Introduction

1.1 Diabetic Nephropathy: A Detailed Exploration

Diabetic nephropathy represents a progressive kidney disorder induced by the damage to the capillaries in the kidney glomeruli, primarily due to prolonged diabetes mellitus. This condition manifests as nephrotic syndrome and nodular glomerulosclerosis, as originally described by Kimmelstiel and Wilson in 1936. Diabetes mellitus is notorious for its association with both microvascular and macrovascular disorders, contributing to conditions like neuropathy, retinopathy, myocardial infarction, and stroke, according to McAlpine et al., 2010. Diabetic nephropathy stands as the foremost cause of kidney failure in developed nations, posing a significant and escalating challenge to medical, social, and economic systems globally. Current treatments can decelerate, but not entirely halt, the disease's progression (Oates, 2010).

1.2 Classification of diabetes:

The World Health Organization Expert Committee (1980, 1985) established several classification systems for diabetes mellitus. The present WHO classification system, developed in collaboration alongside National Diabetes Data Group (USA), is primarily based upon the etiology of D.M (diabetes mellitus).

I. Type I diabetes
In type I diabetes, beta cell damage usually leads to clear insulin deficiency.
(a) Immune mediated
(b) Idiopathic

II. Type II diabetes
It could be mostly secretory defect with insulin resistance or mostly insulin resistance with relative insulin deficiency.

(c) Other specific types

- Beta cell dysfunction due to genetic defects
- Hereditary deformities in insulin activity
- diseases of the pancreas' exocrine system
- Endocrinopathies
- Whether caused by a chemical or a drug
- Infections
- Forms of immune-mediated diabetes that are uncommon

(d) Gestational diabetes

Criteria for the diagnosis of diabetes

1. A1C>6.5%. The procedure for the test ought to be standardized to the DCCT assay and certified by the NGSP in a laboratory.

OR

2. FPG >126 mg/dl (7.0 mmol/l). Fasting is characterized as no caloric admission for somewhere around 8 hrs.

OR

3. OGTT plasma glucose greater than 200 mg/dl (11.1 mmol/l) after two hours. The World Health Organization recommends using a glucose load that contains the equivalent of 75 gm of anhydrous glucose dissolved in water for the test.

OR

4. A random plasma glucose of more than 200 mg/dl (11.1 mmol/l) in a patient with classic hyperglycemia or hyperglycemic crisis symptoms.

1.3 Genetic and Environmental Influences

Diabetic nephropathy is a classic example of a complex trait, where its manifestation in an individual results from the interplay of multiple genetic factors modulated by environmental influences (Adler et al., 2010). The microvascular complications arising from diabetes mellitus lead to significant morbidity and a marked decline in the quality of life.

Dyslipidemia and chronic hyperglycemia heighten oxidative stress, inflammation, and vascular damage, cumulatively causing endothelial dysfunction linked with both microvascular and macrovascular complications. Early detection of microalbuminuria is crucial as it serves as an early indication of diabetic nephropathy and a separate risk factor for heart diseases. This condition is the predominant cause of end-stage nephric disease, with its prevalence on the rise.

1.4 Clinical Studies and Preventative Measures

The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial underscored that mitigating the progression of diabetic nephropathy can not only delay renal complications but also offer significant cardioprotective benefits for Type II diabetes patients. Diabetic nephropathy, a long-term complication of both Type I and Type II diabetes, is a principal cause for end-stage renal disease. It is also associated with increased cardiovascular mortality, with nephropathy responsible for the majority of the extra mortality in people with diabetes.

1.5 Diabetes Mellitus Pathogenesis and Pathophysiology

The principal guilty party for type I diabetes mellitus is immune system destruction of p-cells in the pancreas. An illustration of this auto-immune process includes the presence of insulin autoantibodies, antibodies to islet cells, and antibodies to glutamic corrosive decarboxylase. The factors that start the immune system obliteration of pancreas are obscure; Infections and substance poisons have been proposed as starting specialists of IDDM. Creatures and in people the job of infections, Coxsackie infection B, in the pathogenesis of type I diabetes is extremely clear. Other external factors changes in diet, temperature, and distance from the equator Diabetes risk is increased by ethnicity in the population. The majority of IDDM patients present with one or more side effects of polyuria, polydipsia, over the top craving, and weakness or ketones and glucose levels in the blood and urine increase with weight loss. NIDDM, or juvenile maturity-onset diabetes, may also be present. with classic symptoms, but frequently exhibits no symptoms. NIDDM is the result of insulin action deficiencies caused by the abnormal lack of insulin secretion within the cell or on the cell surface synthesis of these procedures. The essential Reduced glucose-stimulated insulin secretion is the root cause of NIDDM. The Blood glucose levels rise as a result of an insulin reaction deficit and other metabolic disturbances, but not severe lipolysis disturbances enough to cause ketoacidosis or

