EVALUATION ON ANTI-DIABETIC EFFECT OF ETHANOLIC EXTRACT OF WHOLE PLANT OF *NYMPHAEA PUBESCENS* ON STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS

Dissertation submitted to

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI

In partial fulfilment for the award of the degree of

MASTER OF PHARMACY IN PHARMACOLOGY

By

S Vijay

Register No: 261525013

UNDER THE GUIDANCE OF

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DECLARATION

I do hereby declare that the thesis entitled "**Evaluation on Anti-Diabetic effect of Ethanolic Extract Of Whole plant of *Nymphaea pubescens willd* on Streptozotocin induced Diabetes in Wistar rats**" was carried out by me under the guidance and supervision of Dr. P. Amudha., M.Pharm., Ph.D., Asst. professor, department of pharmacology, C.L.Baid Metha College of Pharmacy, Chennai-97. The work embodied in the thesis is original, and is not permitted in part or full for any other degree of this or any other university.

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ACKNOWLEDGMENT

First and foremost I thank and praise to one and only **Almighty God** for his extended grace and blessing to carried out this project. My grateful thanks to my **beloved parents and family members** for their love and support in every step of my life.

It is my great privilege to express my sincere gratitude to my guide **Dr. P. Amudha., M. Pharm., Ph.D.,** Asst. Professor, Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai, for her investigation, valuable suggestion, encouraging, support and constant help throughout the entire course to execute this project successfully.

I extend my deepest gratitude to **Dr. P. Muralidharan., M. Pharm., Ph.D.,** Professor and Head of Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai, for his valuable suggestion, inspiration and encouragement throughout my dissertation work, which ensured the smooth progress of my project.

I am obliged to **Dr. Grace Rathnam., M. Pharm., Ph.D.,** Principal, C. L. Baid Metha College of Pharmacy, Chennai, for her valuable contribution and facilities provided regarding my dissertation work.

I am thankful to **Mr. Srinivasa Ragavan**, Stores in charge, C. L. Baid Metha College of Pharmacy, Chennai, for his timely supply of all necessary chemicals and reagents reguired for the completion of my project work.

I am thankful to **Mr. Ganesh Bahadur**, chief security officer, C. L. Baid Metha College of Pharmacy, Chennai, for providing us the uninterrupted service at college campus during the work period.

I am thankful to **Mr. Natha Ruben** and **Mr. Anand**, lab attenders, Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai, for helping and providing a clean environment during the work period.

I am thankful to all my beloved classmates, department of pharmacology for their valuable help and support.

It would not be fair if I do not regret for all those animals, which lost their lives for the sake of my study, without which I would not have been successful in my study.

DEDICATED TO MY BELOVED PARENTS, TEACHERS AND MY DEAR FRIENDS

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LIST OF ABBREVIATIONS

A	Alpha
Sβ	Beta
Γ	Gama
Δ	Delta
%	Percentage
µ	Micro
µl	Microliter
ºC	Degree Celsius
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
ANOVA	analysis of variance
BMI	Body Mass Index
COX	Cyclooxygenase
CPCSEA	Committee for the purpose of Control and Supervision of Experiments on Animals
Cu	Copper
Ccl_3^-	Trichloromethyl ions
CFA	Complete Freunds Adjuvant
$CuSO_4$	Copper sulphate
Dl	Deciliter

DM	Diabetes Mellitus
DNA	Deoxyribo Nucleic Acid
DNS	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl -1-Picrylhydrazyl
EENP	Ethanolic Extract of Nymphaea pubescens
ER	endoplasmic reticulum
FBS	Fasting Blood Sugar
FFA	Free fatty acids
FT-IR	Fourier transform infrared spectroscopy
GAD	Glutamic Acid Decarboxylase
GC-MS	Gas Chromatography – Mass Spectrometrry
GHS	globally harmonized system
GlcNAc	N-acetyl glucosamine
GLDH	Glutamate Dehydrogenase
GLP-1	Glucagon Like Peptide-1
GLUT2	Glucose Transpoters 2
GLUT4	Glucose Transpoters 4
GPO-PAP	Glycerol Phosphate Oxidase –p- Amino Phenazone
GP_X	Glutathione Peroxidase
PPO	Polyphenoloxidase
GSH	Glutathione
GTT	Glucose tolerance testing

OGTT	oral glucose tolerance test
H_2O	Water
H_2O_2	Hydrogen Peroxide
HDL	High Density Lipoprotein
HGA	Habb-e-Gul-e-Aakh
BUN	Blood urea nitrogen
HOO^-	Hydroperoxy radical
IC50	Inhibition concentration
GFR	glomerular filtration rate
i.p	Intraperitoneal
IZ	inhibition zones
K^+	Potassium ions
K_{ATP}	Potassium ATPase
Kg	Kilogram
KOH	Potassium hydroxide
LADA	Latent Autoimmune Diabetes in Adults
LD_{50}	Lethal Dose – 50%
LDL	Low Density Lipoprotein
M.mol/dl	milli mole per decilitre
MAPK	mitogen-activated protein kinase
MDA	Malonaldehyde
Mg	Milligram

mg/dl	milligrams per decilitre
MHC	Major Histocompatibility Complex
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
MIC	Minimum Inhibitory Concentration
Ml	Milliliter
Mn	Manganese
mol. Wt.	Molecular weight
mtDNA	mitochondrial DNA
Na_2CO_3	Sodium Carbonate
LFTs	liver function transferases
NAD+	Nicotinamide adenine dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide adenine Dinucleotide Phosphate
NF-κB	Nuclear factor Kappa B
HLTE	Hind Limb Tonic Extension
NIDDM	Non Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
NO	Nitric oxide
O_2-	Superoxide
POD	Peroxidase
OECD	Organization for Economic Co-operation and Development

O-GlcNac	β linked N-Acetylglucosamine
O-GlcNacase	Glycoside hydrolase *O*-GlcNac
OH^-	Hydroxide Radical
p.o	*per os* – orally
PARP	polyADP-ribose polymerase
PC	prohormone convertases
PC	pyruvate carboxylase
PC3	prostatic cancer cell lines
PCOS	Polycystic Ovarian Syndrome.
PDGF	Platelet Derived Growth Factor
PKC	Protein Kinase C
PKC-δ/ Prkcd	activates protein kinase C-δ
pNPG	p-nitrophenyl glucopyranoside
$Cr3^+$	element trivalent chromium
pp. cells	Pancreatic polypeptide cells
PPARγ	Peroxisome Proliferator Activated Receptor
TNF-α	tumour necrosis factor- α
RA	Rheumatoid arthritis
RBC	Red Blood Cell
RO^-	alkoxy radical
ROS	Reactive Oxidative Species
SEM	Standard Error Mean

SGLT-2	Sodium Glucose Linked Transportor-2
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
STZ	Streptozotocin
T2DM	Type II Diabetes Mellitus
TC	Total cholesterol
TG	Triglycerides
VLDL	Very low density lipoprotein
WHO	World Health Organization

1. Introduction about herbal plants

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that were regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed the importance, for a while. However, the blind dependence on the synthetics is over and people started returning to the naturals with hope of safety and security. Over the three-quarters of world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crore. It has been estimated that in developed countries like United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China, India and Bangladesh, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India, Bangladesh than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine. Of the 2, 50,000 higher plant species on earth, more than 80,000 are medicinal. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has also adopted a number of plant-derived drugs which form an important segment of modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. diosgenin, solasodine, b-ionone). Not only, that plant-derived drug offers a stable market worldwide, but the plants aslo continue to be an important source for new drugs. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional

medicines which are mainly derived from plant material. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future [1].

1.1 Introduction to Traditional plant medicine

For thousands of years plants and their derivatives are being used for the treatment of diabetes. A large number of animal studies to test the claimed activity have demonstrated the anti-hyperglycemic property of many of these plants. In addition, clinical trials have shown some plants as useful anti-diabetic agents, but the pure chemical compounds isolated from the crude extracts of these plants neither bear structural resemblance to the anti-diabetic drugs in current clinical use nor have similar mechanisms of action. But still the search for a novel anti diabetic drug advocates the utilization of plants as a potential source and can be achieved by application of modern scientific technology and recent knowledge on the physiological changes in case of diabetes[2].

Traditional plant medicines are herbal formulations, which might offer a natural key to unlock diabetic complications. Medicinal plants plays an important role in the management of diabetes mellitus especially in developing countries where the resources are meager. Medicinal plants used to treat anti hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of anti-hyperglycemic activity[3].

Approximately 80% of population are dependent on traditional therapies for their health care and has been sustained by the WHO recommendation to include traditional medicines in

the primary health care level of these countries. Most of the traditional therapies were constituted of plants. When tested using modern methods of evaluation only 18% were found to exhibit some kind of pharmacological activity. According to the review compiled by Bnouham et al[3]., the families of plants with the most potent anti-hyperglycemic effects include: Leguminaceae (11 species), Laminaceae (8 species), Lilliaceae (8 species), Cucurbitaceae (7 species), Asteraceae (6 species), Moraceae (6 species), Rosaceae (6 species), Euphorbiaceae (5 species), Araliaceae (5 species).The commonly studied species are: Opuntia streptacantha, Trigonella fenumgraeicum L, Momordica charantia L, Ficus bengalensis L, Polygala senega L, and Gymnema sylvestra R[6].

The traditional medicine all over the world in now a days revalued by an extensive activity of research on different plant species and their therapeutic principles. Numerous physiological and chemical processes in the human body may produce oxygen centered free radicals and other reactive oxygen species as byproducts. Over production of such free radicals can cause oxidative damage to biomolecules (ex: lipids, proteins, DNA), which leads to many chronic diseases such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans. Plants (fruits, vegetables, medicinal herbs etc.,) may contain a wide variety of free radical scavenging molecules[2,3,4] such as (phenolic compounds, lignans, stilbenes, tannis), nitrogen compounds (alkaloids, amides, betulains), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites which are rich in antioxidant activity[4,5]. The intake of natural antioxidants have been associated with reduced risk of cancer, cardio vascular diseases, diabetes and other diseases associated with aging.

2. Introduction

Diabetes represents a spectrum of metabolic disorders, which has become a major health challenge worldwide[7]. The unprecedented economic development and rapid urbanization in Asian countries, particularly in India has led to a shift in health problems from communicable to non-communicable diseases. Of all the non-communicable diseases, diabetes and cardiovascular diseases lead the list. Diabetes is pandemic in both developed and developing countries. The greatest relative rise is predicted in the developing countries of the Middle Eastern Crescent, Subsaharan Africa and the Indian subcontinent. By the year 2030, over 85 percent of the world's diabetic patients will be in developing countries. In India alone, the prevalence of diabetes is expected to increase from 31.7 million in 2000 to 79.4 million in 2030[8]. These estimates are valid only if the prevalence of obesity remains the same. Since the incidence of obesity is rising at an alarming rate in developed and developing countries, the projections for the number of diabetes could well be a gross underestimation. The prevalence of Type 2 diabetes is 4-6 times higher in the urban areas as compared to rural areas [9]. The prevalence of impaired glucose tolerance (IGT) in the rural population is also high at 7-8%, which indicates presence of a genetic basis for Type 2 diabetes in ethnic Indian population [10]. The Diabetes Control and Complications Trial (DCCT) demonstrated that good metabolic control, resulting from intensive insulin therapy, reduced the risk of progression or development of retinopathy, nephropathy and neuropathy in type 1 diabetes [11]. The United Kingdom Prospective Diabetes Study (UKPDS) showed that intensive glycemic control in type 2 diabetes significantly reduced the risk of development and deterioration of microvascular complications [12]. The target for good glycemic control recommended by the American Diabetes Association (ADA) is glycated hemoglobin A1c (HbA1c) <7.0%. The primary goal of the management of diabetes mellitus is the attainment of near normal glycemia [13,14].

2.1 Definition

According to WHO, Diabetes mellitus is defined as a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action, or both. The

clinical diagnosis of diabetes is often indicated by the presence of symptoms such as polyuria, polydipsia, and unexplained weight loss, and is confirmed by measurement of abnormal hyperglycemia [15].

Diabetes mellitus (DM) is the most severe and challenging metabolic pandemic of the 21st century. This is because it affects essential biochemical activities in almost every cell in the body and increases the risk of cardiac and renal disorders. The worldwide survey reported that DM is affecting nearly 10% of the population[16]. This pandemic is characterized by excessive sugar in the blood due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced to control blood glucose. This disorder affects carbohydrate, protein and fat metabolism[17], and chronic hyperglycemia causes glycation of body proteins that in turn leads to secondary complications that affects eyes, kidneys and nerves[18].

2.2 Epidemiology

The global increase in the prevalence of diabetes is due to population growth, aging, urbanization and an increase of obesity and physical inactivity. The primary determinants of the epidemic are the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity. Unlike in the West, where older populations are most affected, the burden of diabetes in Asian countries is disproportionately high in young to middle aged adults results in long lasting adverse effects on a nation's health and economy, especially for developing countries. Healthcare expenditures on diabetes are expected to account for 11.6% of the total healthcare expenditure in the world in 2010. Estimated global healthcare expenditures to treat and prevent diabetes and its complications are expected to total at least 376 billion U.S. Dollars (USD) in 2010. By 2030, this number is projected to exceed some USD 490 billion [19].

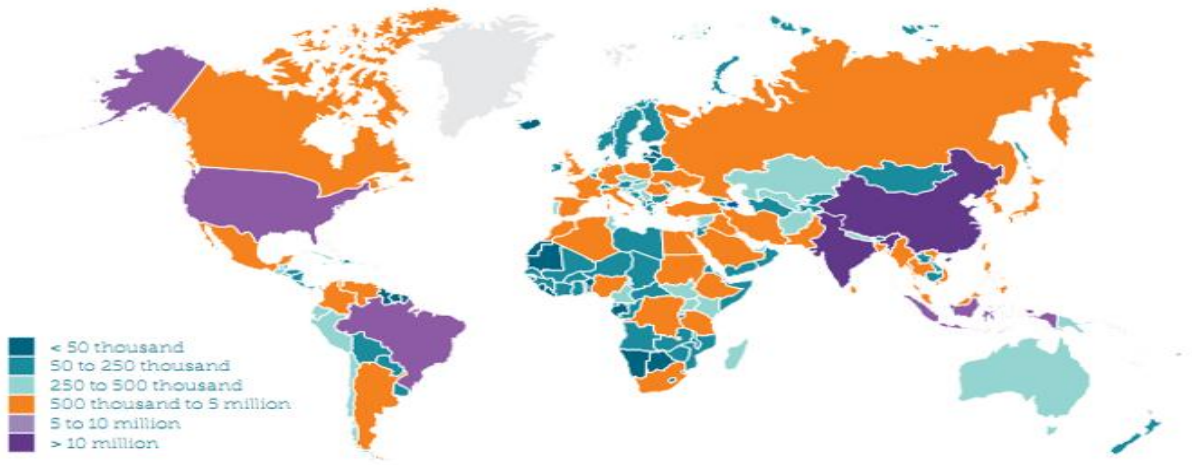
Diabetes is huge and growing problem, and cost to society is high and escalating. In South East Asia half of people with diabetes remain undiagnosed. Total deaths due to diabetes was 12,00,000 out of which 6,80,500 were women , 5,19,500 were men and the 55.1% of the population was found to be under the age of 60yrs [20].

According to International Diabetes Federation, the South East Asia at a glance showed that the adult population in the age between 20-79yrs who are suffering from diabetes are 883 millions in 2013 and by 2035 it may raise to 1217 millions and the Regional prevalence is 8.2% in 2013 which may increase to 10.1% by 2030, similarly comparative prevalence is 8.7% to 9.4% in 2013 and 2035 respectively.

The age between 20-79yrs, Regional prevalence of Impaired Glucose Tolerance in 2013 was found to be 2.7% which may increase to 3.2% by 2035, similarly comparative prevalence from 2.9% to 3%.In between the age of 10-14yrs number of children with type-1 diabetes was found to be 7,79,000 in 2013 and newly diagnosed cases per year was 1,25,000.In between the age of 20-79yrs the number of people with diabetes in 2013 was 72.1 millions by 2035 it will enhance to 123.0 millions. The number of people with Impaired Glucose Tolerance in 2013 was 24.3 millions which may enhance to 38.8 millions.

	2015			2040	
Rank	Country/territory	Number of people with diabetes	Rank	Country/territory	Number of people with diabetes
1	China	109.6 million (99.6-133.4)	1	China	150.7 million (138.0-179.4)
2	India	69.2 million (56.2-84.8)	2	India	123.5 million (99.1-150.3)
3	United States of America	29.3 million (27.6-30.9)	3	United States of America	35.1 million (33.0-37.2)
4	Brazil	14.3 million (12.9-15.8)	4	Brazil	23.3 million (21.0-25.9)
5	Russian Federation	12.1 million (6.2-17.0)	5	Mexico	20.6 million (11.4-24.7)
6	Mexico	11.5 million (6.2-13.7)	6	Indonesia	16.2 million (14.3-17.7)
7	Indonesia	10.0 million (8.7-10.9)	7	Egypt	15.1 million (7.3-17.3)
8	Egypt	7.8 million (3.8-9.0)	8	Pakistan	14.4 million (10.6-20.4)
9	Japan	7.2 million (6.1-9.6)	9	Bangladesh	13.6 million (10.7-24.6)
10	Bangladesh	7.1 million (5.3-12.0)	10	Russian Federation	12.4 million (6.4-17.1)

TOP 10 Countries/Territories for number of people with Diabetes (20-79),2015 and 2040



Number of people living with diabetes who are undiagnosed, 2015

2.3 CLASSIFICATION

Diabetes Mellitus was merely classified into following types. They are

- ✓ Type-1 Diabetes Mellitus
- ✓ Type-2 Diabetes Mellitus
- ✓ Type-3 Diabetes Mellitus
- ✓ Type-4 Diabetes Mellitus

2.3.1 Type 1 Diabetes Mellitus

It is also called "Insulin Dependent Diabetes Mellitus" (IDDM) because in such patients, due to an absolute lack of insulin, regular injections of insulin are needed to save life. Earlier, it was called "juvenile-onset diabetes" because it most commonly develops either before puberty or in youngsters below 20years of age and persists throughout their life. IDDM is an autoimmune disease of the pancreatic beta cells (type-1A) resulting in their degeneration. It could also be idiopathic (type-1B).Viral infection such as echo-virus can also damage pancreatic beta cells (type-1B) .Approximately 10% of diabetics suffer with type 1 DM. These patients have a low degree of genetic predisposition, yet 15-20% of patients reveal a positive family history and the incidence in homozygous twins is about 50%.

