna LK. The national science foundation Sri Lanka, Colombo; Part 1, 2006; 20-21.

- Vaidyaratnam PS. Indian Medicinal Plants. Varier's Arya Vaidya Sala, Orient Longmasn Anna Salai , Madras India , volume 4 : 1995.Abesekara Harald. Osuthuru visithuru. Department of Ayurveda, Sri Lanka, vol: 1, 1994;127

- Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon. With taxonomic updating by Senaratna LK. The national science foundation Sri Lanka, Colombo; part 1, 2006; 20-21.

- Pandua de LS.et.al., Medicinal and Poisonous Plants. Backhuys Publishers Leiden Plant Resources of South East Asia , vol: 1, 1999 ; .431

- Pandua de LS.et.al., Medicinal and Poisonous Plants. Backhuys Publishers Leiden Plant Resources of South East Asia , vol: 1, 1999 ; .431

- Pandua de LS.et.al., Medicinal and Poisonous Plants. Backhuys Publishers Leiden Plant Resources of South East Asia , vol: 1, 1999 ; .431Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon. With taxonomic updating by Senaratna LK. The national science foundation Sri Lanka, Colombo; part 1, 2006; 20-21.

- Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon.
- With taxonomic updating by Senaratna LK. The national science foundation Sri Lanka, Colombo; part 1, 2006; 20-21. Devanarayana et al. UJAHM 2015, 03 (04): Page 10-15 Unique Journal of Ayurvedic and Herbal Medicines, 03 (04), July-August 2015 15

- Pandua de LS.et.al., Medicinal and Poisonous Plants. Backhuys Publishers Leiden Plant Resources of South East Asia, vol: 1, 1999; .431.

- Pandua de LS.et.al., Medicinal and Poisonous Plants. Backhuys Publishers Leiden Plant Resources of South East Asia, vol: 1, 1999; .431.

- Rhinacanthus nasutus . - TD K Ved, Suma Tagadur Sureshchandra, VijayBarve, Vijay Srinivas, Sathya Sangeetha, K. Ravikumar, Kartikeyan R., Vaibhav Kulkarni, Ajith S. Kumar , S.N. Venugopal, B. S. Somashekhar, M.V. Sumanth, Sugandhi Rani, Surekha K.V. and Nikhil Desale. 2002-2014. (envis.frlht.org / frlhtenvis.nic.in). FRLHT's ENVIS Centre on Medicinal Plants, Bangalore.

- "Rhinacanthusnasutus - Snake Jasmine". flowersofindia.net.

- RhinacanthusnasutusImproves the Levels of Liver Carbohydrate, Protein,Glycogen, and Liver Markers in Streptozotocin-Induced Diabetic Rats, hindawi, 20 August 2013 medicinal uses pharmacographicaindicaJames M. Brimson. "Molecules - Free Full-Text - Rhinacanthus nasutusProtects Cultured Neuronal Cells against Hypoxia Induced CellDeath".MDPI.

- Kodama, Osamu. "Isolation and Identification of an Antifungal Naphthopyran Derivative from Rhinacanthus nasutus". Journal of Natural Products. 56: 292– 294. doi:10.1021/np50092a018.

- Puttarak, P. "Antimicrobial activity and stability of rhinacanthins-rich Rhinacanthusnasutus extract". Phytomedicine. 17: 323– 327. doi:10.1016/j.phymed.2009.08.014.

- Tewtrakul S, et al. (2009). "Effects of rhinacanthins from Rhinacanthus nasutus on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha releases using RAW264.7 macrophage cells".nih.gov. 16: 581– 5. PMID 19303271.doi:10.1016/j.phymed.2008.12.022.

- "Induction of Apoptosis by Rhinacanthone Isolated from Rhinacanthusnasutus Roots in Human Cervical Carcinoma Cells". jst.go.jp.

- http://www.hindawi.com/journals/ecam/2013/102901/

- "Antiproliferative Activity of Rhinacanthus nasutus (L.) KURZ Extracts and theActive Moiety, Rhinacanthin C". jst.go.jp.

- Kernan, MR; Sendl, A; Chen, JL; Jolad, SD; Blanc, P; Murphy, JT; Stoddart, CA; Nanakorn, W; Balick, MJ; Rozhon, EJ. "Two New Lignans with Activity against Influenza Virus from the Medicinal Plant Rhinacanthus nasutus". Journal of Natural Products. 60: 635–637. PMID 9214738. doi:10.1021/np960613i.