metabolic acidosis in patients. NIDDM could exhibit the symptoms and typical signs of diabetes, such as thirst, pruritus, polyuria, weight loss, and polyphagia. In addition to hyperglycemia and serum lipid abnormalities, diabetes is related with miniature vascular and full scale vascular complications, which account for the majority of deaths and morbidity in subjects who were diabetic. The procedure is similar. of diabetes complications, both type I and type II, and a high at the time of, the prevalence of nephropathy and retinopathy in type II diabetes finding. Additionally, diabetes raises the chances of cardiovascular diseases, as demonstrated by a number of studies . Atherosclerosis is accelerated by diabetes mellitus, and raises the likelihood of a heart attack. Cells in the eye's lens and retinaare typically damaged in diabetes. It can annihilate the filtration arrangement of the kidney. It cuts off nerves, and feet suffer as a result. lose sensation, making them vulnerable to injury. The obstruction of blood flow Infections spread to a greater extent and wounds heal more slowly. Men become impotent as a result of damage to the circulatory and nervous systems. Problems with congenital anomalies and stillbirths are associated with diabetes during pregnancy (Berelowitz and Eugene, 1996). Most troubling Diabetes can cause vision loss, heart attack, and kidney damage. impotence, limb amputation, and failure. Despite the "Diabetes Management" and "Complications Trial" has found that hyperglycemia is a factor that makes people more likely to improve diabetic entanglements, there are various similarly plausible explanations for the occurrence of problems. These hypotheses include a product of advanced glycation oxidative stress, the aldose reductase hypothesis, and reductive altered lipoprotein metabolism, true hypoxia, carbonyl stress, and stress expanded Protein kinase-C movement and modified development element or cytokine activities. In addition to Ayurveda, the Siddha system of medicine makes use of C. prostratus (CP), whose oil promotes hair growth by acting as a keratogenic agent. Furthermore, a paste prepared from the plant's roots and blossoms is said to have anti-aging qualities, suggesting that it might also have antioxidant qualities. Additionally, a syrup prepared with C. prostratus and Flute player nigrum is recommended in the Unani therapeutic framework for the treatment of draining piles and genital diseases.

1.6 Pathophysiology and Risk Factors

Nephrons, which are hundreds of thousands of filtering units in the kidneys, are made up of glomeruli, which are collections of small blood arteries. The elimination of waste from the body depends on these structures. High blood sugar levels have the potential to gradually

deteriorate these structures, which can result in the albumin protein leaking into the urine. Persistent microalbuminuria and overt nephropathy in diabetic patients have been linked to a number of demographic factors, including American Indian, Hispanic, or African American origin; a family history of nephric disease or high blood pressure; poor blood pressure control; poor glycemic control; early-onset Type I diabetes; and smoking.

1.7 Prediction and Progression

Diabetic nephropathy cannot be predicted in patients based only on blood pressure, glycemic control, or the length of diabetes, albeit these variables are implicated. As a result, even years after being diagnosed with diabetes, a patient with inadequate blood pressure and glucose control may not develop diabetic renal impairment, suggesting the presence of additional contributing factors.

1.8 Prevalence and Disease Progression

With Type I diabetes, the prevalence of nephropathy rises with the length of the disease but reaches a cumulative prevalence of roughly 50% after 15 years. Conversely, the beginning of Type II diabetes is less well-defined, which makes it more difficult to identify comparable patterns. Nephropathy appears to impact only a portion of diabetic people, in contrast to retinopathy, whose frequency rises with the length of diabetes.

1.9 Factors related to the environment

Numerous epidemiological research imply that viruses may be the cause of type I diabetes. seasonal patterns that typically line up with one another. It has long been known that common viral infections are prevalent. New causes detected, coxsackievirus of group B linked to diabetes pancreatic disorders The virus causes mild (3-cell) illness. The injury is followed by a virally altered immune response. Cells with HLA linked susceptibility are β-cells.

1.10 Early Detection and Familial Aggregation

Persistent proteinuria is the primary indicator of diabetic nephropathy, with microalbuminuria signaling early kidney damage that may still be recoverable. High blood pressure usually

accompanies diabetic nephropathy. For both Type I and Type II diabetes, the Diabetes Control and Complications Trial (DCCT) verified the presence of familial aggregation of diabetic nephropathy. Research conducted on a longitudinal basis among Pima Indians revealed that diabetic children whose parents had proteinuria had a greater incidence of proteinuria than diabetic children whose parents did not have proteinuria.

1.11 Genetic Susceptibility and Environmental Factors

The familial aggregation of diabetic nephropathy has also been observed in Brazilian, Italian, & Indian populations. Although hypertension is a known inherited component, the inherited factors that contribute to genetic predisposition to diabetic nephropathy are yet unknown. Compared to parents of diabetic patients with normoalbuminuric conditions, parents of Type I diabetic individuals with nephropathy have greater death rates.

1.12 Birth Characteristics and Adult Disease

Recent studies have provided fresh insights into the vulnerability to diabetic nephropathy by connecting the birth weight and birth anthropometry to adult features and diseases. Low birth weight along with a low ponderal index (being thin at birth) are linked to hyperinsulinemia, poor glucose tolerance, elevated blood pressure, and cardiovascular illness, among other symptoms of the Insulin Resistance Syndrome (IRS). Insulin resistance has also been linked to diabetic nephropathy. The correlation between birth characteristics and diabetic nephropathy suggests a predisposition to insulin resistance, which may stem from intrauterine growth retardation.

1.13 Genetic and Environmental Interplay

The interplay of genetic and environmental factors in the growth of nephropathy is evident. Genetic predispositions, such as low birth-weight linked to maternal and paternal traits, and intrauterine environment factors, like maternal blood pressure throughout gestation period, influence the susceptibility to diabetic nephropathy. People may be predisposed to low birth weight and characteristics of the Insulin Resistance Syndrome, such as diabetic nephropathy, due to common genetic causes.

Diabetic nephropathy remains a significant contributor to mortality and morbidity among Type 2 diabetes patients. Early diagnosis and management of microvascular complications can prevent the progression to more severe macrovascular complications, thereby reducing the economic burden on patients and society. Identifying markers, such as platelet indices, that predict microvascular complications can aid in targeting high-risk individuals for regular monitoring and improved clinical outcomes. Further research is essential to explore the relationship between platelet indices and the severity of diabetic nephropathy, aiming to enhance early detection and intervention strategies.