2.3.2 Type 2 Diabetes Mellitus

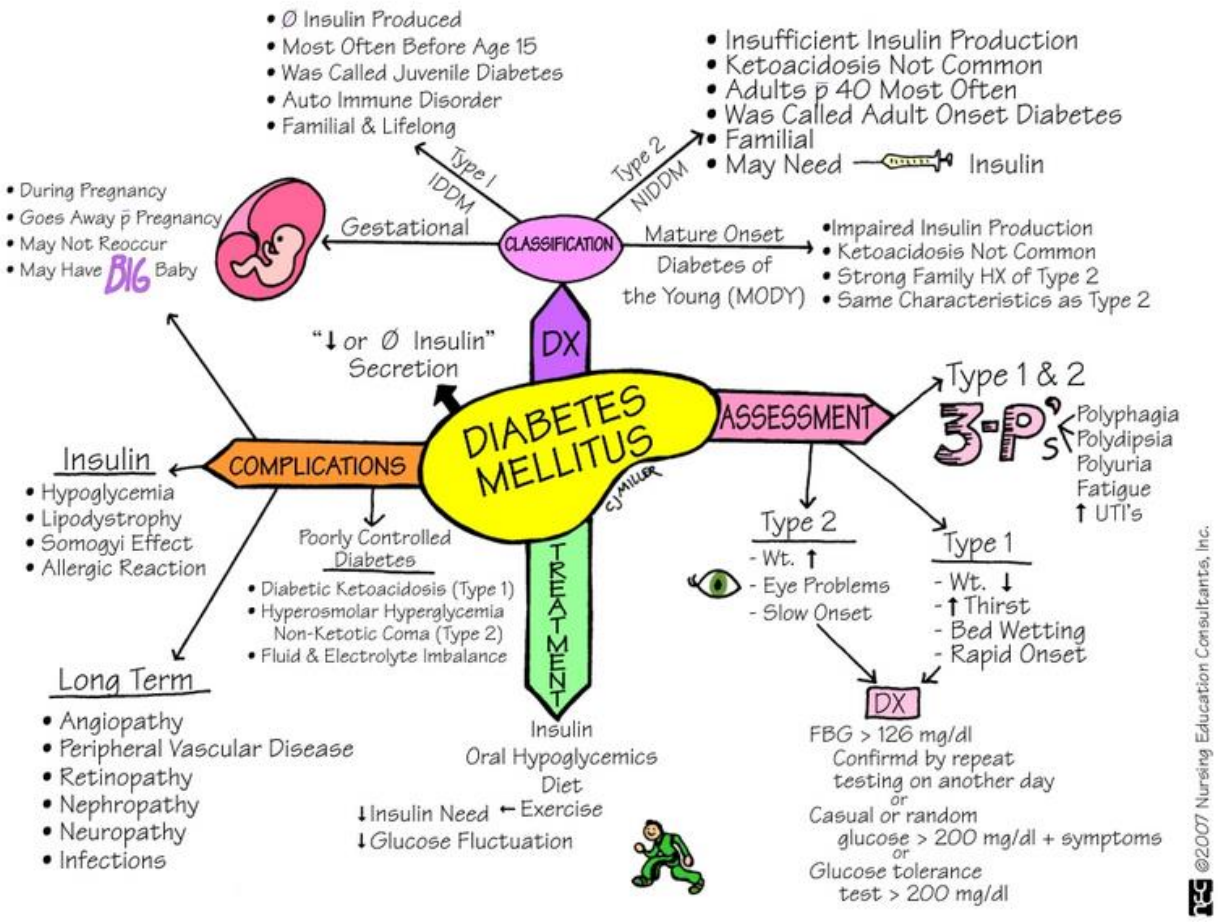
This is also called "Non Insulin Dependent Diabetes Mellitus" (NIDDM) or "Maturity Onset Diabetes" (as it occurs late in life). Approximately, 90-95% of diabetics have type-2 diabetes. It usually occurs in people who are over 40 and over weight. Many type-2 diabetics, however, have a significant amount are elevated levels of insulin. For these people diabetes arises not from shortage of insulin but because their target cells have become relatively insensitive to insulin (peripheral resistance to insulin). Genetic predisposition, in type-2 DM, is important as there is greater than 94% concordance in identical twins.

2.3.3 Type 3 Diabetes Mellitus

In this type, there are other causes of hyperglycemia, e.g., chronic pancreatitis or chronic drug therapy with glucocorticoids, thiazide diuretics, diazoxide , growth hormone and with some protease inhibitors used to treat human immunodeficiency virus infections (e.g., saquinavir).

2.3.4 Type 4 Diabetes Mellitus

It is also called "Gestational Diabetes Mellitus" (GDM). It is observed in approximately 4-5% of all pregnancies. Elevated blood sugar levels are usually observed in second or last trimester of pregnancy and usually resolved during the postpartum period. There is no genetic predisposition. The most plausible cause is that during pregnancy, the placental hormones promote insulin resistance [21].



OVER VIEW OF DM

2.4 Etiology

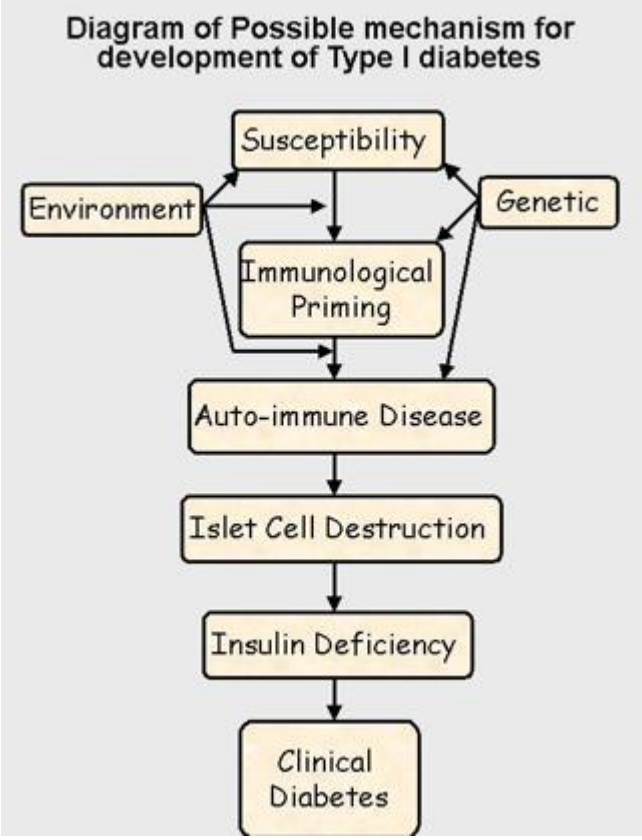
The two main types of diabetes are type 1 diabetes and type 2 diabetes. A third type, gestational diabetes, develops only during pregnancy. Other types of diabetes are caused by defects in specific genes, diseases of the pancreas, certain drugs or chemicals, infections, and other conditions. Some people show signs of both type 1 and type 2 diabetes [22].

2.4.1 Type 1 Diabetes mellitus

Type 1 diabetes is caused by a lack of insulin due to the destruction of insulin producing beta cells in the pancreas. In type 1 diabetes an autoimmune disease the body's immune system

attacks and destroys the beta cells. Normally, the immune system protects the body from infection by identifying and destroying bacteria, viruses, and other potentially harmful foreign substances. But in autoimmune diseases, the immune system attacks the body's own cells. In type 1 diabetes, beta cell destruction may take place over several years, but symptoms of the disease usually develop over a short period of time [23]. Type 1 diabetes typically occurs in children and young adults, though it can appear at any age. In the past, type 1 diabetes was called juvenile diabetes or insulin-dependent diabetes mellitus [24].

Latent autoimmune diabetes in adults (LADA) may be a slowly developing kind of type 1 diabetes. Diagnosis usually occurs after age 30. In LADA, as in type 1 diabetes, the body's immune system destroys the beta cells. At the time of diagnosis, people with LADA may still produce their own insulin, but eventually most will need insulin shots or an insulin pump to control blood glucose levels. Genetic Susceptibility, Autoimmune Destruction of Beta Cells, Environmental Factors , Viruses and infections, Infant feeding practices also leads to DM [25].



Possible mechanism for development of type 1 diabetes.

2.4.2 Type 2 Diabetes mellitus

Type 2 diabetes the most common form of diabetes is caused by a combination of factors, including insulin resistance, a condition in which the body's muscle, fat, and liver cells do not use insulin effectively. Type 2 diabetes develops when the body can no longer produce enough insulin to compensate for the impaired ability to use insulin. Symptoms of type 2 diabetes may develop gradually and can be subtle; some people with type 2 diabetes remain undiagnosed for years. Type 2 diabetes develops most often in middle-aged and older people who are also overweight or obese. The disease, once rare in youth, is becoming more common in overweight and obese children and adolescents. Scientists think genetic susceptibility and environmental factors are the most likely triggers of type 2 diabetes. Genetic Susceptibility , Obesity and Physical Inactivity , Insulin Resistance, Abnormal Glucose Production by the Liver ,Metabolic Syndrome, Beta Cell Dysfunction can lead to DM [26,27].

2.4.3 Other Types of Diabetes

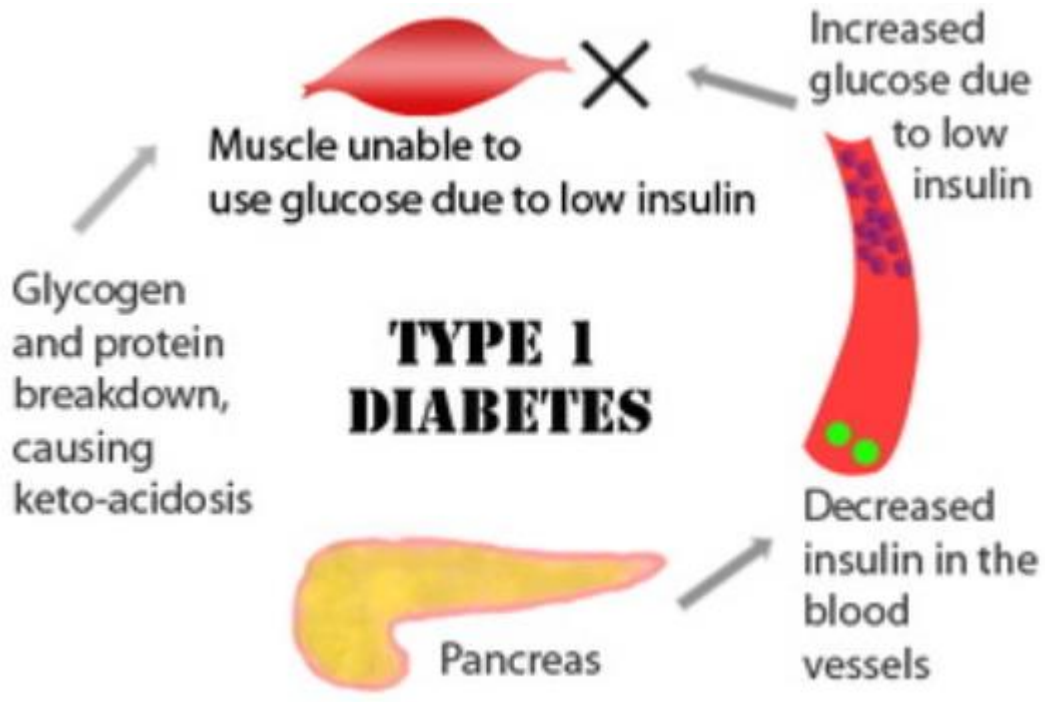
Other types of diabetes have a variety of possible causes. They include Genetic Mutations Affecting Beta Cells, Insulin, and Insulin Action , Other Genetic Diseases, Damage to or Removal of the Pancreas, Endocrine Diseases, Autoimmune Disorders, Medications and Chemical Toxins and Lipodystrophy.

2.5 Pathophysiology

2.5.1 Type 1 Diabetes mellitus

Type-1 DM is an autoimmune disease of the pancreatic beta cells (type-1A) resulting in their degeneration. It could also be idiopathic (type-1B).Viral infection such as echo-virus can also damage pancreatic beta cells (type-1B). Approximately 10% of diabetics suffer with type-1 DM. These patients have a low degree of genetic predisposition, yet 15-20% of patients reveal a positive family history and the incidence in homozygous twins is about 50%.

The clinical features of type-1 DM include hyperglycaemia with polyuria, polydipsia, polyphagia and ketoacidosis. These patients are generally not obese.Diabetic ketoacidosis is the end result of insulin deficiency in uncontrolled type-1 diabetes [28].



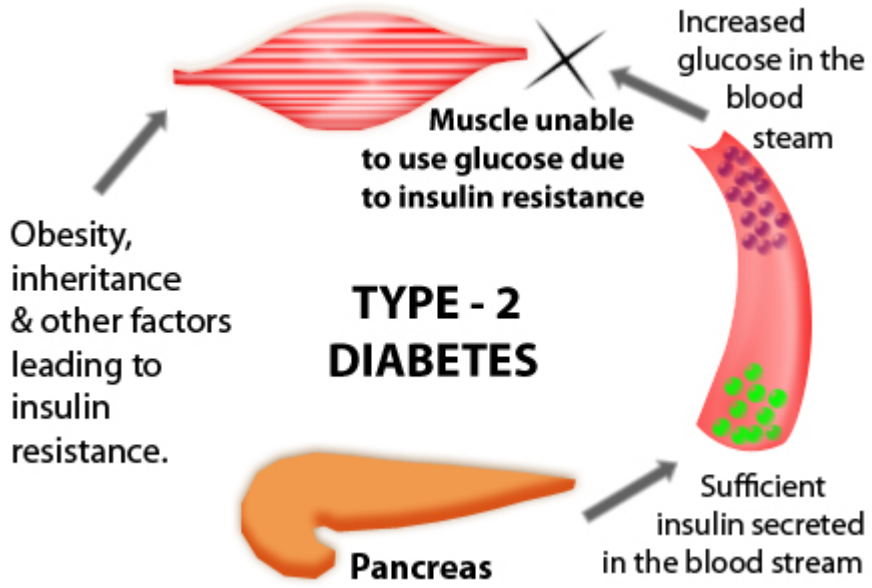
Pathophysiology of Type-I Diabetes

Since insulin is not present to aid the entry of glucose in skeletal muscles and body cells, most cells now use fatty acids to produce ATP to compensate and to provide calories. This accelerated fat breakdown generates Acetyl Co-A. But, due to DM, this acetyl Co-A cannot be removed by Kreb`s cycle (to H2O2 and CO2) and therefore gets accumulated. In absence of aerobic carbohydrate metabolism, two acetyl Co-A molecules join to form acetoacetic acid, beta hydroxyl butyaric acid and acetone, which are collectively called ketone bodies. These metabolic products cause metabolic acidosis or diabetic ketoacidosis, which decreases glucose utilization in brain and decreases pH of the blood leading to coma and death. Renal loses of glucose (glycosuria), nitrogenous substances and ketone bodies (ketoacidosis) promote osmotic dieresis (polyuria) that can result in dehydration and thirst (polydipsia) [28].

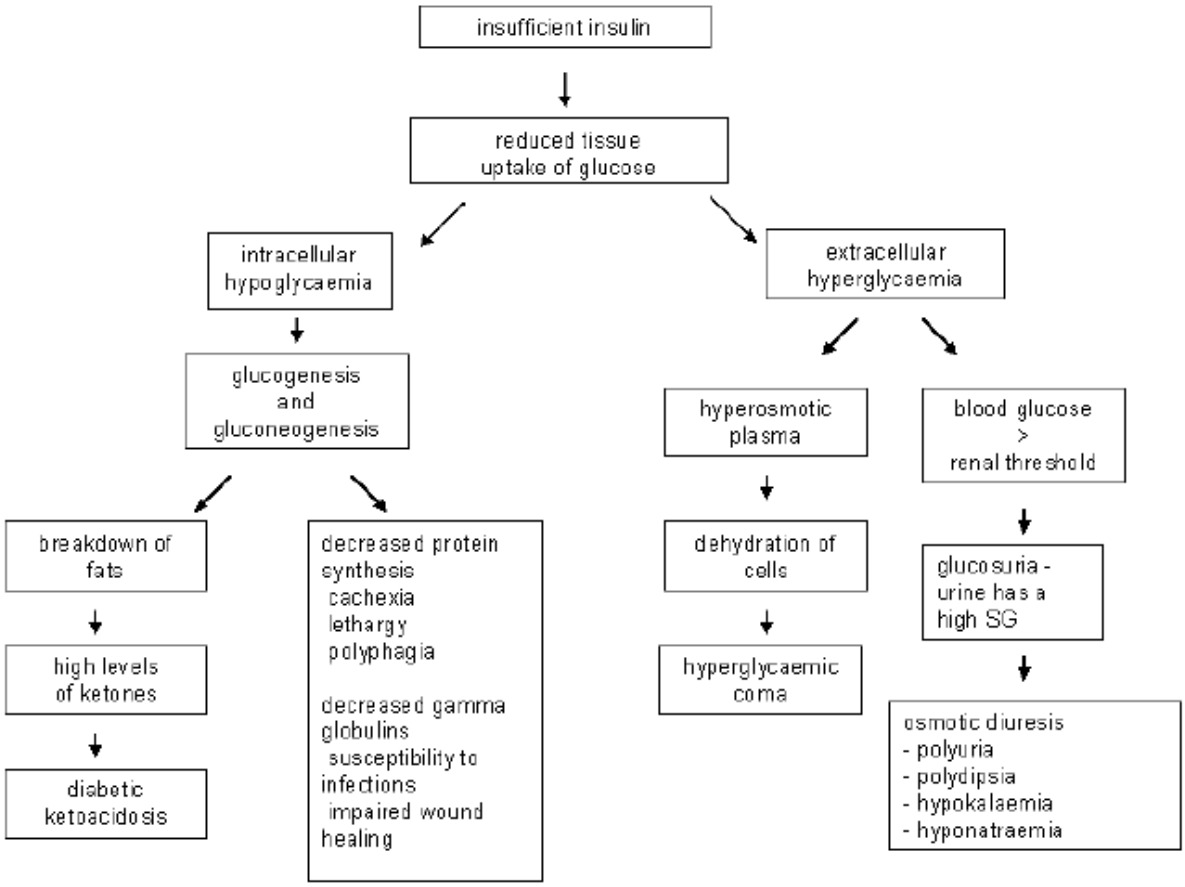
2.5.2 Type 2 Diabetes mellitus

Type 2 diabetes is caused by either inadequate production of the hormone insulin or a lack of response to insulin by various cells of the body. In type 2 diabetes, the body either produces inadequate amounts of insulin to meet the demands of the body or insulin resistance has

developed. Insulin resistance refers to when cells of the body such as the muscle, liver and fat cells fail to respond to insulin, even when levels are high. In fat cells, triglycerides are instead broken down to produce free fatty acids for energy; muscle cells are deprived of an energy source and liver cells fail to build up glycogen stores. This also leads to an overall rise in the level of glucose in the blood. Glycogen stores become markedly reduced and there is less glucose available for release when it may be needed. Obesity and lack of physical activity are thought to be major causes of insulin resistance [29].



Pathophysiology of Type-II Diabetes



2.6 Diagnosis

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following:

- ✓ Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- ✓ Plasma glucose ≥ 11.1 mmol/l (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test.
- ✓ Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
- ✓ Glycated hemoglobin (Hb A1C) ≥ 6.5%.

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic

advantage over the fasting test. Two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus.