1.14 The pathogenesis of diabetes mellitus type-ll.

Type-2 diabetes appears to be associated with epidemiological studies. Genetic defects or problems are the result of a collection of several genetic flaws or problems. Every polymorphism adds to the modified danger and predisposing threat unique to it. Influenced by external influences the insulin secretion in β-cells is deranged. The peripheral tissues' response to insulin decreased. Thus type II diabetes is characterized by two primary metabolic abnormalities.

1.15 Deranged β -cell secretion of insulin:

There is a small chance of developing diabetes type 2. β -cell hyper responsiveness to physiological may be attributed to this. Elevations in the blood glucose levels are common. Observations indicate that there are disturbances in p. The cells react to mild to severe insulin deficiency and hyperglycemia. In type-II diabetes, insulin is produced. As per one viewpoint, every one of the important physical processes are associated. The genetic vulnerability of cells to damage leads to accelerated cell growth.

Resistance to insulin.

There might be a decline in the population with type 2 diabetes. This affects the way insulin signals post-receptor. a state in which the distribution and synthesis of glucose transporters are decreased. The insulin resistance noted in muscle and fat cells is attributed to the GLUTs in muscle and fat cells. It's about obesity. Circulating insulin's mobility guides the disposition. A more persistent hyperglycemia and a higher level of glucose. Therefore, more is achieved. Pancreatic β-cell stimulation for the production of insulin is prolonged. (Rod Kahn, 1997)

1.16 Obesity

The study of obesity shows that life style plays a significant role. Insulin resistance and abdominal obesity may coexist as expressions of each other. The third aspect that is unknown is whether or not they are connected causally. It is necessary to consider that.

1.17 Diabetes management:

Insulin and nutrition are typically used in combination to treat type 2 diabetes.Pharmacological medications, lifestyle modifications, or physical activity. Insulin and antidiabetic medications (e.g., oral) are also used. Changes in diet and lifestyle are possible. Diabetes care includes medical nutrition therapy as a key component. Sadly, a patient's compliance with nutrition guidelines does not equate to their own adherence to them. Medical objectives are the most difficult part of diabetic therapy. The aim of nutrition therapy is blood glucose concentrations.

To return as close to normal as feasible, combine antidiabetic drugs with a balanced food consumption. drug usage along with exercise. The upper maximum is thirty percent. The primary sources of calories per day should be fats, proteins (between 10% and 20%), and carbohydrates. daily caloric balance derived from carbs. Exercise helps, particularly in individuals with modest glycemic control and insulin sensitivity. A high degree of insulin resistance is associated with diabetes.

1.18 Nephropathy:

Mesangium enlargement and basement membrane thickening are traits of diabetic nephropathy., less filtration, renal failure and albuminuria. Type 1 and type 2 renal dysfunction have distinct underlying renal lesions. despite the clinical signs of diabetic nephropathy, diabetes increased blood volume, proteinuria, and a slower glomerular filtration rate similar pressure. In fact, the most significant primary changes in type 1 diabetes occur in the glomerulus, even in the presence of tubules, interstitial and arteriolar sores, and several patients with type 2 diabetes have typical glomerular construction even in the presence of microalbuminuria or proteinuria (30 to 300 mg of egg whites per 24 hrs), despite everything abnormalities of the tubulo-interstitial and arteritis, which can start as early as 5 years after

diabetes was first diagnosed. Patients with glomerular sclerosis at this stage can be more vulnerable to nephropathy. hyper-screening. Glomerular edema, proteinuria, and the onset of nephritic syndrome rate of filtration are clinical features of overt diabetic nephropathy that may progress to end-stage renal disease.

In excess of 65 million American grown-ups have hypertension the National Institutes of Health (NIH) states that The infection is more prevalent among African Americans and prone to escalating complications. As a result, African Americans are more likely to develop the disease, but also to deal with its side effects. More African Americans likely to develop the kind of high blood pressure that can be controlled with salt. restriction. African Americans in particular need to go through screenings for high blood pressure and early treatment. Uncontrolled high blood pressure can harm the blood over time. vessels and nephrons (utilitarian units of the kidneys) in the kidneys. This causes the kidneys to stop removing waste from the body, sodium and blood-derived extra fluids. With no spot to go, the additional liquids and sodium remain in the bloodstream, placing additional strain on the walls of the dilated blood vessels as well as raising blood pressure. This additional stress harms the kidneys much further. According to US reports, the majority The aforementioned issues led to the development of kidney disorders. However, some of the people are also receiving these failures were brought on by drinking polluted water. Diabetes nephropathy is a major problem for patients with either type I or type II diabetes. The influence of a 287 bp addition/erasure (I/D) polymorphism of the quality encoding angiotensin-l changing over chemical (Pro) has been researched and the erasure type is established as a risk factor for this disease's onset.

1.19 Expanded predominance of diabetic nephropathy in South Asians:

Racial contrasts in the predominance of diabetic renal illness have been mentioned. Asians are significantly (p 0.01) more likely diabetic end-stage renal disease (ESRD) prevalence (52.6 percent) when compared to migrant Asian Caucasians (36.2 percent). When ESRD was first discovered, Indians had a 40-fold increased risk. contrasted and the Caucasians. The prevalence of DN in Indian patients with type II diabetes was reported to be 5-9%. Nephropathy patients with diabetes, particularly type II diabetes, a higher risk for cardiovascular disease. The danger to the heart disease (CVD) was three times more common in South Indian NIDDM patients who had nephropathy in comparison to those who are not

nephropathic. As a result, many people with type 2 diabetes may not arrive at end stage renal sickness because of sudden passing from CVD.