2.7 Signs and symptoms

Symptoms of type I diabetes

- ✓ Being very thirsty
- ✓ Feeling hungry
- ✓ Feeling tired all the time
- ✓ Having blurry eyesight
- ✓ Feeling numbness or tingling in your feet
- ✓ Losing weight without trying
- ✓ Urinating more often (including urinating at night or bedwetting in children who were dry overnight before)[30]

Symptoms of type II diabetes

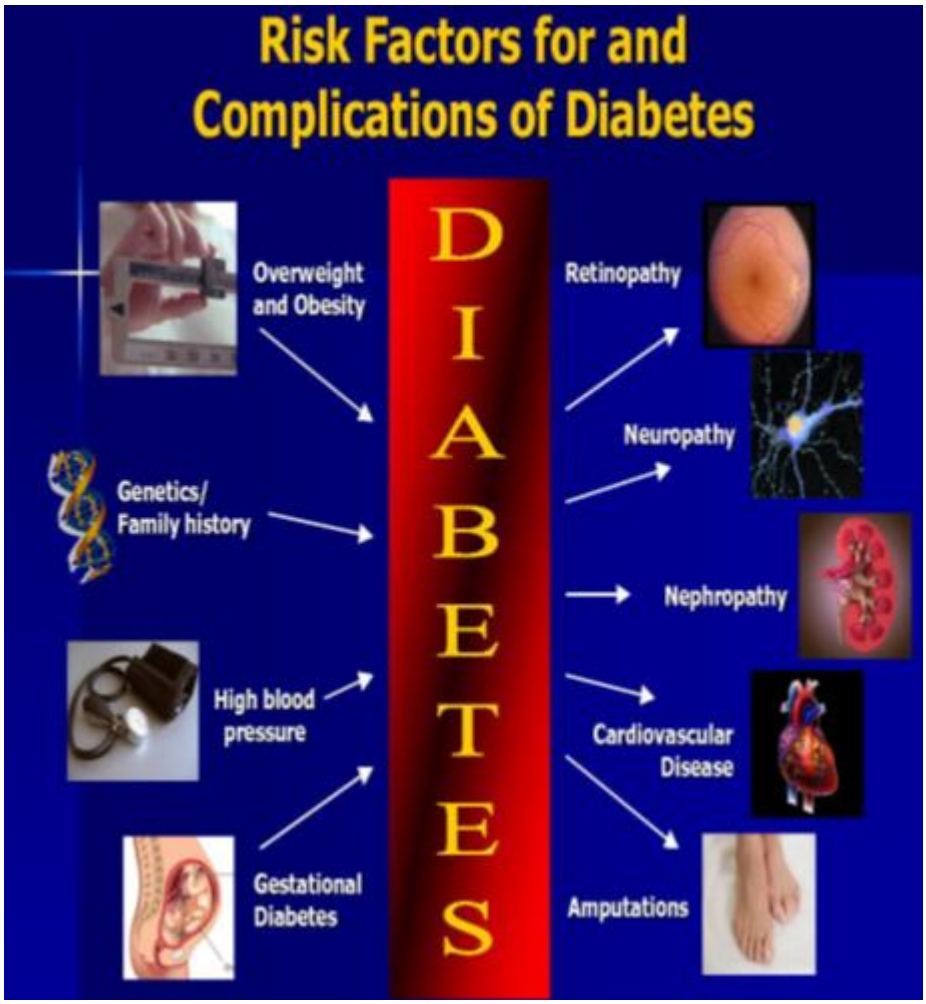
- ✓ Frequent urination
- ✓ Increased thirst
- ✓ Increased hunger
- ✓ Blurred vision
- ✓ Slow-healing wounds or sores
- ✓ Prolonged and unexplained fatigue
- ✓ Numbness or tingling or burning sensation in the legs or feet
- ✓ Gynecological fungal infections in women
- ✓ Sexual impotence in men
- ✓ Muscle cramps [31]

Symptoms of retinopathy are minimal until advanced disease ensues with loss or blurring of vision. Signs of non-proliferative retinopathy include microaneurysms, venous loops, retinal hemorrhages, hard exudates, and soft exudates. Proliferative retinopathy can include new vessels in the eyes or vitreous hemorrhage. The earliest sign of nephropathy is hypertension. Development of hypertension often coincides with the development of microalbuminuria. As nephropathy worsens, patients can develop edema, arrhythmias associated with hyperglycemia,

and/or symptoms related to renal failure. Signs and symptoms of neuropathy are dependent on the type of neuropathy that develops. Most commonly, patients develop symptomatic distal polyneuropathy. Signs include depression or loss of ankle jerks and vibratory sensation, with hyperalgesia and calf pain in some patients. The deficit is in a stocking glove distribution. Wasting of the small muscle of the hands and feet can also occur. Patients may present with focal neuropathies due either to mononeuritis or entrapment syndromes. These produce focal neurologic deficits confined to a single nerve. A rare but severe form of diabetic neuropathy is diabetic amyotrophy, which begins with pain followed by severe weakness and spreads from unilateral to bilateral. It resolves spontaneously in 18-24 months. Patients with coronary artery disease can present with stable angina pectoris, unstable angina pectoris, or myocardial infarction. Many patients have unrecognizable symptoms and can present with dysrhythmias. Patients with cerebral vascular disease can present with a sudden onset of a focal neurologic deficit such as facial droop, hemiparesis, or isolated weakness of an arm or leg. Dizziness, slurred speech, gait difficulties, and visual loss can also be presenting symptoms. Stroke symptoms that last <24 hours are referred to as atransient ischemic event. Peripheral vascular disease is recognized by exertional leg pain that can progress to rest pain and ischemic ulcers. Most cases are asymptomatic.

2.8 Risk factors and complications

The major cause of the high morbidity and mortality rate associated with diabetes is a group of microvascular and macrovascular complications affecting multiple organ systems. People with diabetes have a greatly increased risk for blindness, kidney failure, myocardial infarction, stroke, necessary limb amputation, and a host of other maladies. The onset and progression of these complications is strongly linked to the presence of sustained hyperglycemia. The complication rate and the severity of complications increase as the duration of diabetes increases. Other disorders (such as hypertension and dyslipidemia) commonly seen in people with diabetes increase the risk for microvascular and macrovascular complications. There may also be genetic determinants of risk for diabetic complications [32].



RISK FACTOS AND COMPLICATIONS OF DIABETES

2.9 Prevention

There is no known preventive measure for type 1 diabetes. Type 2 diabetescan often be prevented by a person being a normal body weight, physical exercise, and following a healthy diet. Dietary changes known to be effective in helping to prevent diabetes include a diet rich in whole grains and fiber, and choosing good fats, such as polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help in the prevention of diabetes. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well.

2.10 Management of diabetes

The mainstay of non-pharmacological treatment of diabetes is diet and physical activity. Other methods of treatment includes acupuncture, hydrotherapy, mineral supplementation and conventional drugs which includes exogenous insulin, oral hypoglycemic agents and transplantation.

Oral glucose lowering drugs belong to five classes of oral agents approved for the management of diabetes mellitus. Oral therapy was indicated in any patient in whom diet and exercise fail to achieve acceptable glycemic control. Although initial response may be good, oral hypoglycemic drugs may lose their effectiveness in a significant percentage of patients. The drug category includes sulfonylurea, biguanide, α-glucosidase inhibitor, thiazolidinedione and meglitinide and these drugs have various side effects. For instance; sulfonylurea causes weight gain due to hyperinsulinemia, biguanide cause body weakness, fatigue, lactic acidosis and alpha glucosidase inhibitor may cause diarrhea while thiazolidinediones may increase LDL-cholesterol level[33]. Insulin is the commonly included in an oral agent when glucose control is sub-optimal at maximal dose of oral medication. Weight gain and hypoglycemia are the most common side effects of insulin. Vigorous insulin treatment may also carry an increase in atherogenesis[34]

Oral glucose-lowering agent sulfonylurea, tolbutamide and glyburide acts by enhancing insulin secretion from the pancreatic β cells[35]. These act on liver cells stimulating breakdown of glucose in glycolytic pathway and inhibiting glucose generation. Sulphonylureas acts by inhibiting the KATP channels in plasma membranes of pancreatic β cells. The inhibition works to stimulate the secretion of insulin which is similar to that produced by glucose in the body but is of a distinct mechanism. They may be used as first-line drugs in a case where oral hypoglycemic medication is required particularly in patients who cannot tolerate metformin. Newer drugs in this category such as glipizide and glimipramide appear to afford similar efficacy than older drugs such as gliclazide[36].

All sulphonylureas drugs have a sulphonic acid-urea nucleus, and different chemical moieties are added at various positions on the nucleus to make different drugs. The action of the resultant drugs may have the desired effect, however, the potency and efficacy may differ significantly[37].

Sulphonylureas drugs are typically not indicated for type 1 diabetic patients since they require the functioning of the β cells to produce the desired effect on blood glucose. They have been found to be most effective in non-obese patients with mild maturity onset diabetes and whose high glucose levels have not responded appropriately to diet alterations.

Biguanides such as metformin acts by increasing glucose transport across cell membrane of the skeletal muscle. They act in the presence of endogenous insulin, and are effective only where there are residual functioning pancreatic islet cells. Metformin is widely used in treatment of patients with insulin resistance because it can be used safely as an adjunct to diet therapy in obese patients to control their high glucose levels especially those who are not responsive to other therapies. The exact mode of action of metformin is disputable. Zhou et al indicated that it activates adenosine monophosphate protein kinase (AMPK) in liver cells leading to increased fatty acid oxidation and glucose uptake by cells[38]. An overall reduction in lipogenesis and hepatic glucose production is normally observed. Metformin has antioxidant properties which are useful in its use in treatment of diabetes and cardiovascular disease. It has been demonstrated to inhibit xanthine oxidase and phosphodiesterase, advanced glycation end product formation and decreased production of tumour necrosis factor[39].

The main problem with metformin is the risk of lactic acidosis which is particularly common in patients with renal insufficiency, cardiovascular disease, peripheral vascular disease, liver disease, pulmonary disease and in individuals over 65 years. Weakness, fatigue, shortness of breath, nausea, dizziness and kidney toxicity are the side effects.

Thiazolidinediones are known to act by increasing the sensitivity of peripheral tissues to insulin by affecting the expression of specific genes. They achieve this by binding and activating γ peroxisome proliferator-activated receptor (PPAR-γ), a nuclear receptor. Some of the effects of this gene expression include the increase in the expression of the glucose transporters, decreased hepartic glucose output as well as the increased differentiation of pre-adipocytes into adipocytes. The high affinity of this drug to PPAR-γ is important in the management of insulin resistance since large adipocytes that differentiate from smaller ones produce TNF-α which increase insulin resistance. Thiazolidinediones therefore suppresses the expression of these adipokines involved in insulin resistance[40].

Alpha-glucosidase acts by inhibiting alpha glucosidase enzyme in the brush border of the small intestine. This delays the absorption of glucose by decreasing the breakdown of complex carbohydrates by enteric digestive enzymes. Some of the most commonly used α-glucosidase inhibitors like acarbose have severe gastrointestinal side effects such as diarrhoea, flatulence and abdominal pains. This raises the need for other sources of these inhibitors that have fewer side effects. The most obvious choice for these alternatives would be plants with ethnomedical uses in management of diabetes.

2.11 Introduction of Streptozotocin

Streptozotocin is a permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *Streptomyces achromogenes* (gram positive bacterium) with broad spectrum of antibacterial properties. Streptozotocin is an unusual aminoglycoside containing a nitrosoamino group discovered in 1959 as an antibiotic, now marketed as a generic drug. The nitrosoamino group enables the metabolite to act as a nitric oxide (NO) donor. NO is an important messenger molecule involved in many physiological and pathological processes in the body. Streptozotocin is widely used to induce diabetes in rodent models by inhibition of β-cell O-GlcNAcase.

Streptozotocin features four important biological properties as evidenced by its antibiotic, β-cell (beta)-cytotoxic, oncolytic, as well as oncogenic effects. This product is an antineoplastic antibiotic and is used mainly in the treatment of pancreatic (islet cell) tumors. It is used for the treatment of malignant insulinoma. Current use of STZ is mostly as an investigational drug for diabetes research due to its specific toxicity associated with pancreatic β-cells. Low affinity glucose transporter- GLUT2 of βcells transports STZ into the cell and causes alkylation of DNA and irreversible necrosis of β cells. DNA synthesis in mammalian and bacterial cells is inhibited by action of STZ. STZ is widely used to induce both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). STZ is an antibiotic and antitumor agent, induces diabetes mellitus via reduction of nicotinamide adenine dinucleotide in pancreatic β-cells *in vivo*. This review will summarize the chemistry of STZ and its β-cell toxicity through the link between STZ and free radicals. In addition, dosage, route of administration and metabolism of STZ in experimental animal models to study diabetes will be addressed[41].

2.12 Mechanism of action of Streptozotocin

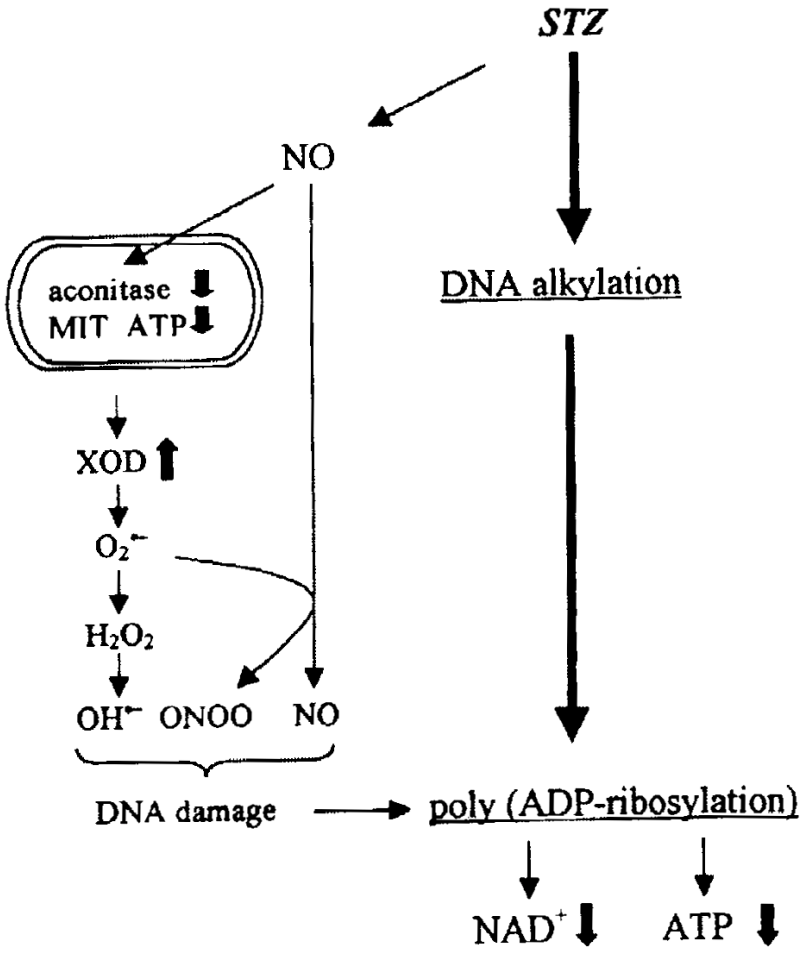
The range of the STZ dose is not as narrow as in the case of alloxan. The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w., but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40 mg/kg b.w. may be ineffective . For instance, when 50 mg/kg b.w. STZ are injected intravenously to fed rats, blood glucose (determined 2 weeks after treatment) can reach about 15 mM STZ may also be given in multiple low doses. Such treatment is used predominantly in the mouse and the induction of IDDM is mediated by the activation of immune mechanisms. Streptozotocin action in B cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin. About six hours later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease. These changes in blood glucose and insulin concentrations reflect abnormalities in B cell function. STZ impairs glucose oxidation and decreases insulin biosynthesis and secretion. It was observed that STZ at first abolished the B cell response to glucose. Temporary return of responsiveness then appears which is followed by its permanent loss and cells are damaged.

STZ is taken up by pancreatic B cells via glucose transporter GLUT2. A reduced expression of GLUT2 has been found to prevent the diabetogenic action of STZ. Intracellular action of STZ results in changes of DNA in pancreatic B cells comprising its fragmentation Recent experiments have proved that the main reason for the STZ-induced B cell death is alkylation of DNA. The alkylating activity of STZ is related to its nitrosourea moiety, especially at the O6 position of guanine. After STZ injection to rats, different methylated purines were found in tissues of these animals.

Since STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, it was proposed that this molecule contributes to STZ-induced DNA damage. Pancreatic B cells exposed to STZ manifested changes characteristic for NO action, i.e. increased activity of guanylyl cyclase and enhanced formation of cGMP. STZ is, however, not a spontaneous nitric oxide donor. This molecule is liberated when STZ is metabolized inside cells, but NO synthase is not required for this effect. On the other hand, the lowering of NO concentration in pancreatic islet cells by inhibition of the inducible form of nitric oxide synthase partially counteracted DNA cleavage induced by STZ. A similar effect can be attained by NO scavengers. However, the results of several experiments provide the evidence that NO is not the only molecule responsible for the cytotoxic effect of STZ. STZ was found to generate reactive oxygen species, which also contribute to DNA fragmentation and evoke other deleterious changes in the cells. The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase.It was demonstrated that STZ inhibits the Krebs cycle and substantially decreases oxygen consumption by mitochondria. These effects strongly limit mitochondrial ATP production and cause depletion of this nucleotide in B cells. Restriction of mitochondrial ATP generation is partially mediated by NO. This molecule was found to bind to the iron-containing aconitase inhibiting enzyme activity. Augmented ATP dephosphorylation increases the supply of substrate for xanthine oxidase (B cells possess high activity of this enzyme) and enhances the production of uric acid – the final product of ATP degradation. Then, xanthine oxidase catalyses reaction in which the superoxide anion is formed. As a result of superoxide anion generation hydrogen peroxide and hydroxyl radicals are formed. The inhibition of xanthine oxidase by allopurinol restricts the cytotoxic effect of STZ in vitro. Pretreatment of B cells with this inhibitor prevented the STZ-induced decrease of insulin secretion. It can be stated that potent alkylating properties of STZ are the main reason of its toxicity. However, the synergistic action of both NO and reactive oxygen species may also contribute to DNA fragmentation and other deleterious changes caused by STZ. NO and reactive oxygen species can act separately or form the highly toxic peroxynitrate (Figure). Therefore, intracellular antioxidants or NO scavengers substantially attenuate STZ toxicity. STZ-induced DNA damage activates poly ADP ribosylation. This process leads to depletion of cellular NAD+, further reduction of the ATP content and subsequent inhibition of insulin synthesis and

secretion. The concept of unfavorable consequences of augmented poly ADP-ribosylation as a result of STZ action was confirmed by experiments revealing that the inhibition of this process prevents the toxicity of this diabetogenic agent. It was found that 3-aminobenzamide, a strong inhibitor of poly(ADP-ribose) synthase, protected against the action of STZ in rats, even when this substance was administered 45-60 min after STZ. Another inhibitor of poly(ADP-ribose) synthase, nicotinamide, which is also scavenging oxygen free radicals, exerted best protection when it was administered shortly after STZ. The failure of protective action of nicotinamide administered after STZ is probably due to a potent reduction of the cellular ATP content by STZ since nicotinamide uptake is ATP-dependent. The protective effect of 3-aminobenzamide and nicotinamide was also confirmed in vitro.

It has been suggested that some inhibitors of poly ADP-ribosylation may also exert a protective effect due to their hydroxyl radical scavenging properties. However, in the case of STZ, recent investigations in poly(ADP-ribose) polymerasedeficient mice demonstrated that the inhibition of poly ADP-ribosylation itself prevents STZ-induced B cell damage and hyperglycemia. Thus, it can be stated that the activation of poly ADP-ribosylation is of greater importance for the diabetogenicity of STZ than generation of free radicals and DNA damage per se. Calcium, which may also induce necrosis, does not seem to play a significant role in the necrosis evoked by STZ since calcium channel antagonists do not protect B cells against streptozotocin, as they do in the case of alloxan[42].



The mechanism of streptozotocin (STZ)-induced toxic events in B cells of rat pancreas. MIT –mitochondria; XOD – xanthine oxidase.

3.1 Description

Nymphaea pubescens(Nymphaeaceae) large perennial aquatic herb with short, erect, roundish, tuberous rhizomes; leasves floating, peltate, sharply sinuate-toothed, flowers large, floating, solitary, variable in colour from pure white to deep red; fruits spongy many seeded berries, seeds minute, grayish black when dry with longitudinal striations. Nymphaea pubescens found throughout the warmer parts of India, in tanks, ponds and ditches. *Nymphaea pubescens* of rhizome is cooling, sweet, bitter and tonic, and is useful in diarrhoea, dysentery, dipsia and general debility.The flowers are astringent and cardio tonic. The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative. They are useful in vitiated conditions of pita, dipsia, diarrhea and dermatopathy[43].