1.20 Mechanisms that are abnormal and involved in diabetic nephropathy:

The specific reason for diabetic nephropathy is obscure, yet different Hyperglycemia (which results in increased filtering) and age, cytokine activation, and renal injury Hyperglycemia increments the glomerular expression of strengthening growth factor beta (TGFB) and of matrix proteins that this cytokine specifically stimulates. TGFR might add to both the cell hypertrophy and upgraded collagen synthesis that can be seen in diabetic nephropathy. In a southern study, According to Vijay et al. (2005), TGF- G.1 levels are elevated in India. subjects with type II diabetes, Insulin and angiotensin II therapy, angiotensin receptor blocker (ARB) or converting enzyme inhibitor (ACEI) appears to decrease TGF- R1 levels. Also, hyperglycemia may activate PKC, which may be a factor in kidney disease and other vascular complexities in diabetes. In individuals with obvious diabetic nephropathy (dipstick positive proteinuria and a decline in GFR), systemic hypertension also occurs in addition to changes in renal hemodynamics, notably a drop in renal plasma and glomerular filtration rate stream. All progressive renal illnesses are negatively impacted by high blood pressure, but it seems to be more so in this case. in kidney damage caused by diabetes. The negative effects of high blood pressure are coordinated at the full scale and microvasculature.

1.21 Pathophysiology of diabetic nephropathy:

Genetic vulnerability is thought to be a major contributor in the development and progression of diabetic nephropathy, however there are other elements involved in the pathophysiology of the condition. Two crucial Diabetes has been linked to a variety of contributing factors. nephropathy, metabolic and hemodynamic. Three significant histologic In diabetic nephropathy, the glomeruli undergo changes (1) mesangial development is straightforwardly actuated by hyperglycemia, maybe by means of expanded lattice creation or glycosylation of grid proteins, (2) Thickening of glomerular storm cellar layer (3) Intraglomerular hypertension causing glomerular disease It would appear that these various histologic patterns have similar significance for predictions. Diabetes results in high-quality and quantitative changes in the organization of the fine cellar layer and this changed material goes through sped up glycosylation and additional rearrangement that results in advanced glycosylation end products (AGE), which invigorate protein union , further decrease in the

basement membrane's ability to degrade, boost its permeability, and result in dysfunction in the endothelium. dysfunctional endothelial cells measured as abnormal flow and elevated levels of endothelin-1 South Indian type 2 diabetics have demonstrated dilatation.

1.22 Factors controlling diabetic nephropathy:

1.22.1 Genetical:

Diabetic patients may be influenced by family history. nephropathy. Some ethnic groups, particularly black Americans, Native Americans and Hispanics may be specifically susceptible to renal involvement as a diabetes-related complication. (Vijay and colleagues, 1999) Researchers led a review to decide familial conglomeration of diabetic nephropathy in South Indian patients with type II diabetes. It turned out that 50% of the patients had proteinuria, while 26.7 percent had microalbuminuria. siblings of people who have diabetes and nephropathy. Contrarily, the prevalence of proteinuria & microalbuminuria in siblings with diabetes of normoalbuminuric probands were 3% and 0%, respectively (P=0.057). to treat microalbuminuria). There is some evidence that polymorphism in the angiotensin-converting enzyme (ACE) gene plays a role in either predisposing individuals to nephropathy or accelerating its progression. In a review from south India (Vijay et al., 2001), it was shown that a positive ACE's "D" allele, or ID and DD genotype, is related to each other. proteinuria and polymorphism in South Indian type 2 diabetics. Notwithstanding, authoritative hereditary markers still can't seem to be distinguished. In outline, Several hemodynamic and metabolic processes are initiated by hyperglycemia. abnormalities that occur in the standard clinical and histologic picture that is observed in diabetic nephropathy patients.

1.22.2 Polymorphisms in genes:

Late progressions in atomic hereditary methods have provided fresh perspectives on the role that genetic variation plays in renal disorders. regarding the likelihood of developing renal disease, the course illness and the advantages of renoprotective treatment. A lot of work has been put into figuring out how genetic polymorphisms work. Changes in the Human genomes are frequently changed. The results they have can vary. A The first possibility is that the affected children die naturally or becomes weighed down by obvious clinical symptoms of disease. By this component It is difficult to easily transmit the mutation to subsequent generations. These are called pressure on selection. On the other hand, if the mutation does

not negative effects, descendants can survive and appear relatively healthy. Subjects occasionally get sick but also have advantages, such as sickle cell disease patients who are less susceptible to malaria Polymorphisms in genes as a new indicator of disease susceptibility The complex interaction that occurs between multiple environmental factors and genetic polymorphisms passed down through generations may lead to the ability to prevent or alter the course of certain diseases, by each having a small but combined effect. Recently, it was suggested that genetic polymorphisms can increase or decrease a person's risk of osteoporosis, Alzheimer's disease, hypertension, and diabetes affliction (Yoshida et al., 1996). Nonetheless, the way to take apart the effect of Genetic polymorphisms are difficult to understand. Each polymorphism does appears to have little effect on the phenotype, allowing for the identification of Identifying these susceptibility genes is much easier than finding them. of major chromosomal abnormalities or mutations in a single gene. These are due to the fact that even the presence of multiple susceptibility alleles at various loci does not always result in obvious clinical symptoms, as hypothesized to caused by a small difference in how much influence the environment has on them. (2000, Holtzman and Marteau)