Nymphaea pubescens

3.2 Regional and other names

Common name	-	Hairy water lilly
Tamil	-	Vellambal. Neerambal
Sanskrit	-	Kumuda
Hindi	-	Kokaa

Malayalam - Neerambal

3.3 Classification

Kingdom	-	Plantae
Phylum	-	Tracheophyta
Class	-	Magnoliopsida
Order	-	Nymphaeales
Genus	-	Nymphaea
Family	-	Nymphaeaceae
Species	-	N. pubescens

Nymphaea pubescens is known under a number of different synonyms, the most common of which is Nymphaea rubra for the reddish variant known under the commercial name Red water lily, which often has also purplish leaves.

3.4 Geographical range

Bangladesh; Cambodia; India; Indonesia; Lao People's Democratic Republic; Malaysia; Myanmar; Nepal; Pakistan; Philippines; Sri Lanka; Thailand; Viet Nam

3.5 Habitat and etiology

Plants of this species are found commonly in shallow pools and ponds. The hairy water lily is found cultivated and also in the wild. It prefers non-acidic water and does not tolerate temperature below 15° C.

3.6 Ethanomedical uses

Nymphaea pubescenes revealed that the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids. The hypoglycaemic effect of Nymphaea pubescens tuber was found to be inducing insulin release from pancreatic cells of diabetic. *Nymphaea pubescens* tuber extract can reduce the levels of serum urea and creatinine and confirms the protection of vital tissues (Kidney and liver) including the pancreas, thereby reducing the causation of diabetes in the experimental animals. Ethanol extract of Nymphaea pubescens tuber offers a promising therapeutic value in prevention of diabetes. These effects could be mainly attributed to its antioxidant properties as shown by significant quenching impact on the extract of lipid peroxidation along with, enhancement of antioxidant defense systems in pancreatic tissue. The antioxidative property of Nymphaea pubescens extract certainly is due to its chemical constituents. Phytochemical investigations of Nymphaea pubescens have demonstrated the presence of flavonoids and phenolic compounds as main active ingredients having potent antioxidant activities. Further studies will be needed in future to determine the main active ingredient having the beneficial antidiabetic, hypolipidaemic and antioxidant effects. *Nymphaea pubescens* was used as blood purifier and in the treatment of jaundice.

4. Literature review about the work so far done in the plant *Nymphaea pubescens* (willd)

4.1.1. Inhibitory Effect Of *Nymphaea pubescens* Willd. Flower Extract On Carrageenan-Induced Inflammation And Ccl_4-Induced Hepatotoxicity In Rats.

Nymphaea pubescens Willd. is used as ingredient of ethnic diet and folk medicine in South-East Asia. The water (NPW), methanol (NPM) and chloroform (NPC) extracts of N. pubescens flowers were investigated for NO·, $O_2{\cdot}^-$ and DPPH radical scavenging and iron chelating activities in vitro. NPW was found to be the most potent free radical scavenger (EC_{50}<100 µg/mL) whereas NPC did not show EC_{50} at 500 µg/mL. Therefore, NPW was selected for further studies on anti-inflammatory and hepatoprotective activities, using standard in vitro and in vivo models. NPW exhibited inhibition of nitrogen radical generation in LPS-activated macrophages (IC_{50}=75.5 µg/mL) through suppression of iNOS protein, with no associated toxicity in the cells. Further, 500 mg/kg of NPW reduced rat paw edema by ~50% after 6h of carrageenan administration. Hepatoprotective activity of NPW was also evaluated in vivo on CCl4-induced hepatotoxicity model in rats. NPW treatment (500 mg/kg/day for ten days) attenuated CCl4-induced increase in serum enzymes, viz. alanine and aspartate aminotransferases (ALT and AST) and bilirubin. Also, glutathione and superoxide dismutase (SOD)-levels were restored towards normalcy in the liver of CCl4-treated rats, indicating the hepatoprotective role of NPW, which was found to contain a fair amount of flavonoids, phenolics, and saponin constituents[44].

4.1.2. Antiproliferative Activity Of Ethanolic Flower Extract From *Nymphaea pubescens* Willd Against Human Cervical And Breast Carcinoma In Vitro.

Nymphaea pubescens Willd (Nymphaeaceae) is a fascinating aquatic plant mentioned in siddha system of medicine, in the treatment of bleeding piles, diabetes and as cardiotonic in palpitation of the heart. Nymphaea species was traditionally used for treating cancer. The present study was designed to evaluate the invitro antiproliferative activity of *Nymphaea pubescens* Willd. The ethanolic extract of different parts such as rhizome, leaf, flower and fruit

was subjected for MTT assay. The ethanolic extract of flower part was found to be cytotoxic against human cervical carcinoma Hela cell lines and human breast carcinoma MCF cell lines. The IC50 value of ethanolic flower extract was 91.57µg/ml against Hela cell lines and 99.6µg/ml against MCF-7 cell lines. Significant results were observed thereby justifying the use of plant in the traditional system of medicine[45].

4.1.3. Antihepatotoxic Efficacy Of *Nymphaea pubescens* (Willd.) On Acetaminophen Induced Liver Damage In Male Wistar Rats.

The antihepatotoxic efficacy of aqueous flower extracts of *Nymphaea pubescens*(NP) and Silymarin were investigated against acetaminophen induced liver damage in rats. Acetaminophen at the dose of 3gm/kg body weight orally produced liver damage in rats as manifested by the significant rise in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, cholesterol and decreases the protein level compared with control. Oral administration of flower extracts of *Nymphaea pubescens*(100, 200, 400mg/kg) and silymarin (25mg/kg) once daily for 28 days to acetaminophen treated rats shows lowered significantly the afore mentioned clinical parameters where as protein level increased. The extract alone treated rats did not adversely affect the serum biochemical estimation. A significant antihepatotoxic efficacy of *Nymphaea pubescens* extracts was reported[46].

4.1.4. Antihyperglycaemic, Antihyperlipidaemic And Antioxidant Assays(In Vivo) Of *Nymphaea pubescens* Leaf Extract.

The present study was designed to investigate the possible antidiabetic, hypolipidaemic and antioxidant effects of methanol extract of *Nymphaea pubescens* leaves.Diabetes was persuaded in albino rats by injecting of alloxan monohydrate .The methanol extract of *Nymphaea pubescens* leaves at a dose of 250mg/kg and 500mg/kg body weight were administered at single dose per day to diabetes induced rats for a period of 28 days. The effect of methanol extract of *Nymphaea pubescens* leaves extract on blood glucose(48), glycosylated haemoglobin (HbA1C) (3), Alanine aminotransferase (ALT) (38.5) and Aspartate aminotransferase (AST) (56) level. serum lipid profile total cholesterol (TC) (56.5), triglyceride

(TG) (44), low density lipoprotein- cholesterol (LDLC) (10.5), high density lipoprotein-cholesterol (HDL-C) (39) and catalase (CAT) (0.51), lipoprotein peroxidation (LPO) (0.134), blood reduced glutathione (GSH) (0.169) were measured in the diabetic rats. The methanol extracts of *Nymphaea pubescens* leaves elicited significant ($p<0.05$) Declines of blood glucose, lipid parameters except HDLCholestrol, serum enzymes and significantly increased HDL-C and antioxidant. In conclusion, the methanol extract of *Nymphaea pubescens* leaves offers promising antidiabetic and hypolipidaemic effects compared with control drug that may be mainly attributed to its potent antioxidant potential. Additional research will be needed in future in order to conclude which one of its active components have the main antidiabetic and hypolidaemic effects[47].

4.1.5. Phytochemical Analysis And Antimicrobial Properties Of Leaf Extracts Of *Nymphaea pubescens*.

Objective:

To evaluate the antimicrobial activity of *Nymphaea pubescens* extracts against some bacteria and fungi. The study also investigated the phytochemical constituents of the plant.

Methods:

The phytochemical constituents of the dried powdered plant was extracted using; Organic solvents (Hexane: petroleum ether, ethyl acetate and methanol). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both bacteria and fungi using the Agar well diffusion method.

Results:

Results of the phytochemical screening revealed the presence of Alkaloids, Terpenoids, Steroids, Anthocyanidins, Phenolic compounds and flavonoids and the methanol, ethyl acetate extracts were found to be more potent than hexane: petroleum ether extract.

Conclusions:

Nymphaea pubescens has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for possible treatment of gonorrhea, pneumonia, urinary tract and some mycotic infections[48].

4.1.6. Tapping of Aquatic Plant *Nymphaea pubescens* WILLD. For Synthesis of Silver Nanoparticles and Its Antimicrobial Evaluation.

Nymphaea pubescens Willd. is a traditional medicinal plant used in Ayurvedic formulation from ancient times. Today biological synthesis of nanoparticles emerges as an eco-friendly and cost effective approach in the field of nanotechnology. In the present study, we report the biosynthesis of silver nanoparticles (SNPs) using plant leaf extract for the reduction of aqueous Ag+ ions into Ag0 particles, characterization and its antimicrobial activity on clinically isolated microorganisms. Stable silver nanoparticles were formed by treating aqueous solution of $Ag(NO3)_2$ with the plant leaf extract. These nanoparticles are monitored by using UV -Visible spectroscopy, FTIR, AFM and EDAX analysis for their synthesis confirmation, size and shape distribution. Further these nano particles are evaluated for antibacterial activity against clinically isolated bacterial pathogens like, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus. The UV-Vis spectroscopic shows a broad peak at 404 nm indicates the formation of SNPs. FT-IR studies reveals broad peaks at 3336 cm-1 assigned for O-H bond of phenols and 1637 assigned for N-H bond of primary amines of proteins. AFM studies clearly indicate the particles are spherical in shape having the size range from 66 to 91 nm. EDAX spectrum of synthesized nanoparticles shows 5.24 weight percentage Ag metal in the reaction mixture indicates the purity of SNPs. Further antimicrobial studies on bacterial pathogens shows potential activity indicates the N. pubescens is an ideal plant for the synthesis of SNPs and these biologically synthesized SNPs have an advantage over conventional antibiotics[49].

4.1.7. Invitro Antioxidant And Free Radical Scavenging Activity Of *Nymphaea pubescens*

In vitro antioxidant effects of the ethanolic extract of whole plant of *Nymphaea pubescens* were investigated in the present study. 1,1-diphenyl-2-picryl hydroxyl (DPPH) quenching assay, Hydroxyl Radical Scavenging Activity, Nitric Oxide Scavenging activity using established assay procedures were performed for its antioxidant activity. The generation

of free radicals DPPH, nitric oxide and hydroxyl radicals were effectively scavenged by the ethanolic extract of *Nymphaea pubescens* . The extract showed strong antioxidant activity in a dose dependent manner. The results from the present study clearly indicates that N. pubescens scavenges free radicals, ameliorating damage imposed by oxidative stress in different disease conditions and act as a novel source of natural antioxidant[50].

4.1.8. Potential antidiabetic, hypolipidaemic and Antioxidant effects of *Nymphaea pubescens* extract in alloxan induced diabetic rats.

The present study was designed to investigate the possible antidiabetic, hypolipidaemic and antioxidant effects of ethanol extract of *Nymphaea pubescens* tuber. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150 mg/kg, body weight i.p). The ethanol extract of *Nymphaea pubescens* tuber at a dose of 200mg/kg and 500mg/kg body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Nymphaea pubescens* tuber extract on blood glucose, plasma insulin, urea creatinine, glycosylated haemoglobin, serum lipid profile (total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), high density lipoprotein-cholesterol (HDL-C)and phospholipids (PL)), serum protein, albumin, globulin, serumenzymes (Serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphate (ALP)), lipoprotein peroxidation (LPO), blood reduced glutathione (GSH), oxidative glutathione (GSSG), GSH/GSSG ratio, erythrocytes glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione S-transferase (GST) were measured in the diabetic rats. The ethanol extracts of *Nymphaea pubescens* tuber elicited significant ($p<0.05$) reductions of blood glucose, lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant. The extract also caused significant increase in plasma insulin ($p<0.05$) in the diabetic rats. In conclusion, ethanol extract of *Nymphaea pubescens* tuber offers promising antidiabetic and hypolipidaemic effects that may be mainly attributed to its potent antioxidant potential. Further studies will be needed in future in order to determine which one or more of its active constituents have the main antidiabetic and hypolipdaemic effects[51].

4.1.9. Effect of *Nymphaea pubescens* Leaf Extract on Growth of Nile Tilapia (Oreochromis niloticus)

The use of herbal plants has been conducted for the treatment of various ailments since the ancient civilizations, but their uses in fish culture are still limited. Plant-based feed supplementation may provide the elements or bioactive compounds that would help to enhance fish growth and to improve feed utilization. The aims of this present study were therefore, to investigate the use of *Nymphaea pubescens* leaf extract as a feed additive on survival rate and growth performance of Nile tilapia (Oreochromis niloticus). N. pubescens leaves were extracted using 70% ethanol. One hundred fingerlings were divided into 5 groups: each consisting of 20 fish. In a feeding trail of 60 days, Group 1-4, fish were fed diets containing 0, 0.05, 0.1 and 1% of N. pubescens leaf extract, respectively. Group 5 received 0.05% of oxytetracycline and served as a positive control group. Fish were fed two times a day for 60 days. The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. It was found that the N. pubescens leaf extract supplementation produced a marked significant increases in weight gain and specific growth rate and caused a significant decrease in feed conversion ratio when compared to the control diet ($P<0.05$). In addition, there were no significant changes in the cumulative mortality of the fish fed with the plant extract compared to the control and positive control groups ($P>0.05$). Fish health and feeding behavior were the same as the control animals, which suggesting that N. pubescens leaf extract had no toxic effect on Nile tilapia. Thus, the results of the present study demonstrate that N. pubescens leaves are apparently safe and may be used as a natural feed additive in fish diets to enhance fish growth[52].

4.1.10. Phytochemical Investigation of *Nymphaea pubescens* and Study of its Antimicrobial Activities.

Nymphaea pubescens Wild (Nymphaeaceae) is a fascinating aquatic plant used in Bangladesh for the treatment of diabetes, liver disorders, urinary problems, menorrhagia, blenorragia; also used as tonic and aphrodisiac. The roots used by the traditional healers of the Tripura, Marma and Murong tribes to treat dysuria, urinary tract infections and leucorrhea. Also, used for indigestion, heart diseases, stomachaches, cancer, and as anti-hemorrhagic. Due to its large traditional use, N. pubescens was the plant of choice of our study. Extraction of *Nymphaea pubescens* leaves (700 g) at room temperature by maceration with methanol yielded 122 g (17.43% of dry weight) extracts respectively. The methanol extract of *Nymphaea pubescens* was subjected to Vacuum liquid chromatography and five different fractions were collected using five different solvents; Fractions were- n-hexane Fraction- Fraction -1, DCM fraction-Fraction-2, Ethyl-acetate fraction- Fraction-3, Acetone fraction- Fraction-4 and Methanol fraction-Fraction-5. Their weights were Fraction-1=7.38 gm, Fraction-2=3.28 gm, Fraction-3=34.66 gm, Fraction-4=79.3 gm and Fraction-5=14.27 gm. Among the five fractions n-hexane fraction and Ethyl-acetate fraction were subjected to column chromatography. The n-hexane fraction or Fraction-1 and ethyl acetate fraction or Fraction-3 of *Nymphaea pubescens* were separated by open column chromatography with silica gel and the fractions were collected monitoring the TLC. Fraction-1-40 yielded a colorless crystal, NPH-40. This compound was UV inactive and charring with methanol and H2SO4 (9:1) gave characteristic red color indicating the compound is a fatty acid derivative. 1HNMR report of NPH-40 informs that may be this is a mixture of four fatty acids and those are myristic acid, palmitic acid, lauric acid and 4-methyl-4-tetradecenoic acid. Further analysis such as 13CNMR, Mass spectroscopy etc is required for definite result. Fraction-3-22 yielded another colorless crystal, NPE-22. This compound was also UV inactive and charring with methanol and H2SO4 (9:1) gave characteristic brown color indicating the compound is a triterpenoid derivative. 1HNMR report of NPE-22 informs that may be this compound is oleanolic acid. Further analysis such as 13CNMR, Mass spectroscopy etc is also required here for definite result. According to the literature review done so far in this research paper, this is the first time that Oleanolic acid has been isolated from *Nymphaea pubescens*[53].

4.2 Some of the recent Literature reviews about the anti-diabetic activity experimented using various medicinal plants

4.2.1 Tadesse Bekele Tafesse et.al (2017), Antidiabetic Activity And Phytochemical Screening Of Extracts Of The Leaves Of Ajuga Remota Benth On Alloxan-Induced Diabetic Mice. The aqueous extract (500 mg/kg) showed the highest percentage reduction in blood glucose levels and the ability of A. remota extracts in reducing blood glucose levels presumably due to the presence of antioxidant constituents such as flavonoids. The effect of the extract supported the traditional claim of the plant[54].

4.2.2 Muhammad Ajmal Shaha et.al (2017), A-Glucosidase Inhibitory Effect Of Rhinacanthins-Rich Extract From Rhinacanthus Nasutus Leaf And Synergistic Effect In Combination With Acarbose. This present study provides the first evidence that RRE containing rhinacanthin-C as the major compound, could find application as an α-glucosidase inhibitor[55].

4.2.3 Martha Thomson et.al (2016), assessed the Anti-Diabetic And Anti-Oxidant Potential Of Aged Garlic Extract (AGE) In Streptozotocin-Induced Diabetic Rats. Treatment with AGE positively reversed the diabetic changes in the targeted parameters to levels significantly lower than those measured in the CD group and the degrees of attenuation were almost dose dependent especially with the two higher doses. AGE exhibits a dose-dependent ameliorative action on indicators of diabetes in STZ-induceddiabetic rats[56].

4.2.4 Wang J et.al (2016), evaluated the Antidiabetic and Antinephritic Activities of Paecilomyces hepiali Water Extract (PHC) in Diet-Streptozotocin-Induced Diabetic Sprague Dawley Rats. PHC promotes glucose metabolism by enhancing insulin, pyruvate kinase activity, and increasing the synthesis of glycogen. PHC normalized the disturbed levels of superoxide dismutase, methane dicarboxylic aldehyde, and glutathione peroxidase in kidney. The inhibitory effects on the levels of interleukin-2, interleukin-6, interleukin-10, and tumor necrosis factor-α in serum and kidney revealed the protection of PHC against diabetic nephropathy. Compared with nontreated diabetic rats, four-week PHC treatment resulted in a decrement on nuclear factor

kappa B expression in kidney. These results show that Paecilomyces hepiali possesses antidiabetic and antinephritic effects which are related to the modulation of nuclear factor kappa B activity[57].

4.2.5 Moodley K et.al (2015), Antioxidant, antidiabetic and hypolipidemic effects of Tulbaghia violacea Harv. (wild garlic) rhizome methanolic extract in a diabetic rat model. TVL treatment improved body weights, significantly reduced fasting blood glucose levels, improved glucose tolerance and significantly increased plasma insulin and liver glycogen content. TVL treatment also reduced liver thiobarbituric acid reactive substances (TBARS) levels, increased liver superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and increased plasma nitric oxide (NO) levels. Furthermore, TVL administration reduced serum triglycerides, VLDL, total-cholesterol levels and increased HDL-cholesterol levels. TVL also reduced serum levels of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [58].

4.2.6 Babin D Reejo et.al (2014), evaluated the Antidiabetic Activity Of Samanea Saman (Jacq.) Merr. Methanolic extract of Samaneasaman(Jacq.) Merr. at the doses of 250 mg/kg p.o and 500mg/kg p.o significantly reduces the increased blood glucose level as compared to the disease control group ($p<0.001$) at1 and 2, ($p<0.05$) 1/2 hours respectively inepinephrine induced diabetic rats. Also shows significant α- amylase inhibition in concentrations such as 50µ g/ml <100µ g/ml <150µ g/ml <200µg/ml<250µ g/ml. Evaluation of active compounds from the methanolic extract of Samaneasaman(Jacq.) Merr. for their antidiabetic activities may paw the way for the identification of a new class of phytochemical for the treatment of diabetes mellitus [59].