1.22.3 The angiotensin-converting enzyme's insertion/deletion (I/D) polymorphism:

The renin-angiotensin aldosterone framework (RAAS) plays a critical part in both cardiovascular and renal pathophysiology (Dzau, 1994; Wolf, 1998). Angiotensin II (angll), the main organic dynamic item, is produced through a process that involves a number of precursor peptides and enzymes, some of which are controlled by specific genes. The most popular The ability of angll to stifle vascular smooth muscle cells and to direct tubular cells and cause fluid and sodium retention to be induced and by encouraging the production of aldosterone. However, in recent times Angll's other potential actions have been explained. Numerous studies have demonstrated that angll promotes vascular smooth muscle in vitro, growth of cells in the renal tubules and mesangia of the glomerulus (Dzau, 1994; Wolf, 1998). Angll additionally has all the earmarks of being associated with the aggregation of collagen by inhibiting collagen degradation while simultaneously stimulating collagen synthesis. Yoshida and colleagues (1996) As a result, it is abundantly clear that genetic polymorphisms the RAAS have acquired interest in the quest for hereditary elements that may influence how long-term renal failure progresses and how to treatment to renoprotective regimens. A zinc metallopeptidase, angiotensin-I converting enzyme (ACE), is widely

dispersed on the epithelial and endothelial cell surfaces. By Angiotensinogen is transformed into angiotensin I when renin is stimulated. ACE after that, it changes angiotensin-I into angiotensin-II, which is the primary active product of the RAS Relationship to the ACE

1.22.4 polymorphism:

Nephropathy caused by diabetes, typically preceded by hypertension and 30 to 50 percent of patients with the condition eventually develop persistent albuminuria. diabetes that is either insulin-dependent (NIDDM) or insulin-dependent (IDDM) one of the most usual causes of end-stage nephric failure in diabetes mellitus, most nations that are industrialized. A variety of drugs, including a-blockers, ACEI, ARB, calcium channel blockers, and other medications can be used to hypertension. But getting everyone to start treatment isn't the most important thing. one class of drugs, but effectively treating with the best drug for individual patients. Despite calcium, a-blockers, and 3-blockers channel blockers stay significant enemy of hypertensive specialists in diabetic patients, they ought to be taken as second- or third-line medications in together with ARB and ACEI. based on the conclusion that both diabetic nephropathy (Seaquist et al, 1989) and hypertension (Krolewski et al., 1988) tend to live in families, so it is reasonable to assume that genetic It is possible to identify markers that would enable earlier detection of this. devastating condition that affects diabetics. The renin-angiotensin system is important for controlling blood pressure and renal function as a key regulator of sodium homeostasis, as well as additionally as a vascular tone and structure modulator. Angiotensin II, which is released, primarily mediates these effects. by the sequential actions of renin and angiotensin- from angiotensinogen enzyme that converts (ACE). The significance of renin and angiotensin framework in the advancement of diabetic nephropathy is upheld by several studies that show that inhibiting this system has a positive effect on the progression and development of this problem. The genes that code for the parts of the renin- As a result, the angiotensin system's genes are excellent candidates for a role in diabetic nephropathy's onset or progression. Late examinations have distinguished a variation of angiotensin-converting enzyme gene (ACE) with a 289-deletion intronic deletion (D). Alu sequence at bp, which has been linked to more plasma and this enzyme's involvement in tissue activity. This hereditary variation has been ensnared as a gamble factor for left ventricular stroke and hypertrophy of the heart, but these results are by no means conclusive. In IDDM, patients reported that the presence of the Pro D variation was related with a chances proportion (or) of 3.88 to get diabetic nephropathy. Since this first appeared,

Numerous researchers have looked into this problem, both with patients and with IDDM and NIDDM, resulting in varying outcomes. The essential goal of As a result, the purpose of this review was to investigate the reported between diabetic nephropathy and the ACE-D variant in an effort to make sense of the questionable outcomes in the distributed writing. This subject is suitable for a meta-analytic approach due to the fact that all studies assume the nephropathy and the ACE-D variant share the same relationship. Also, every Similar methods have been used in studies to test this hypothesis, comparing nephropathic patients' allelic or genotype frequencies to those in control subjects without nephropathies.

Chapter 2- Plant Introduction

2. Convolvulus pluricaulis:

Convolvulus pluricaulis finds extensive use as a primary medicinal plant in regions of northern, central, and western India. Meanwhile, Evolvulus alsinoides is more common in Karnataka, and Clitoria ternatea is frequently utilized in Kerala. Regardless of the source plant, this drug demonstrates significant therapeutic effects on central nervous system (CNS)

disorders. Comparative studies on the efficacy and usage indicate that Convolvulus pluricaulis serves as the main source, with Evolvulus alsinoides and Clitoria ternatea acting as alternative sources for Shankhapushpi. The Indian Council of Medical Research (ICMR) has also set quality standards for Convolvulus pluricaulis in its publications.

Fig 2.0 Convolvulus pluricaulis (leaf & flower)

2.1 Clinical and Pre-Clinical Applications of Convolvulus pluricaulis:

Convolvulus pluricaulis, known commonly as Shankhpushpi, has undergone extensive research due to its numerous pharmacological properties. Notably, it exhibits a range of neuropharmacological effects, including nootropic (cognitive-enhancing), antistress, anxiolytic (anxiety-reducing), antidepressant, anticonvulsant (seizure-preventing), tranquillising, and sedative activities. These attributes support its traditional use in Ayurvedic medicine for treating central nervous system (CNS) disorders. Additionally, Shankhpushpi possesses antimicrobial, antipyretic (reducing-fever), analgesic (relieving-pain), anti-inflammatory, diuretic (promoting urine production), antidiabetic, and insecticidal properties.