5. SCOPE OF WORK

Management of Diabetes mellitus is a global problem, successful treatment is very important for preventing or at least delaying the onset of long term diabetic complications like diabetic neuropathy, nephropathy, retinopathy, erectile dysfunction, hypertension and injury caused by ischaemic and reperfusion. Through nature in the form of herbal medicines or drugs with very minimal adverse effects are preferred when compared to the available synthetic drugs to treat such chronic disease and disorders. Herbal drugs as therapeutic agents are a nature's boon when compared to the severe adverse effects of the allopathic medical practice for diabetes, despite the fact that the search for a complete and permanent cure for the disease is being pursued uncompromisingly by eluding physicians and researchers. These herbal remedies which exemplifies the process of symbiosis still remains unfamiliar up to data technical advances, which has fashioned a marvelous scope for folk lore or traditional medicines .It is supposed that the tradition medicines used for the treatment of diabetes mellitus satisfy the sequence of complication of the disease.

Even though, the traditional medicinal plants are used to cure the disease from human origin, scientific validation of such medicinal plants are necessary and also a scientific research to prove its pharmacological and therapeutic efficacy is became vital.

Nymphaea pubescens willd is having the anti-diabetic activity. As per the literature review still no anti-diabetic activity has been reported on this whole plant. Hence, this study has been taken to explore the anti-diabetic potential of *Nymphaea pubescens* willd on streptozotocin induced diabetes in wistar albino rats.

6. PLAN OF WORK

1. Collection of *Nymphaeae pubescens.*
2. Authentication of plant.
3. Preparation of ethanolic extract of *Nymphaeae pubescens*. (EENP)
4. Experimental Animals
5. Preliminary Phytochemical screening
6. Acute toxicity studies (OECD 423guidelines)
7. Induction of diabetes mellitus in experimental animals
8. Effect of EENP on blood glucose level on STZ induced diabetic rats for 21 days
9. Effect of EENP on Serum glutamic oxaloacetic transaminase (SGOT)
10. Effect of EENP on Serum glutamic pyruvic transaminase (SGPT)
11. Effect of EENP on serum lipid profiles
    a. Total cholesterol in serum
    b. HDL-cholesterol in serum
    c. Triglycerides in serum
    d. Very low density lipoprotein (VLDL)
    e. Low density lipoprotein (LDL)
12. Effect of EENP on serum biomarker profiles
    a. Serum total protein
    b. Serum creatinine
13. Histopathology
14. Statistical analysis by one way ANOVA followed by Dunnet's test

7. Materials and Methods

7.1 Collection of Plant *Nymphaea Pubescesns*:

The whole plant of *Nymphaea Pubescesns willd* used for the present study was obtained from Nandivaram, Guduvancherry, Chennai, Tamil Nadu, India.

7.2 Authentication of plant:

The plant material was identified and authenticated by Dr.P.Jayaraman, Retd. Professor, Presidency College, Chennai–600005, Tamilnadu. [PARC/2017/3473]. A voucher specimen was submitted at C.L.Baid Metha College of Pharmacy, Chennai- 600097

7.3 Sample Extraction:

The sample was washed with distilled water to remove any adherent particles, shade dried and powdered. 25g of each sample was weighed and extracted with 300ml of ethanol by continuous hot percolation with the help of soxhlet apparatus for 10hrs of time. On completion the extract was filtered and concentrated using rotary evaporator under reduced pressure and controlled temperature of 500C – 600 C. The concentrates were stored in the refrigerator for further use.

7.3.1 Percentage yield

The percentage yield of hydro alcoholic extract was 6.84 % w/v and it was preserved in refrigeration for further use.

7.4 Chemicals

All the Chemicals used in the study were of analytical grade. The following chemicals were used for the experimental study.

Table1: Name of the chemicals and their source

S.No	Materials	Sources
1.	Absolute alcohol	S.d.fine chemicals Ltd, Mumbai
2.	Chloroform	S.d.fine chemicals Ltd, Mumbai
3.	Copper sulphate	Qualigens fine chemicals, Mumbai
4.	Creatinine kit	Span diagnosis Ltd, Bangalore
5.	DNS (3,5-dinitrosalicylic acid)	Sigma chemical Co., USA
6.	Ethanol	S.d.fine chemicals Ltd, Mumbai
7.	Glucose test strips	One touch Horizon test strips
8.	HDL kit	Span diagnosis Ltd, Bangalore
9.	Hydrochloric acid	Qualigens fine chemicals, Mumbai
10.	Hydrogen peroxide solution	Qualigens fine chemicals, Mumbai
11.	Sodium hydrogen carbonate	S.d.fine chemicals Ltd, Mumbai
12.	LDL kit	Span diagnosis Ltd, Bangalore
13.	Petroleum ether	Sigma chemical Co., USA
14.	p-nitrophenyl glucopyranoside	S.d.fine chemicals Ltd, Mumbai

15.	Pyrogallol	S.d.fine chemicals Ltd, Mumbai
16.	SGOT kit	Span diagnosis Ltd, Bangalore
17.	SGPT kit	Span diagnosis Ltd, Bangalore
18.	Sodium hydroxide	Qualigens fine chemicals, Mumbai
19.	Total protein kit	Span diagnosis Ltd, Bangalore
20.	Total cholesterol kit	Span diagnosis Ltd, Bangalore
21.	Triglycerides kit	Span diagnosis Ltd, Bangalore
22.	Urea kit	Span diagnosis Ltd, Bangalore
23.	VLDL kit	Span diagnosis Ltd, Bangalore

7.5 Experimental design

Adult Male Wistar rats of weighing 180-230 gms were used for this study. The inbred animals were procured from the animal house of C.L. Baid Metha College of Pharmacy, Thoripakkam, Chennai- 97. They were housed five per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C with 12 hr light/dark cycle. The animals were acclimatized to laboratory conditions one week and provided with standard pellet chow and water *ad libitum*. Ethical committee clearance was obtained from IAEC of CPCSEA. (IAEC/L/07/CLBMCP/2017)

7.6 Phytochemical analysis[60]

7.6.1. TEST FOR TANNINS:

1ml of sample was taken, to that few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

7.6.2. TEST FOR SAPONINS:

1 ml of sample was taken, to that 2 ml of water was added .The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins.

7.6.3. TEST FOR FLAVONOIDS:

1 ml of sample was taken, to that few drops of Sodium hydroxide solution was added. Formation of intense yellow colour, which becomes colourless on further addition of diluted hydrochloric acid, indicated the presence of flavanoid

7.6.4. TEST FOR ALKALOIDS:

1 ml of sample was taken, to that few drops of dragandoff reagent was added. Prominent yellow precipitates indicate the test as positive.

7.6.5. TEST FOR PROTEIN:

1 ml of sample was taken, to that few drops of Millon's reagent was added. A white precipitate indicates the presence of Protein.

7.6.6. TEST FOR STEROIDS:

1 ml of sample was taken, to that two drops of concentrated sulphuric acid was added and observed for brown colour.

7.6.7. TEST FOF ANTHRAQUINONES:

1 ml of sample was taken, to that aqueous ammonia was added and observed for change in colour. Pink, red, or violet colour in aqueous layer indicates the presence of anthraquinoness.

7.6.8. TEST FOR PHENOL:

1 ml of sample was taken, to that 3 ml of 10% lead acetate solution is added a bulky white precipitate indicates the presence of phenolic compounds.

7.7 Acute toxicity studies :

Acute oral toxicity study was performed as per CPCSEA guidelines (acute toxic class method).The group of six animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. In case if the mortality was observed in two out of three animals, then the dose administered would be assigned as toxic dose. If mortality was observed in one animal, then the same dose would be repeated again to confirm the toxic dose. If mortality was not observed, the procedure would be repeated for higher doses such as 250, 500, 1000 and 2000 mg/kg body weight[61].

7.7.1 Procedure:

Adult male wistar albino rats weighed 180- 230gms were used for the study. The starting dose level of EENM Was 2000mg/kg body weight p.o. Most of the crude extracts possess LD_{50} value more than 2000 mg/kg, p.o. so the starting dose which used was 2000mg/g p.o. Food was

withheld for a further 3-4 hrs after administration (p.o) of drugs and observed for the signs of toxicity.

Body weights of rats before and after administration were observed for morbidity and mortality. Any changes in skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behaviour pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted.

7.8.Induction of diabetes in experimental animals

Animals were allowed to fast for 12 h and were administered freshly prepared streptozotocin (STZ) at the concentration of 55 mg/kg bodyweight, i.p. in 0.1 mol/L cold citrate buffer, pH 4.5. The STZ-treated animals were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. Rats which were having persistent glycosuria and hyperglycaemia with a fasting blood glucose >250 mg/dL on the third day after the STZ injection were considered diabetic and which were used for further experimentation[62].

7.9.Experimental Design

The animals were divided into 5 groups each constituting 6 rats. Group I were normal rats received water, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic rats which acts as diabetic control group. Group III STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with Glibenclamide 5mg/kg b.w/p.o Group IV STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with EENP 200mg/kg b.w/ p.o Group V STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with EENP 400mg/kg b w/p.o for 21 days[63].

Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured by using the glucose oxidase peroxidase reactive strips and a glucometer (One touch glucometer).

7.10 Biochemical parameter studies:

At the last day animal was sacrificed by decapitation, blood samples were collected and serum was separated using centrifuge to study the biochemical parameters. The estimation of protein was carried out using the method of Lowry[64]. The extraction of serum lipids were carried out by the method of Folch [65] and the serum cholesterol estimation was carried out by the method of Zlatkis[66] Serum triglycerides were estimated by the method of Foster and Dunn and HDL cholesterol was estimated by the method of Burstein[67]. The VLDL cholesterol was evaluated using the formula, TG/5 mg/dl. The serum LDL cholesterol was estimated by the method of Friedwald[68] SGOT and SGPT were measured by the method of Reitman and Frankel (Colorimetric method) [69] the plasma creatinine was measured by Jaffe's method[70] Serum urea was measured by the diacetyl monoxime method[71] and Histopathology studies of liver and pancreas were carried out by using standard procedure.

7.11 Histopathology

At the end of the study, all the animals were sacrifices under light ether anesthesia. The rats were sacrificed by decapitation and blood samples were collected by bleeding of retro-orbitol plexus using micro capillary technique from all the groups of overnight fasted rats and serum was separated to study the biochemical parameters. The relevant organs like pancreas, liver and kidney were removed and dissected out and washed with ice-cold saline. The organs were preserved in 10% formalin solution for histopathological studies.

7.12 Statistical analysis

The data were expressed as mean ± standard error (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's test p values lees than 0.05 were considered as significance.

8.1 Preliminary Phytochemical analysis of Ethanolic extract of *Nymphaea pubescens* willd (EENP)

The result of preliminary phytochemical analysis of Ethanolic extract of *Nymphaea pubescens* willd (EENP) showed presence of various phytochemical constituents such as, flavonoids, alkaloids, tannins, proteins, steroids and phenol with absence of saponins and anthroquinones. The results were shown in **Table-1**

8.2 Acute Oral Toxicity Study:

Acute oral toxicity study was performed as per CPCSEA guidelines (acute toxic class method).The group of six animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 2mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 250, 500, 1000 and 2000 mg/kg body weight. . The results were shown in **Table-2**

8.3 Effect of EENP on body weight of STZ induced diabetic rats

-It was found that the body weight was decreased significantly ($p<0.001$) when the comparison was made between group I with group II, group III, group IV and group V.

-The bodyweight in group II was compared with group III, group IV and group V were increased significantly ($p<0.001$). The results were shown in **(Table-3) (Histogram-1)**

8.4 Effect of EENP on blood glucose level of STZ induced diabetic rats

- The blood glucose levels were compared between group I with group II, group III, group IV and group V and it was found that blood glucose levels were significantly increased ($p<0.001$).

-The blood glucose levels in group II was compared with group III, group IV and group V were significantly ($p<0.001$) decreased. The results were shown in **(Table-4) (Histogram-2)**

8.5 Effect of EENP on serum cholesterol level of STZ induced diabetic rats

- The serum cholestrol levels were compared between group I with group II, group III, group IV and group V and it was found that serum cholesterol levels were significantly increased ($p<0.001$).

-The serum creatinine level in group II was compared with group III, group IV and group V were decreased significantly ($p<0.001$). The results were shown in **(Table-5) (Histogram-3)**

8.6 Effect of EENP on serum triglyceride level of STZ induced diabetic rats

- The serum triglycerides levels were compared between group I with group II, group III, group IV and group V and it was found that serum triglycerides levels were significantly increased ($p<0.001$).

-The serum triglyceride level in group II was compared with group III, group IV and group V ($p<0.001$) were decreased significantly. The results were shown in **(Table-5) (Histogram-4).**

8.7 Effect of EENP on serum HDL level of STZ induced diabetic rats

- The serum HDL levels were compared between group I with group II, group III, group IV and group V and it was found that serum HDL levels were significantly decreased ($p<0.001$).

- The HDL level in group II was compared with group III group IV and group V ($p<0.001$) increased significantly. The results were shown in **(Table-5) (Histogram-5).**

8.8 Effect of EENP on serum LDL level of STZ induced diabetic rats

- The serum LDL levels were compared between group I with group II, group III, group IV and group V and it was found that serum LDL levels were significantly increased ($p<0.001$).

-The serum LDL level in group II was compared with group III group IV and group V ($p<0.001$) were significantly decreased. The results were shown in **(Table-5) (Histogram-6).**

8.9 Effect of EENP on serum VLDL level of STZ induced diabetic rats

- The serum VLDL levels were compared between group I with group II, group III, group IV and group V and it was found that serum VLDL levels were significantly increased ($p<0.001$).

-The serum VLDL level in group II was compared with group III, group IV and group V were significantly decreased ($p<0.001$). The results were shown in **(Table-5) (Histogram-7).**

8.10 Effect of EENP on serum total protein level of STZ induced diabetic rats

-The serum total protein level in group I was compared with group II ($p<0.01$), group III (ns), group IV (ns) and group V (ns) were increased significantly.

-The serum total protein level in group II was compared with group III, (0.001) group IV and group V ($p<0.01$) were decreased significantly. The results were shown in **(Table-6) (Histogram-8).**

8.11 Effect of EENP on serum creatinine level of STZ induced diabetic rats

-The serum creatinine level in group I was compared with group II group III group IV and group V ($p<0.001$) and it was found that the levels were increased significantly.

-The serum creatinine level in group II was compared with group III, group IV and group V ($p<0.001$) were decreased significantly. The results were shown in **(Table-7) (Histogram-9).**

8.13 Effect of EENP on SGOT level of STZ induced diabetic rats

-The SGOT level in group I was compared with group II group III group IV and group V ($p<0.001$) and it was found that the levels were increased significantly.

-The SGOT level in group II was compared with group III, group IV and group V were decreased significantly ($p<0.001$). The results were shown in **(Table-8) (Histogram-10).**

8.14 Effect of EENP on SGPT level of STZ induced diabetic rats

-The SGPT level in group I was compared with group II group III group IV and group V ($p<0.001$) and it was found that the levels were increased significantly.

-The SGPT level in group II was compared with group III, group IV and group V were decreased significantly ($p<0.001$). The results were shown in **(Table-9) (Histogram-11).**

8.15 Histopathology

8.15.1 Histopathology of pancreas

The histopathology of rat pancreas of control groups showed normal acini and islets whereas diabetic control groups showed damaged and atrophy islets with acini. Diabetic animal treated with glibenclamide (5mg/kg b.w/p.o) has showed preserved normal islets in pancreas, whereas EENP 200mg/kg/p.o treated animals has showed small pancreatic islet and EENP 400mg/kg/p.o treated animals showed hyperplastic.

8.15.2 Histopathology of Liver

Histopathological examinations of diabetic animals showed centrilobular necrosis accompanied by fatty changes and ballooning degeneration which were observed in the remaining hepatocytes in the liver of rats treated with STZ (55mg/kg b.w/p.o) were much of intensity and which were recovered with the treatments using EENP.

9. Tables and figures

Table- 1. Phytochemical screening of EENM

Table: The Phytochemical studies of the sample TEST	Sample
TANNINS	+
SAPONINS	-
FLAVONOIDS	+
ALKALOIDS	+
PROTEINS	+
STEROIDS	+
ANTHROQUINONES	-
PHENOL	+

Table-2. Acute oral toxicity studies of EENM (OECD 423 guideline)

Si. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	EENM	2g/kg	200	210	No signs of toxicity	Nil	Nil	14 days
2.	EENM	2g/kg	180	195	No signs of toxicity	Nil	Nil	14 days
3.	EENM	24g/kg	190	200	No signs of toxicity	Nil	Nil	14 days

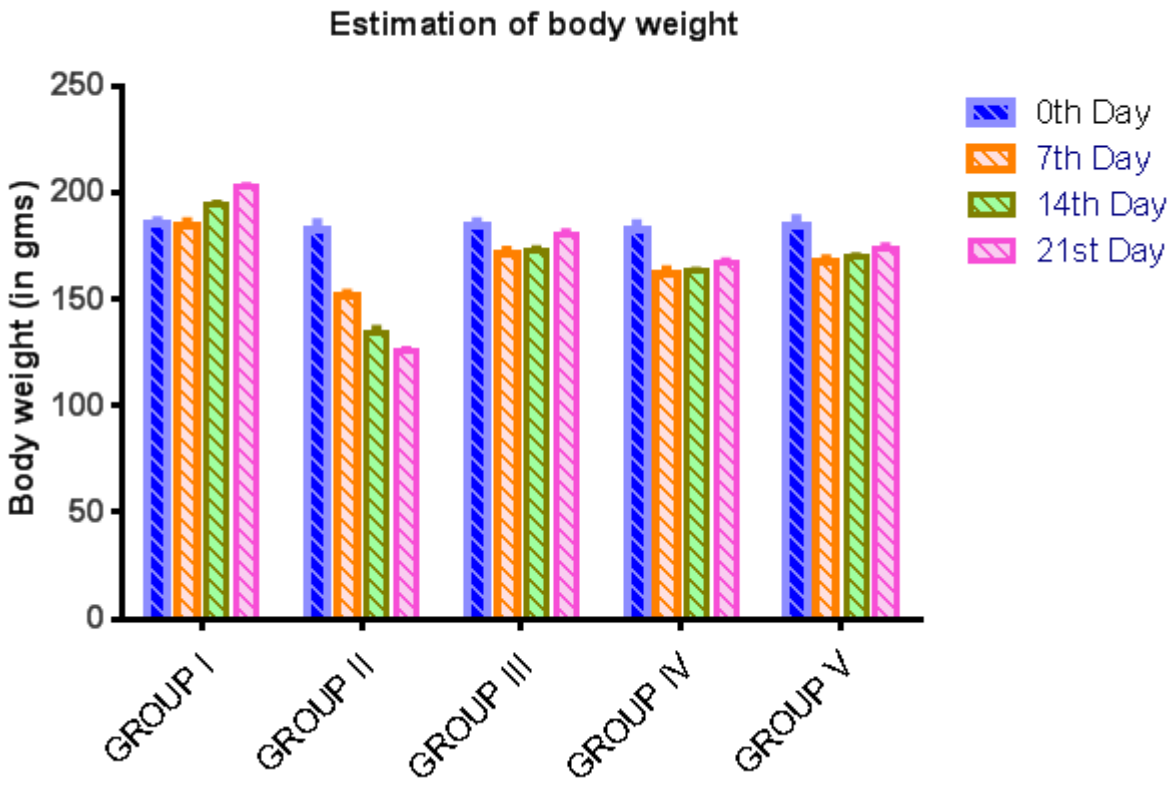
Table-3. Effect of EENM on body weight of STZ induced diabetic rats

Group	Body weight (gm)			
	Day – 0	Day – 7	Day – 14	Day – 21
I	185.2±1.930	184.2±2.271	194.5±0.7548	202.3±1.173
II	182.2±4.010	151.5±1.482 a***	134.5±1.988 a***	125.7±0.892 a***
III	182.34±2.186	171.2±2.012 a***b***	172.5±1.222 a***b***	180.0±1.780 a***b***
IV	182.12±3.615	161.7±2.236 a***b^{ns}	162.8±0.9458 a***b***	167.2±0.938 a***b***
V	184.8±3.137	167.3±1.914 a***b***	169.8±0.9098 a***b***	173.8±1.337 a***b***

- Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets 't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.