2.2 Shankhapushpi: An Ayurvedic Marvel

Shankhapushpi, an esteemed herb in Ayurveda, falls under the category of "Medhya Rasayana," meaning it rejuvenates the intellect or brain. This herb, part of the Convolvulaceae family, is a prostrate, spreading perennial wild plant usually found on rocky

or sandy terrains under dry conditions in northern India. Shankhapushpi blooms from September to October, showcasing flowers that range from white to light pink. The name "Shankhapushpi" derives from the shape of its flowers, which resemble a conch (shankh). The plant's branches extend over 30 cm, with elliptic leaves (about 2 mm) positioned alternately along the branches or flowers. Every part of this herb is known for its therapeutic benefits, particularly in enhancing cognitive functions like learning, memory, and recall. Shankhapushpi is celebrated as a remarkable gift of nature.

Despite its widespread use, there is ongoing debate regarding its precise botanical identity. Ayurvedic texts and various literatures suggest multiple plant species are referred to as Shankhapushpi. According to literature and observations in drug markets, these species includes:

- **Evolvulus alsinoides Linn,**
- **Clitorea ternatea Linn,**
- **Convolvulus pluricaulis Choisy,**
- **Canscora decaussate Schultz.**

2.3 Scientific classification

The following illustrates C. pluricaulis's scientific classification:

Table 2.3 Scientific classification (*C. pluricaulis)*

Division-	Magnoliophyta
Super-division	Spermatophyta
Kingdom-	Plantae
Sub-kingdom	Tracheobionta
Class-	Magnoliopsida

Sub-class	Asteridae
Order-	Solanales
Family-	Convolvulaceae
Species	*pluricaulis*
Genus-	*Convolvulus*

2.4 Vernacular names

The following is a description of C. pluricaulis' vernacular names:

Table 2.4 vernacular names of *C. pluricaulis*

Hindi-	*Shankhapushpi, Aparajit*
Sanskrit:	*Sankhapuspa*
Gujarathi-	*Shankhavali*
Bengal:	*Sankhapuspi*
Kannada-	*Bilikanthisoppu*
Punjabi:	*Shankhapuspi*
Marathi-	*Shankhavela*
Telugu:	*Shankhapushpi*
Tamil-	*Sanghupushpam, kakkurattai*

It was C. pluricaulis, a perennial plant with a morning glory-like appearance. It can have branches longer than 30 cm. Branching branches or flowers alternate with elliptic leaves (2 mm) and blue flowers (5 mm). In India, aloe weed is widely distributed, especially in the state of Bihar.

2.5 Chemical Constituents of Shankhapushpi:

Shankhapushpi comprises several significant chemical constituents. It has carbohydrates such as D-glucose , sucrose, starch, maltose and rhamnose. The plant also includes amino acids, proteins, and the alkaloid shankhapushpine ($C_{17}H_{25}NO_2$), which has a melting point of 162 - 164°C. Notably, tropane alkaloids are present, with convolamine being identified.

Volatile oils, fatty alcohols, fatty acids , and hydrocarbons, including straight-chain hydrocarbon hexatriacontane, linoleic acid (2.3%), palmitic acid (66.8%), and myristic acid (30.9%) are all found in the fresh plant. The whole plant Convolvulus pluricaulis is rich in ceryl alcohol, β-sitosterol, and scopoletin. There were also notable concentrations of the steroids β-sitosterol and phytosterol, as well as the flavonoid kaempferol. Tetratriacontanoic acid, 29-oxodotriacontanol, and 20-oxodotriacontanol are among the plant's chloroform fraction components that have been shown to be effective insect antifeedants.

Table 2.5 Chemical constituents of *C. pluricaulis.*

Class	**Chemical constituents**
Alkaloids-	Convolamine, shankhapushpine, convolidine, convoline, convolvine, convosine, confoline
Phenolic /glycosides /triterpenoids /steroids	Scopoletin, steroids-phytosterols, β-sitosterol, 20-oxodotriacontanol, ceryl alcohols, tetratriacontanoic acids, flavonoid-kampferol,
Carbohydrates	D-glucose, rhamnose, maltose, starch , sucrose & other carbohydrates

Fatty acids /fixed oil /volatile acids	Volatile oils, palmitic acids , fatty alcohols,fatty acids, hydrocarbons,myristic acids, and linoleic acids
Proteins & amino acids	Proteins &amino acids

2.6 Traditional Medicinal Applications of Convolvulus Pluricaulis: Key Plant Components and Preparation Methods

C. pluricaulis (CP) has been used in the Siddha medical system in addition to Ayurveda. This plant's oil is utilized in this technique as a keratogenic agent to encourage the creation of new hair. Its possible antioxidant action is further supported by the belief that a paste prepared from its roots and petals functions as an anti-aging agent. Additionally, bleeding piles and sexual disorders are treated with a syrup made with C. pluricaulis and Piper nigrum in the Unani medical system. These are all listed uses of C. pluricaulis in ethnomedicine.