Histogram-1. Effect of EENM on body weight

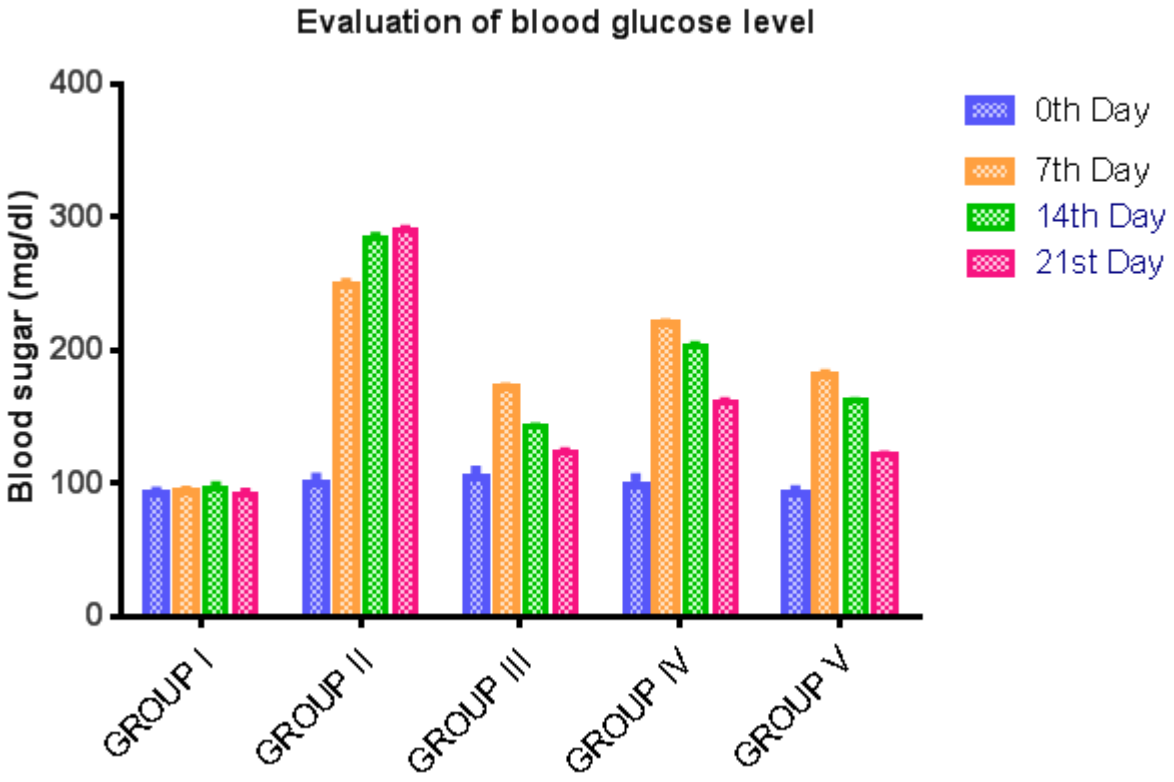
Table-4. Effect of EENM on blood glucose level in STZ induced diabetic rats

Group	Blood glucose (mg/dl)			
	Day – 0	Day – 7	Day – 14	Day – 21
I	91.35±3.130	93.50±1.660	95.83±3.646	91.33±2.996
II	100.178±4.649	249.07±1.922a a***	284.2±2.110 a***	290.7±1.510 a***
III	104.216±5.890	171.01±1.549 a***b***	141.89±1.461 a*** b***	122.795±1.783 a*** b***
IV	98.03±6.260	219.159±1.497 a***b***	202.53±2.057 a***b***	160.7±1.515 a***b***
V	92.131±3.170	181.371±1.438 a***b***	161.26±1.316 a***b***	121.049±1.150 a**b***

- Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets ‘t’ test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.



Histogram-2. Effect of EENM on blood glucose

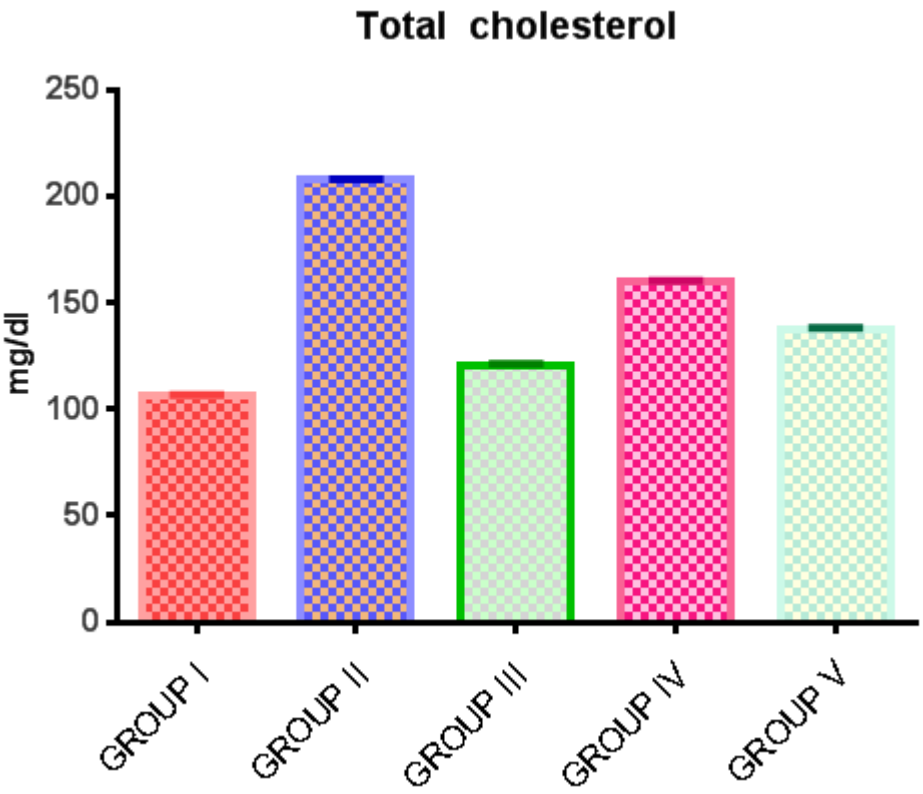
Table-5.Effect of EENM on total cholesterol, Triglycerides, HDL, LDL, VLDL

Groups	Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Control	106.3±0.352	86.4±0.38	61.03±0.37	41.4±0.48	18.69±0.49
II	Diabetic control 55mg stz	207.6±0.319 a***	170.2±0.44 a***	34.56±0.46 a***	139.43±0.74 a***	40.17±0.61 a***
III	STZ+ Glibenclamide	120.7±0.576 a***b***	92.1±0.49 a***b***	56.71±0.69 a**b***	86.59±0.42 a**b***	26.69±0.37 a***b***
IV	STZ+EENM 200 mg	159.83±0.29 a***b***	129.4±0.29 a***b***	42.03±0.37 a***b***	113.21±0.49 a***b***	30.46±0.29 a***b***
V	STZ+EENM 400 mg	137.783±0.390 a***b***	100.1±0.18 a***b***	53.21±0.53 a***b***	92.84±0.51 a***b***	24.84±0.24 a***b***

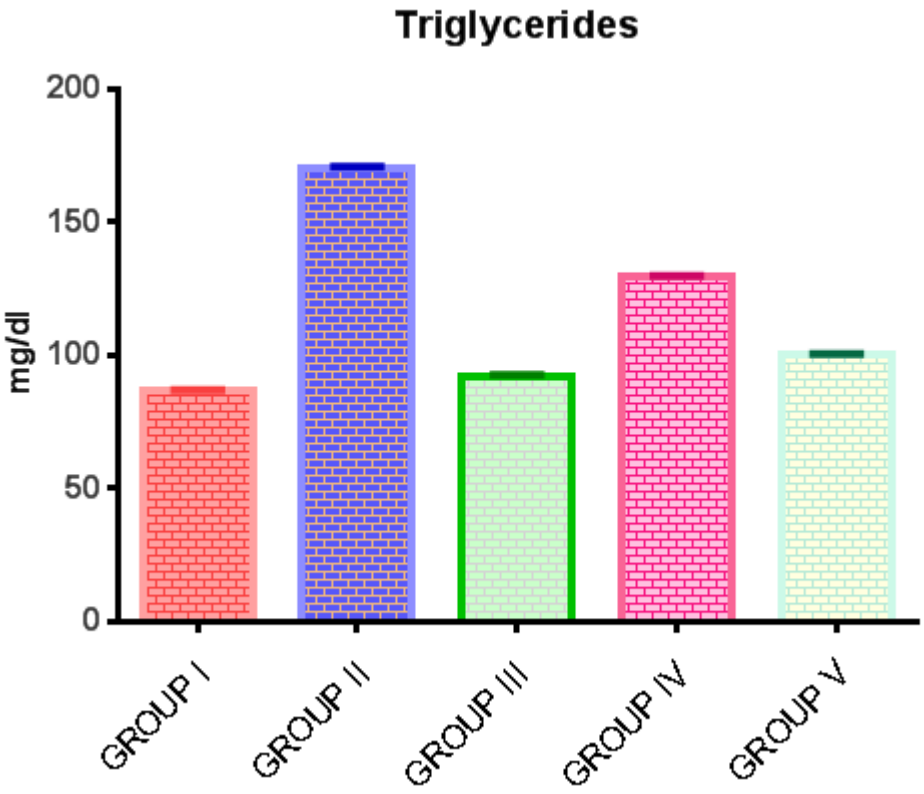
- Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

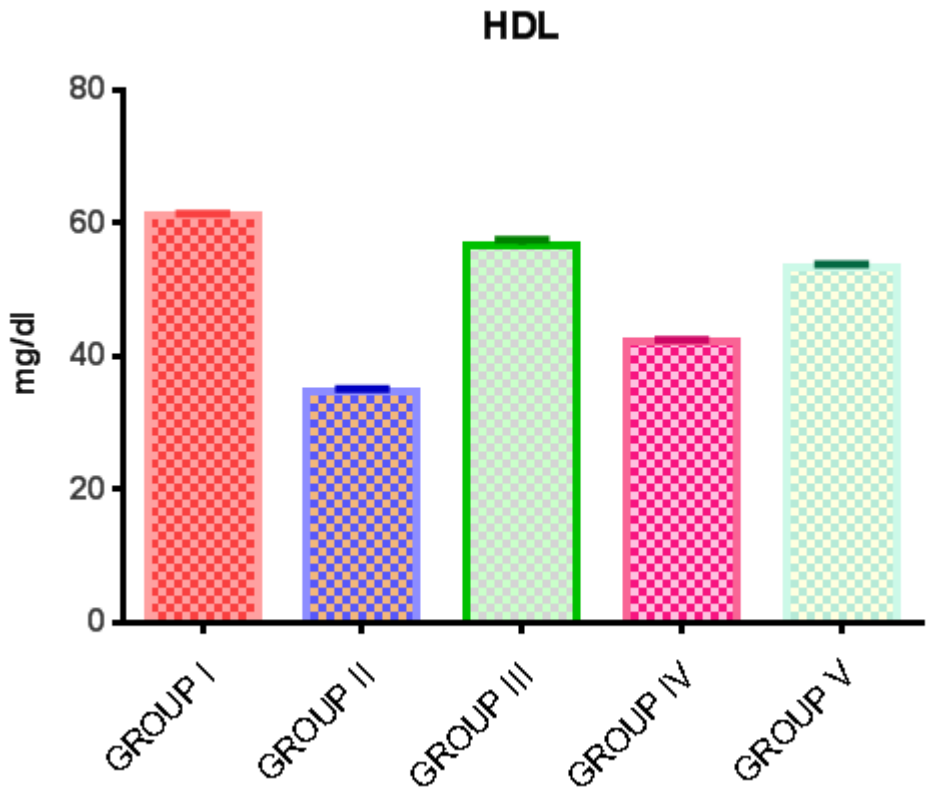
- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets 't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.



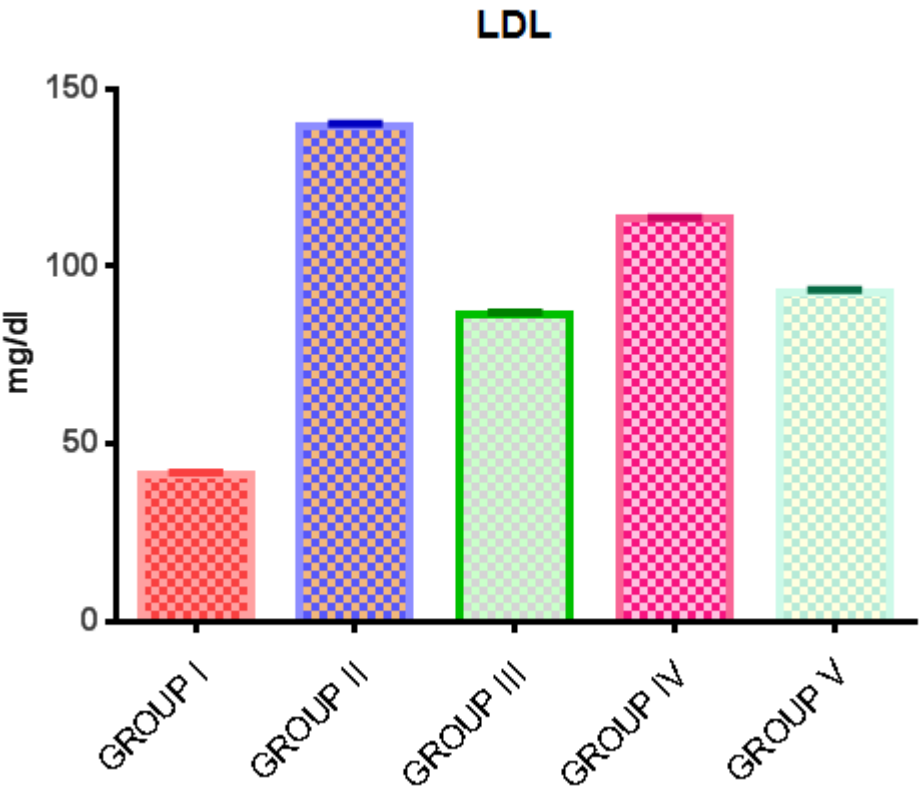
Histogram-3. Effect of EENM on Total cholestrol



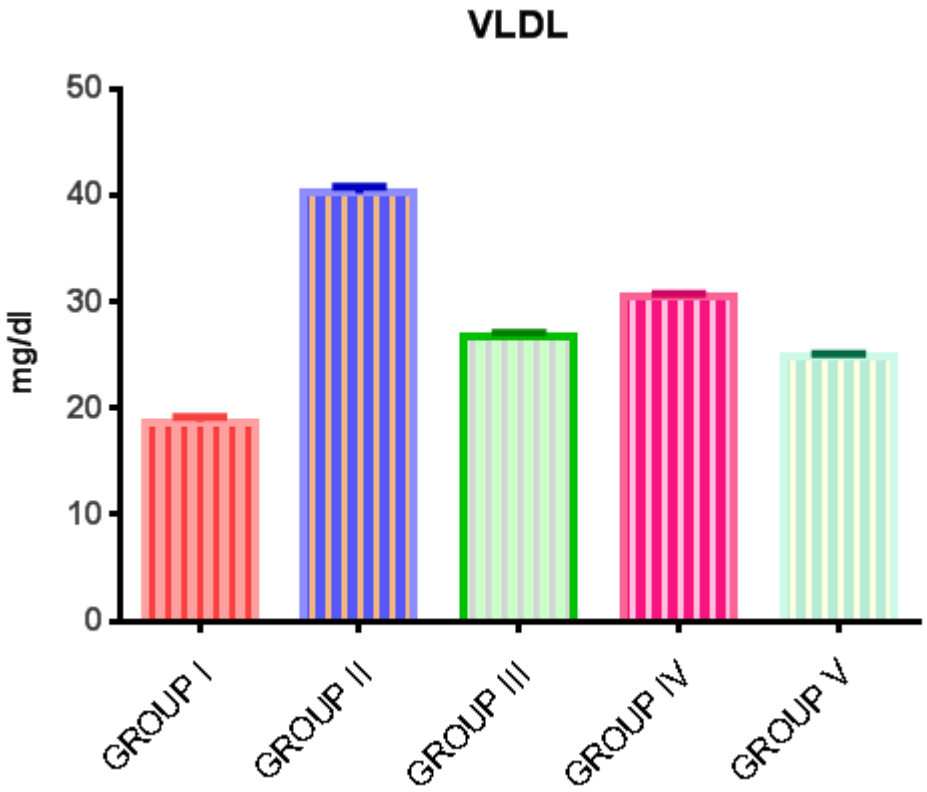
Histogram-4. Effect of EENM on Triglycerides



Histogram-5. Effect of EENM on HDL



Histogram-6. Effect of EENM on LDL



Histogram-7. Effect of EENM on VLDL

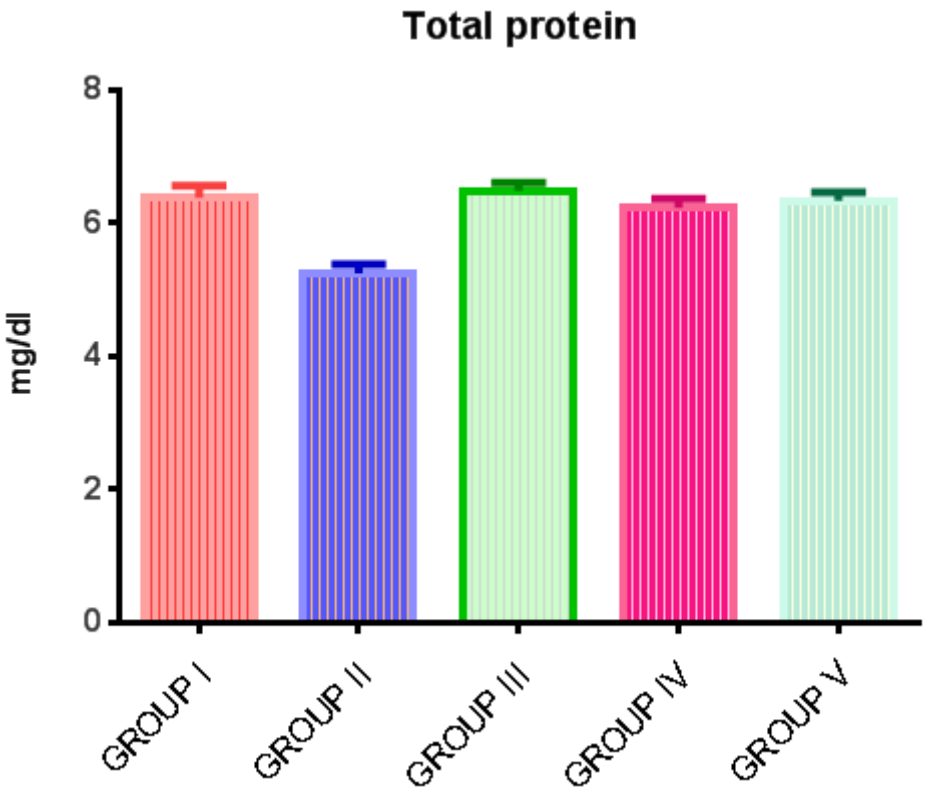
Table-6. Effect of EENM on Total protein in STZ induced diabetic rats

Groups	Treatment	Total protein (mg/dl)
I	Control	6.38±0.1767
II	STZ (55mg/kg b.w., i.p) induced diabetic rats	5.24±0.1344 a**
III	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with glibenclamide (5mg/kg b.w., p.o)	6.48±0.1186 a^{ns} b***
IV	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (200mg/kg b.w., p.o)	6.23±0.1271 a^{ns} b**
V	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (400mg/kg b.w., p.o)	6.33±0.1308 a^{ns} b**

- Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets 't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.