Table 2.6 Uses of *Convolvulus Pluricaulis*

Diseases Targeted	**Section(s) used**	**Preparation Techniques and Recommended Dosages (if any)**
For Amnesia	Entire plant	This herb's decoction is used with milk.
For Anorexia	Leaf	Intact portion
For Anxiety	Leaf, Flower	Used for treating neurosis associated with anxiety.
For Arthritis	Entire plant	Entire plant for treating rheumatoid arthritis and osteoarthritis
For Asthma	Leaf	Intact portion

For Blood disorders	Entire plant	The entire plant is utilized
For Bone fracture	Leaf, Flower	Paste made of flowers and leaves that is used to treat fractures
For Bronchitis	Leaf	Intact portion
For Burning sensation	Entire plant	The entire plant is utilized
For Calculi	Leaf	Intact portion
For Constipation	Leaf, Stem	Intact portion used for the management of constipation
For Cough	Leaf	Intact portion used as such
For Dementia[†]	Root, Flower	Administer a paste made of flowers and roots to cure dementia
For Diabetes	Leaf	Leaves paste (100 g) and 3-4 grains of black pepper should be taken once a day.
For Dyspnoea	Leaf	Intact portion
For Dysuria	Entire plant	Plant's jelly (10 grams) and honey (10 grams) are to be consumed three times daily.
For Edema	Entire plant	The entire plant is utilized
For Emesis	Entire plant	The entire plant is utilized

For Enuresis	Entire plant	Plant's powder, Hyoscyamus niger, Prunella vulgaris (1-2gm), and milk (one hundred milliliters) should be taken twice daily.
For Epilepsy[†]	Entire plant	Three times a day, C.P plant’s paste, cumin seeds (l g), and milk
For Gonorrhoea	Entire plant	Plant's jelly (10 grams) and honey (10 grams) are to be consumed three times daily.
For Haemoptysis	Leaf	leaves juice has to be taken three times a day at a quantity of 10 mL.
For Haemorrhoid	Entire plant	The entire plant is utilized
For Headache[†]	Entire plant	Take 3 grams of plant powder, 5 grams of sugar, and 20 milliliters of milk twice a day.
For Hematemesis	Entire plant	The entire plant is utilized
For Hysteria[†]	Entire plant	Intact portion
For Insomnia[†]	Entire plant	The entire plant is utilized
For Leprosy	Entire plant	The entire plant is utilized
For Leucoderma	Entire plant	The entire plant is utilized
For Menorrhagia	Entire plant	Plant's paste consumed with milk.

For Neurological disorders†	Entire plant	A decoction of this herb, combined with cumin and milk, is traditionally employed for treating various neurological and psychological conditions including stress disorders, emotional stress, depression, mental hypersensitivity, memory loss, mental debility, mild convulsions, attention deficit hyperactivity disorder (ADHD), and schizophrenia.
For Polydipsia	Leaf, Stem	Intact sections used to treat extreme thirst
For Pyrexia	Root, Leaf	Intact sections used for relieving fever
For Pyrosis	Leaf, Stem	Intact sections used to treat pyrosis or heartburn
For Scrofula	Entire plant	Decoction used along with cumin and milk
For Sexual disability	Entire plant	Plant (100 g) is mashed with little water and consumed once everyday with sugar or honey, For 21 days.
For Snake bite	Entire plant	The entire plant is utilized
For Stomach Ache	Entire plant	consume one tea spoonful of powdered dried herb.
For Syphilis	Entire plant	Decoction used along with cumin and milk
For Ulcer	Root	Intact portion
For Urinary diseases	Leaf	Intact portion
For Vertigo†	Entire plant	Plant is made into a syrup that is used to treat vertigo.

For Worm infestation	Entire plant	The entire plant is utilized
For Wound	Entire plant	The entire plant is utilized

Neurological conditions potentially treatable with Convolvulus pluricaulis administration

Home grown medication is encountering a noteworthy resurgence because of the mind-boggling results of engineered medication, and the restorative methodology is moving towards elective medication. Plants are utilized as helpful specialists in both coordinated (Ayurveda, Unani) and disarranged (people, ancestral, local) structures. Shankhapushpi, also known as Convolvulus pluricaulis Choisy, CP, is a potent herb that has been used to treat diabetes since ancient times. In the traditional medical system, numerous scientific journals have reported therapeutic activity experimentally and clinically using a variety of dosage forms and derived products. Shankhapushpi is viewed as Medhya Rasayana (memory enhancer) in Ayurvedic texts and has been utilized as a rejuvenator, hostile to maturing, mental energizer, and sedative. The Ayurvedic Pharmacopoeia of India states that the medicinal use of all parts of CP (Convolvulaceae) is permitted. In a publication, the Indian Council of Medical Research established quality standards for CP medication. Even though a lot of research has been done over the past few decades, very little information about the medicinal benefits of CP is available, so it's important to keep track of it. As a result, the current review attempts to examine ancient Ayurvedic literature that has portrayed its medicinal benefits and validated them in light of current experimental and clinical studies.

AIM & OBJECTIVE

Aim

The Aim of this study to protective effect of Convolvulus pluricaulis leaves on streptozotocin and high fat diet induced diabetic nephropathy in wistar albino rat.

Objective

The following goals of the current study are centred on the creation of herbal-based medication delivery systems.

1.To investigate the potential protective effect of Convolvulus pluricaulis leaves against diabetic nephropathy induced by STZ & HFD in wistar albino rats.

2. To determine the effect of Convolvulus pluricaulis on glucose metabolism, lipid profile, renal function, and oxidative stress markers in rat model.

3. The ultimate goal of the research is to determine the potential therapeutic value of Convolvulus pluricaulis in preventing or treating diabetic nephropathy.

Plan of work

The work schedule was established with the following goals and objectives in mind:

- Plant Extract
- Preliminary Phytochemical Screening.
- Administration of a chemical combined with a high-fat diet induces T2DN.
- Experimental design.
- Blood Collection.
- Parameter –
    1. To determine blood glucose level
    2. Determination of body weight
    3. Estimation of serum creatinine and albumin level
    4. Determination of urine
    5. Determination of lipid profile
    6. Determination of oxidative stress
- Examination of histopathology.
- Statistical evaluation.
- Submission of thesis.

MATERIAL AND METHODOLOGY

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7. Method

7.1 COLLECTION OF PLANT EXTRACT

Hiya India Biotech Pvt Ltd., B-51, Okhla Industrial Area, Phase -1, New Delhi –110020, provided the Convolvulus pluricaulis extract that was purchased.