Histogram-8. Effect of EENM on Total Protein

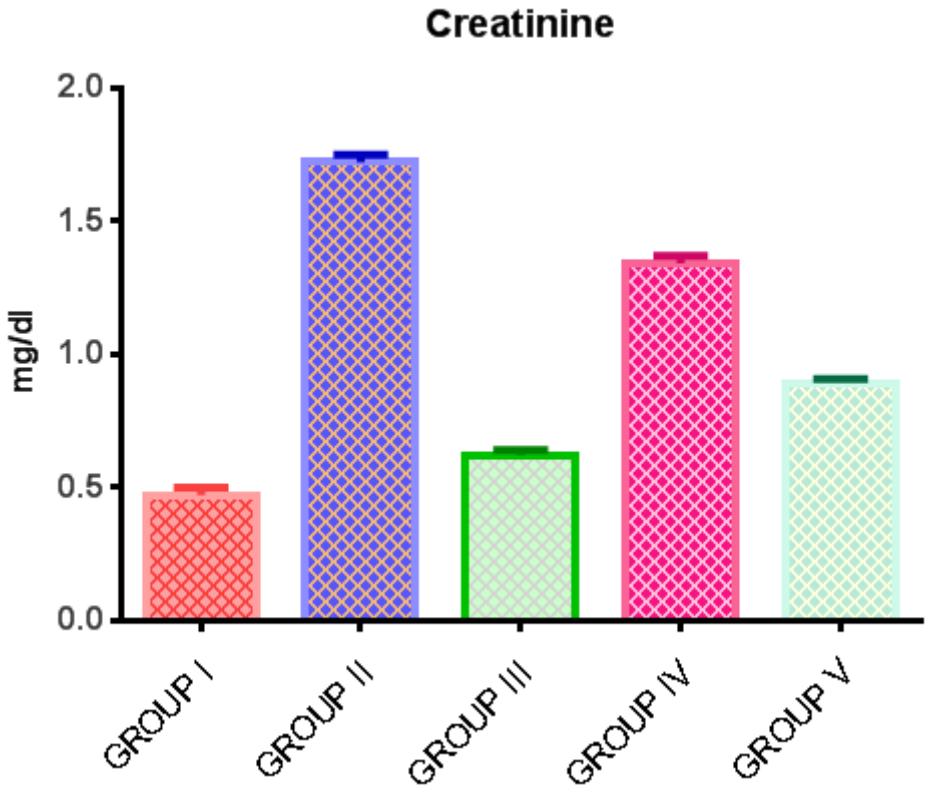
Table-7. Effect of EENM on Creatinine in STZ induced diabetic rats

Groups	Treatment	Creatinine (mg/dl)
I	Control	0.47±0.0281
II	STZ (55mg/kg b.w., i.p) induced diabetic rats	1.72±0.0277 a***
III	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with glibenclamide (5mg/kg b.w., p.o)	0.62±0.0185 a***b***
IV	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (200mg/kg b.w., p.o)	1.34±0.0296 a***b***
V	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (400mg/kg b.w., p.o)	0.89±0.0170 a***b***

- Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets 't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.



Histogram-9. Effect of EENM on Creatinine

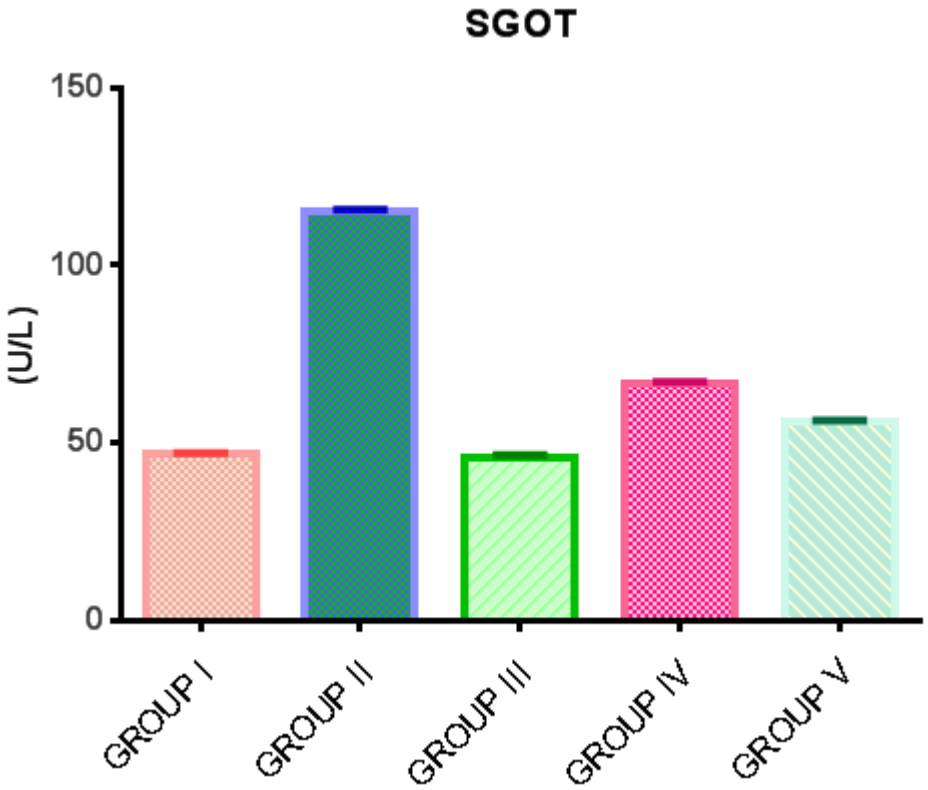
Table-8. Effect of EENM on SGOT in liver tissue of STZ induced diabetic rats

Groups	Treatment	SGOT (U/L)
I	Control	47.01±0.12
II	STZ (55mg/kg b.w., i.p) induced diabetic rats	115.03±0.64 a***
III	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with glibenclamide (5mg/kg b.w., p.o)	45.86±0.58 a***b***
IV	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (200mg/kg b.w., p.o)	66.83±0.29 a***b***
V	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (400mg/kg b.w., p.o)	55.86±0.36 a***b***

- Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets 't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.



Histogram-10. Effect of EENM on SGOT

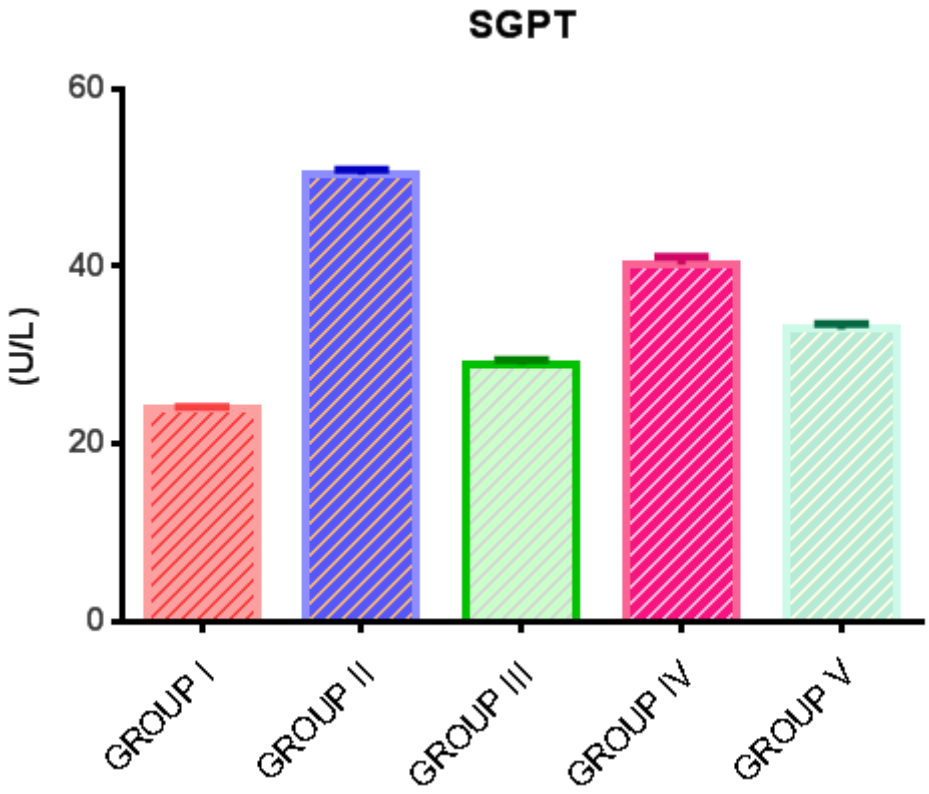
Table-9. Effect of EENM on SGPT in liver tissue of STZ induced diabetic rats

Groups	Treatment	SGPT (U/L)
I	Control	24.01±0.12
II	STZ (55mg/kg b.w., i.p) induced diabetic rats	50.29±0.54 a***
III	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with glibenclamide (5mg/kg b.w., p.o)	28.86±0.58 a***b***
IV	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (200mg/kg b.w., p.o)	40.13±0.89 a***b***
V	STZ (55mg/kg b.w., i.p) induced diabetic rats treated with EENM (400mg/kg b.w., p.o)	32.86±0.66 a***b***

- Values are expressed as mean ± SEM of 6 animals.

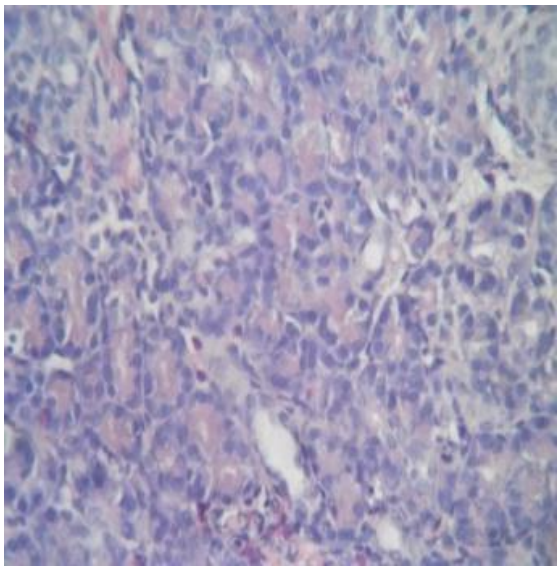
Comparisons were made between the following:

- a - Group I vs. II,III,IV and V, b - Group II vs.III, IV, and V.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets ‘t’ test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.

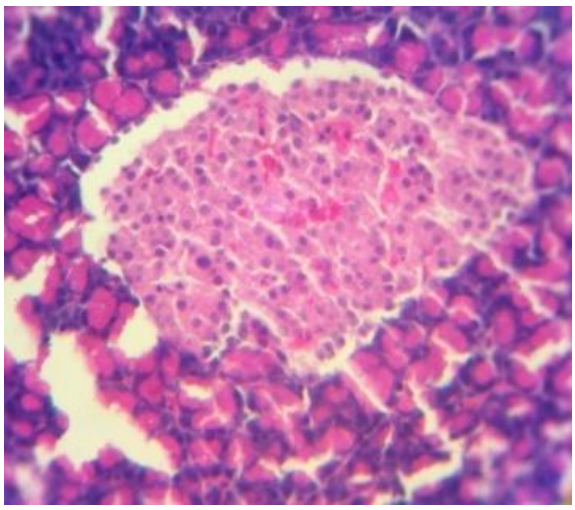


Histogram-11. Effect of EENM on SGPT

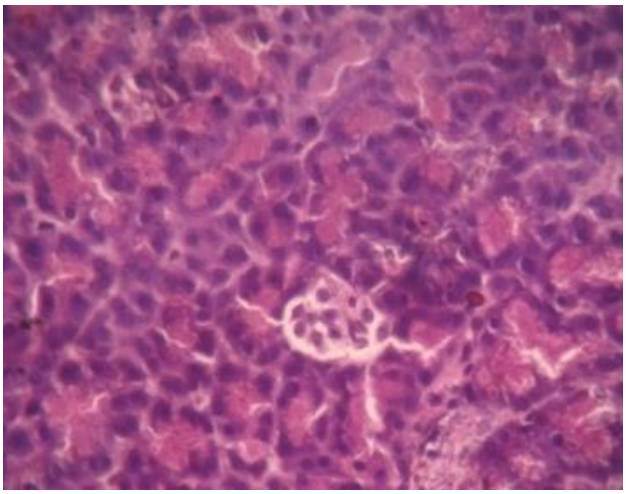
HISTOPATHOLOGICAL ANALYSIS OF PANCREAS



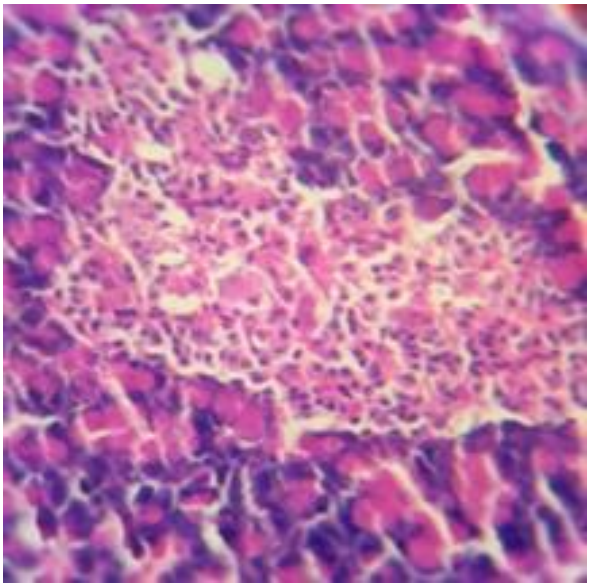
Histopathology of Pancreas- Group-I



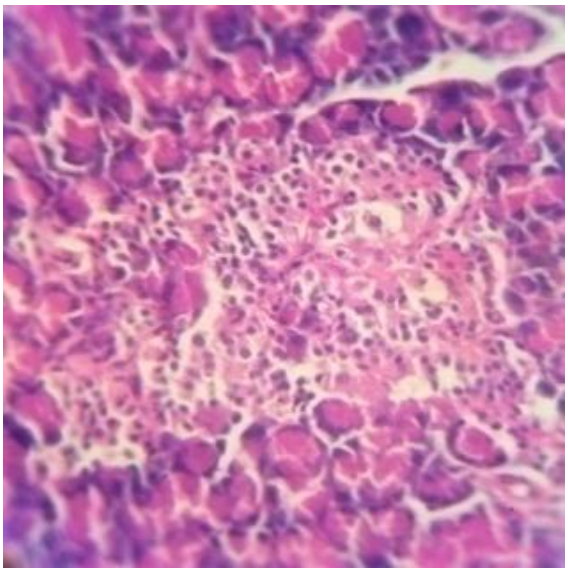
Histopathology of Pancreas- Group-II



Histopathology of Pancreas- Group-III

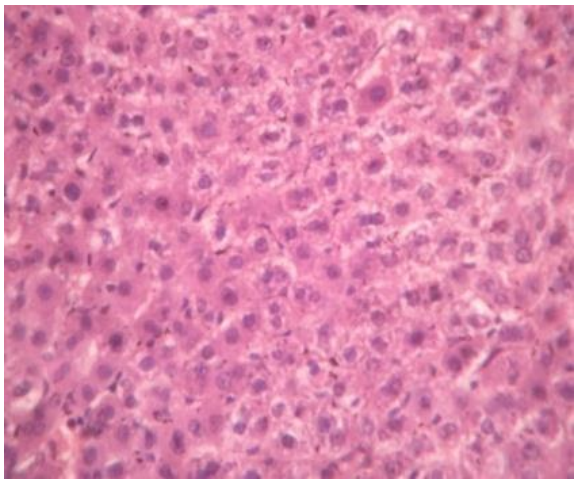


Histopathology of Pancreas- Group-IV

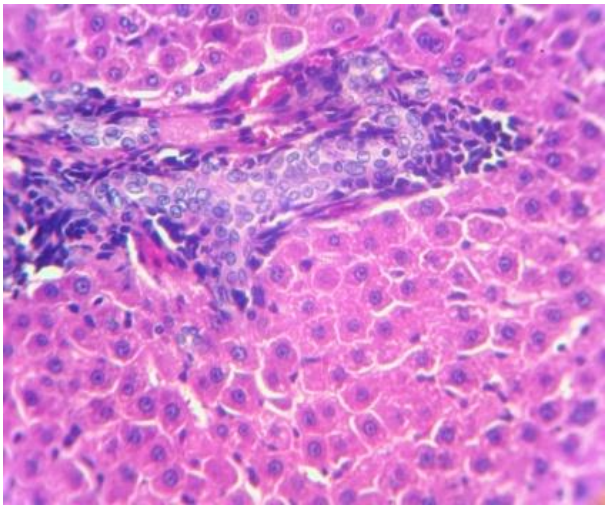


Histopathology of Pancreas- Group-V

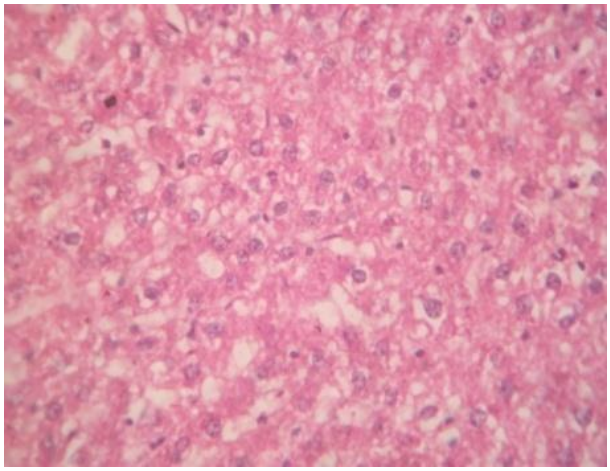
HISTOPATHOLOGICAL ANALYSIS OF LIVER



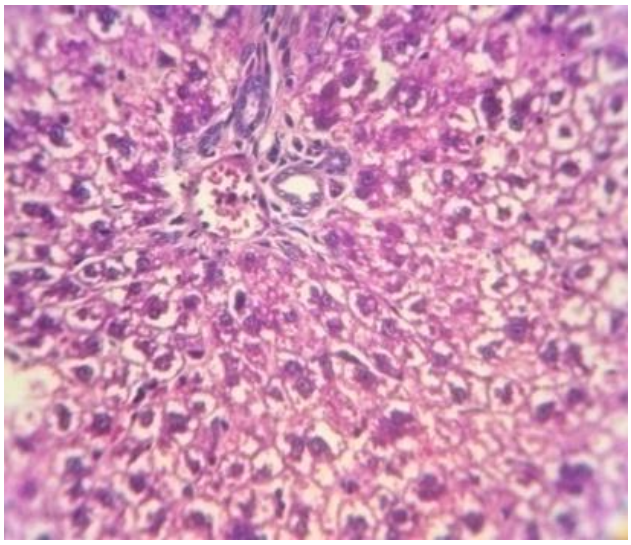
Histopathology of Liver- Group-I



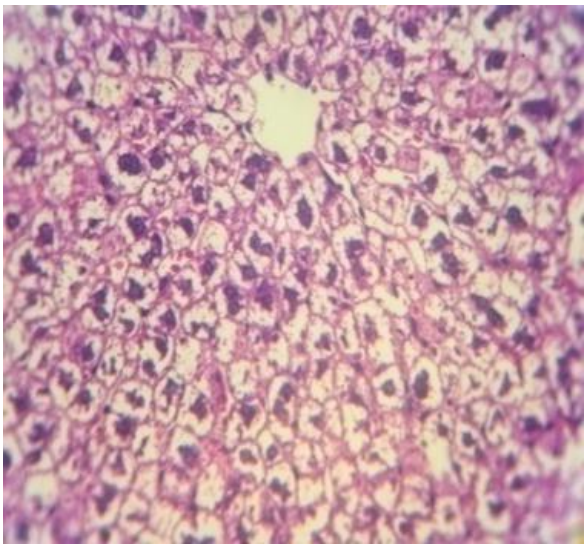
Histopathology of Liver- Group-II



Histopathology of Liver- Group-III



Histopathology of Liver- Group-IV



Histopathology of Liver- Group-V

10. DISCUSSION & CONCLUSION

10.1 Discussion

The ethanolic extract from whole plant of Nymphaea Pubescens were subjected to preliminary phytochemical analysis which shown presence of flavonoids, alkaloids, tannins, proteins, steroids and phenol with absence of saponins and anthroquinones.

Acute oral toxicity studies of EENP did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w/p.o, in experimental rats.

The anti-hyperglycemic effects of the plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence, treatment with herbal drugs has an effect on protecting b cells and smoothing out fluctuation in glucose levels[72] The present study evalulation of anti-diabetic activity of whole plant of *Nymphaea pubescens* STZ induced diabetic rats.

Experimental induction of hyperglycemia with STZ is associated with the characteristic loss of body weight which is due to loss or degradation of structural proteins it leads to increased muscle wasting and due to loss of tissue protein, as the structural proteins are known to contribute to body weight. Diabetic rats treated with glibenclamide and EENP showed increased body weight when compared to untreated diabetic animals. It may be due to increased insulin secretion and glycemic control of EENP.

Reduced glucose transport or absorption from the gut, extra pancreatic action probably by stimulation of glucose utilization in peripheral tissues, increase in glycogenic or glycolytic enzyme activities in peripheral tissues, decrease in the secretion of counter-regulatory hormones like glucagon, growth hormones are the possible mechanisms involved with suppressing blood glucose levels. The glibenclamide, stimulating insulin secretion from pancreatic β cells principally by inhibiting ATP sensitive K_{ATP} channels in the plasma membrane and decreases the blood glucose level[73]. Blood glucose level decreased significantly in glibenclamide and

EENP treated diabetic rats and the histopathology of pancreas showed normal islets in pancreas with normal anatomy compared with normal rats which may be due to the anti-diabetic activity.

Hyperglycaemia is accompanied with dyslipidemia under normal circumstances; insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hyper triglyceridemia, and insulin deficiency is also associated with hyper cholesterolemia due to metabolic abnormalities. The dyslipidemia is characterized by increase in TC, LDL, VLDL, TG and fall in HDL which is observed in STZ induced diabetic rats[74]. The diabetic rats treated with glibenclamide and EENP showed reduced severity of dyslipidemia with decrease in TC, LDL, VLDL, TG and increase in HDL.

Both SGOT and SGPT enzyme levels get elevated during liver damage which is more in diabetic rats[75]. The diabetic rats treated with glibenclamide and EENP reduced the SGOT and SGPT level. The liver histopathology of STZ induced diabetic rats showed centrilobular necrosis accompanied by fatty changes and ballooning degeneration in the hepatocytes treatment with EENP reversed in diabetic rats treated with glibenclamide and EENP which indicates that the liver damage is reduced in EENP treated group.

The diabetic hyperglycaemia induces elevation of the serum levels of creatinine which are significant markers of renal dysfunction, The treatment of EENP in rats showed marked decrease in serum creatinine levels in diabetic animals.

10.2 Conclusion

The anti-diabetic activity of Whole pant of *Nymphaea pubescens* Willd is evidenced by blood glucose level, estimation of lipid profile activity of *Nymphaea pubescens* and the evident reduction in SGOT, SGPT in liver and creatinine in serum also proves that the *Nymphaea pubescens* reduced the Pancreas, liver damage which is common in diabetes.

Thus, it may be concluded that *Nymphaea pubescens* produced significant antidiabetic activity in streptozotocin induced diabetic rats. The efficacy of the *Nymphaea pubescens* was comparable to that of Glibenclamide. Further work was necessary to elucidate the mechanism of action involved in the anti-diabetic activity of *Nymphaea pubescens* with special references to phytochemicals.

1.Ivorra MD, Paya M, Villar A. A review of natural products and plants as potential anti diabetic drugs. J Ethnopharmacol 1989;27: 243.

2.Shukia R, Sharma SB, Puri D. Medicinal plants for treatment of diabetes. Indian J Clin Biochem 2000; 15:169.

3.Sowmya PR, Rout KA, Chowdary DM. Plants as source of Novel Anti- Diabetic Drug: Present Scenario & Future Perspectives. Current Trends in Biotech & Pharmacy 2009;3: 37-55.

4.Swarnalatha Saraf, Mahendra Singh Ashawat. Flavonoids: A Nutrional Protective against oxidative and UV induced cellular damages. Pharmacognosy Reviews 2007;1:30-38.

5.Fabricant DS, Farn Swarth NR. The value of plants used in traditional medicine for Drug Discovery. Environ Health Perspectives 2007;109:69-75..

6.Newmann DJ, Gragg GM. Natural Products as sources of New drugs over the last 25 years. J Nat Products 2007;70:461-77.

7.King H, Aubert RE, Herman WH. Global burden of diabetes,1995 -2025 -Prevalence, numerical estimates and projections. Diabetes Care 1998;21:1414-1431.

8.Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.

9.Viswanathan M, McCarthy MI, Snehalatha C, Hitman GA, Ramachandran A. Familial aggregation of type 2 diabetes mellitus in South India. Diab Med 1996; 31: 232-237.

10.Lt Gen SR Mehta, Col AS Kashyap, Lt Col S Das et al.Diabetes Mellitus in India: The Modern Scourge 2009;65:50-54.

11. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 1993;329:977–986.

12. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837–853.

13. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan DM, Peterson CM. Tests of glycemia in diabetes. Diabetes Care 2004;27(Suppl 1):S91–S93.

14. A Ramachandran, A K Das, SR Joshi et al.Current Status of Diabetes in India and Need for Novel Therapeutic Agents june 2010;58:7-9.

15. Definition, Diagnosis and classification of diabetes mellitus and its complications, Report of a WHO consultation 10. IDF Diabetes Atlas, 4th edition. International Diabetes Federation, 2009.

16. Siddharth N.S.Containing the global epidemic of diabetes. Journal of Diabetology. 2001 3:11.

17.Davis S.N. and Granner D.K. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas. In: Goodman I S; Gilman AG, eds, The Pharmacological Basis of Therapeutics, 9th Ed. New York: Mc-Graw Hill; pp. 1996 1487-1517.

18. Sharma A.K. Diabetes mellitus and its complications: An update, 1st Ed. New Delhi: mhbMacmillan India Ltd. pp.1993 92-205.

19. IDF Diabetes Atlas, 4th edition. International Diabetes Federation, 2009.

20. IDF Diabetes Atlas, 6th edition. International Diabetes Federation, 20014.Available from http://www.idf.org/diabetesatlas (Acessed january 31,2014).

21. HL. Sharma, KK Sharma, insulin, Oral hypoglycaemics and other related agents, chapter 49.principles of pharmacology 2007:edition 1;646-647 .

22. T.R Harisons, W.R Resnick, M.M Wintrobe et al., diabetes mellitus, chapter 344. Principles of internal medicine 2012:edition 18;2968-3002.Schectman JM, Nadkarni MM, Voss JD. The association between diabetes metabolic control and drug adherence in an indigent population. Diabetes Care. 2002;25(6):1015-1021.

23. Dimri S, Tiwari P, Basu S, Parmar VR. Drug use pattern in children at a teaching hospital. Indian Pediatrics. 2009;46(2):165-167.

24. Mogensen C, Christensen C. Predicting diabetic nephropathy in insulin dependent diabetics. New England Journal of Medicine 1984;311:89-93.

25. DeFronzo RA. Lilly Lecture: The triumvirate: β-cell, muscle, liver: A collusion responsible for NIDDM. Diabetes 1988;37:667-687.

26. DCCT (Diabetes Control and Complications Trial) Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England Journal of Medicine 1993;329:977-989.

27. Dowse GK. Incidence of NIDDM and the natural history of IGT in Pacific and Indian Ocean populations. Diabetes Research in Clinical Practice 1996;34 Suppl:S45-50.

28. DeFronzo RA. From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 2009;58:773-95.

29. Committee WHO. Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: Report of a WHO consultation; 1999.

30. http://www.nytimes.com /health/guides/disease/type-1-diabetes/overview.html

31. Pratley RE, Weyer C, Bogardus C, Metabolic abnormalities in the development of noninsulin-dependent diabetes mellitus. Diabetes mellitus, ed 2. Philadelphia: Lippincott-Raven Publishes, 2000;548-557

32.Medical pubs, Disease management http://www.clevelandclinicmeded.com/ medicalpubs/diseasemanagement/endocrinology/diabetes-mellitus/#(Assessedon 08/05/2014).

33.Pandey A., Tripathi P., Pandey R., Srivatava R. and Goswami S. Alternative therapies useful in the management of diabetes: A systematic review. Journal of Pharmacy and Bioallied Sciences.2011, 3(4): 504-12.

34.Ribes G., Sauvaire Y., Da Costa C., Baccou J.C. and Loubatieres-Mariam M.M. Antidiabetic effects of subfractions from fenugreek seeds in diabetic dogs. Proceedings of Society of Experimental Biology and Medicine.1986, 182: 159-66.

35.Kelly G. Squalene and its potential clinical uses. Alternative Medicine Review: A Journal of Clinical Therapeutic.1999, 4: 29-36.

36.Foye W.O., Lemke T.L. and Williams D.A. Principles of Medicinal Chemistry, 4th Edition. New York: Wilkins and Wilkins.1995.

37.Malender A.Kinetics-effect relations of insulin releasing drugs in patients with type II diabetes. Diabetes.2004, 53: 151-55.

38.Zhou G., Myers R., Li Y., Chen Y., Shen X., Fenyk-Melody J., Wu M., Ventre J., Doebber T., Fujii N., Musi N., Hirshman M.F., Goodyear L.J. and Moller D.E. Role of AMP-activated

protein kinase in mechanism of metformin action. Journal of Clinical Investigation.2001, 108: 1167-1174.

39.Rahimi R., Nikfan S., Larijani B. and Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomedicine and Pharmacotherapy.2005, 59: 365-373.

40.Sharma A.M. and Staels B. Review: Peroxisome proliferator activated receptor gamma and adipose tissue- understanding obesity related changes in regulation of lipid and glucose metabolism. Journal of Clinical Endocrinology and Metabolism.2007 92: 386-395.

41. Busineni Jayasimha Goud et al, Streptozotocin -A Diabetogenic Agent in Animal Models, . Ijppr.Human, 2015; Vol. 3(1): 253-269.

42. T. SZKUDELSKI. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas, Physiol. Res. 50: 536-546, 2001

43.Muthulingam M. Antihepatotoxic Efficacy Of Nymphaea Pubescens(Willd.) On Acetaminophen Induced Liver Damage In Male Wistar Rats. International Journal Of Current Research. 2010, 3: 012 -016.

44. Debnath S, Ghosh S, Hazra B. Inhibitory Effect Of Nymphaea pubescens Willd. Flower Extract On Carrageenan-Induced Inflammation And Ccl_4-Induced Hepatotoxicity In Rats, Food Chem Toxicol. 2013,59:485-91

45. Selvakumari E, Shantha S, Purushoth Prabhu T, Sreenathkumar C, Antiproliferative Activity Of Ethanolic Flower Extract From *Nymphaea pubescens* Willd Against Human Cervical And Breast Carcinoma In Vitro, International Research Journal Of Pharmacy 2012, 3 (1), ISSN 2230-8407

46. Muthulingam M. Antihepatotoxic Efficacy Of *Nymphaea pubescens* (Willd.) On Acetaminophen Induced Liver Damage In Male Wistar Rats. International Journal Of Current Research. 2010, 3: 012 -016.

47. Kiran Kumar Angadi, Ammani Kandru, Abdul Rahaman. Antihyperglycaemic, Antihyperlipidaemic And Antioxidant Assays(In Vivo) Of *Nymphaea pubescens* Leaf Extract. Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 624 – 630.

48. Kiran Kumar Angadi, Ravi Kumar Gundampati, Medicherla V. Jagannadham, Ammani Kandru. Phytochemical Analysis And Antimicrobial Properties Of Leaf Extracts Of Nymphaea pubescens, Asian Pacific Journal Of Tropical Biomedicine (2012)1-5.

49. Koya Siva Prasad, Nataru Savithramma. Tapping of Aquatic Plant Nymphaea pubescens WILLD. For Synthesis of Silver Nanoparticles and Its Antimicrobial Evaluation. Int. J. Pharm. Sci. Rev. Res., 33(2), July –August 2015; Article No. 12, Pages: 63-66.

50. Rushender, C. Rajan; Eerike, Madhavi; Madhusudhanan, N.; Rao Konda, Venu Gopala. Invitro Antioxidant And Free Radical Scavenging Activity Of Nymphaea pubescens . Journal of Pharmacy Research;2012, Vol. 5 Issue 7, p3804.

51. Shajeela.P.S, Kalpanadevi.V and Mohan.V.R. Potential antidiabetic, hypolipidaemic and Antioxidant effects of Nymphaea pubescens extract in alloxan induced diabetic rats. Journal of Applied Pharmaceutical Science 02 (02); 2012:83-88.

52. Phukphon Munglue. (Conference: 8th Ubon Ratchathani Research Conference, At Ubon Ratchathani University, Ubon Ratchathani, Thailand. Effect of Nymphaea pubescens Leaf Extract on Growth of Nile Tilapia (Oreochromis niloticus).

53. AM Tunan,Phytochemical Investigation of Nymphaea pubescens and Study of its Antimicrobial Activities, EWU Publication.

54. Tadesse Bekele Tafesse, Ariaya Hymete, Yalemtsehay Mekonnen And Mekuria Tadesse. Antidiabetic Activity And Phytochemical Screening Of Extracts Of The Leaves Of Ajuga Remota Benth On Alloxan-Induced Diabetic Mice,BMC Complementary And Alternative Medicine (2017) 17:243.

55. Muhammad Ajmal Shaha, Ruqaiya Khalil, Zaheer Ul-Haq, Pharkphoom Panichayupakaranant. A-Glucosidase Inhibitory Effect Of Rhinacanthins-Rich Extract From Rhinacanthus Nasutus Leaf And Synergistic Effect In Combination With Acarbose, Journal Of Functional Foods Volume 36, September 2017, Pages 325-331.

56. Martha Thomson , Khaled K. Al-Qattan, Divya JS, And Muslim Ali. Anti-Diabetic And Anti-Oxidant Potential Of Aged Garlic Extract (AGE) In Streptozotocin-Induced Diabetic Rats. BMC Complementary And Alternative Medicine (2016) 16:17.

57. Wang J, Teng L, Liu Y, Hu W, Chen W, Hu X, Wang Y, Wang D. Studies on the Antidiabetic and Antinephritic Activities of Paecilomyces hepiali Water Extract in Diet-Streptozotocin-Induced Diabetic Sprague Dawley Rats, Journal of Diabetes Research.Volume 2016.

58. Moodley K, Joseph K, Naidoo Y, Islam S, Mackraj I. Antioxidant, antidiabetic and hypolipidemic effects of Tulbaghia violacea Harv. (wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complementary and Alternative Medicine. 2015;15:408.

59. Babin D Reejo, P. Natarajan, A. Thangathirupathi. Evaluation Of Antidiabetic Activity Of Samanea Saman (Jacq.) Merr International Journal Of Research In Pharmaceutical And Nano Sciences. 3(4), 2014, 352- 356.

60. Raman N. Phytochemical Technique. 2006; New Indian Publishing Agencies: New Delhi p.19

61. Kumar A et al. Anti-diabetic activity of Syzygium cumini and its isolated compound against streptozotocin-induced diabetic rats. Journal of Medicinal Plants Research 2008; 2(9): 246-249.

62. Yogesha Mohan, Grace Nirmala Jesuthankaraj, and Narendhirakannan Ramasamy Thangavelu.Antidiabetic and Antioxidant Properties of Triticum aestivum in Streptozotocin-Induced Diabetic Rats. Advances in Pharmacological Sciences Volume 2013 (2013), Article ID 716073, 9 pages

63. Vishwanath Jannu, Sai Vishal D, Ranjith Babu V, Harisha B, Ravi Chandra Sekhara Reddy D. Antidiabetic activity of hydro-alcoholic extract of Cissampelos pareira linn. Leaves in streptozotocin induced diabetic rats. International Journal Of Pharmacy&Technology ISSN: 0975-766X

64. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., Protein measurement with Folin-phenol reagent. J. Biol. Chem. 1951. 193, 265–275.

65. Folch, J., Lees, M., Solane, S.G.H.,. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 1957, 26, 497–509.

66. Zlatkis, A., Zak, B., Boyle, A.J., A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 1953. 41, 486–492.

67. Burstein, M., Scholnichk, H.R., Morin, R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res.1970, 11, 583–595.

68. Friedwald, W.T., Levy, R.I., Fredrickson, D.S., Estimation of the concentration of LDL-cholesterol in plasma without the use of the preparative ultracentrifuge. Clin. Chem. 1972.18, 499–502.

69. Reitman, S., Frankel, S. Colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 1957, 28, 56–63.

70. Slot, C., Plasma creatinine determination. Jaffe's A new and specific reaction method. Scand. J. Clin. Lab. Invest. 1965. 17, 381–387.

71. Wybenga, D.R., Di Giorgio, J., Pileggi, V.J. Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem. 1971.17, 891–895.

72. Elder, C.Ayurveda for diabetes mellitus: a review of the biomedical literature. Altern. Ther. Health Med. 2004, 10 (1), 44–50.

73. Padmini K, Chakrabarti CH. Effects of bitter ground seed and glibenclamide in streptozotocin induced diabetis mellitus. Indian j.Exp.Biol. 1982; 20:232-235.

74. Bierman EL, Amaral JAP, Belknap BH. Hyperlipidemia and diabetes. Diabetes. 1996; 15: 675–679

75. Ellils G, Goldberg DN, Spooner RI. Serum enzyme test in disease of the liver and billiary tract. Annales J Clinical Pathology. 1978; 17: 248-258

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AUTHENTICATION CERTIFICATE

Based upon the Organoleptic ✓ /macroscopic ✓ /~~microscopic~~ examination of fresh ✓ /~~market~~ sample, it is certified that the specimen given by S.Vijay, M.Pharm [Pharmacology] C.L.BAID METHA COLLEGE OF PHARMACY. is identified as below:

Binomial: Nymphaea pubescens Willd.

Family: Nymphaeaceae

Synonym(s): N. lotus auct.non L.; N. lotus L. Var pubescens - [Willd.] Hook.f.& Thoms.

Regional names: Ta:- Allitamarai, Vellambal.

Reg.No of the certificate: PARC/2017/3473

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: Pg: 09 .1983. ✓

Henry, A.N. *et al.* Ibid. _____ II: _____ .1987.

Ibid. _____ III: _____ .1989.

Ed:S.P.Ambasta,
The Useful Plants of India,
CSIR- Publication, 1986.

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