7.2 Pre-liminary phytochemical screening

An examination of the extract's phytochemical makeup was done

7.2.1. Test for alkaloid -

Before being filtered, a small amount (3-5 g) of the leaves of a plant extract was treated with a few droplets of diluted hydrochloric acid (HCl). After the filtrate was treated with Mayer's reagent (mercuric chloride and potassium iodide), the emergence of a yellowish buff colored precipitate indicated a positive test for alkaloids.

After a little amount of plant leaf extract was treated with a few droplets of diluted HCl (basic bismuth carbonate,sodium iodide, ethyl acetate, and glacial acetic acid), Dragendroff's reagent was added to the filtrate. An orange-brown precipitate indicated the presence of alkaloids.

A reddish brown precipitate formed after Wagner's reagent (Iodine &
potassium iodide) was applied to a little amount of plant leaf extract after being treated with a few drops of diluted HCL, indicating the presence of alkaloids.

When a little amount of diluted HCl was added to plant leaf extract and the mixture was filtered, Hager's reagent (an aqueous solution of picric acid) was added, and a reddish precipitate formed that showed the presence of alkaloids

7.2.2 Test for amino acids –

In a second experiment, a tiny volume of plant leaf extract was treated using Ninhydrin (tri-ketohydrindene hydrate) at a pH of 4 to 8 after being diluted in a few milliliters of distilled water. The lack of purple coloring suggested the absence of amino acids.

7.2.3 Test for flavonoids and glycosides –

A tiny amount of leaf extract was dissolved in ethanol, and after that, it was hydrolyzed with 10% sulfuric acid (H2SO4) and allowed to cool. The mixture was extracted using diethyl ether, divided into three portions, and then put into three separate test tubes. Ammonia solution, 0.1M sodium hydroxide, and 1ml of diluted sodium carbonate were each added to the first, second, & third test tubes. As the amount of flavonoids in each test tube rose, they all became yellow.

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7.2.4 Test for steroids and triterpenoids –

Libermann-Burchard reaction: 10 mg of the plant extract were dissolved in 1 ml of chloroform, and the mixture was then added to 2 ml of concentrated H2SO4 and 1 ml of acetic anhydride. The presence of steroids and triterpenoids was indicated by a reddish violet band at the junction of the two distinct layers.

Salkowski test: When conc, H2SO4 was added to a chloroform sol. (1 ml) of the extract, a reddish-blue color appeared in the layer of chloroform and green fluorescence in the acid layer, confirming the presence of steroids.

7.2.5 Test for reducing sugar –

Before being filtered, a very little amount of plant extract—between 2 and 5 mg—was diluted in distilled water. When the filtrate was heated briefly and combined with an equivalent volume of Benedict's reagent, a brick-red precipitate was produced, indicating the presence of reducing sugars. In a test tube that had been briefly heated, a little quantity of plant extract (between 2 and 5 mg) was dissolved in the least amount of distilled water, filtered, and added to an equivalent volume of Fehling's solutions. As a result, a brick-red color developed, signifying the existence of reducing sugars.

7.2.6 Test for gums –

Small amounts of plant extract were filtered after being severely diluted in distilled water. The filtrate was treated first with an equivalent amount of concentrated H2SO4 and then with a 15% alcoholic solution of naphthol (Molish's reagent). The junction of the extract and sulphuric acid layer produced a reddish-violet ring, indicating a favorable result for gums according to the Molish experiment.

7.2.7 Test for tannins –

To demonstrate the presence of tannins, a 10% aqueous potassium dichromate solution was added to a tiny quantity of plant extract, which was then diluted in a small amount of distilled water, filtered, and the filtrate was then treated with the precipitate, which became yellowish-brown. When the aforementioned filtrate was allowed to react with a 10% aqueous lead acetate solution, a yellow precipitate appeared, signifying that the tannin test was successful. Once more, when 1 ml of 5% ferric chloride solution was added to the previously stated extract filtrate, the reaction produced a greenish-black color that showed the presence of tannins.

7.2.8 Test for saponins –

After a little quantity of plant extract was dispersed in a small amount of distilled water, the mixture was stirred for fifteen minutes in a graduated cylinder. Sluggish foam development suggests the presence of saponins.

7.3 DPPH Assay for Antioxidant Activity Evaluation (Diphenyl picryl hydrazyl)

Principle:

DPPH (Diphenyl picryl hydrazyl) is a stable free radical that has a noticeable ESR signal. Its interaction with antioxidants may be the cause of the 517 nm absorbance drop. It is well accepted that when DPPH accepts an electron or hydrogen radical, it changes into a stable diamagnetic molecule. Because of its odd electron, the ethanol sol. of DPPH in the purple hue solution shows a notable absorption at 517 nm. When suitable reducing chemicals react with

DPPH radicals, the color of the solution is lost stoichiometrically proportionate to the quantity of electrons taken up.

DPPH + AH — DPPH-H + A

Instruments:

Shimadzu UV Visible spectro photometer, Model 1800

Reagents:

DPPH solution: 0.1 mM in ethanol or methanol

Procedure:

1. Prepare DPPH stock solution (4 mg/100 mL ethanol)
2. Mix 1 mL of test sample (various concentrations) with 4 mL DPPH solution
3. Prepare control: 1 mL ethanol + 4 mL DPPH solution
4. Incubate mixtures in dark for 30 minutes
5. Measure absorbance at 517 nm
6. Calculate inhibition percentage: **% inhibition = [(Control - Test) / Control] x 100**
7. Determine IC50 using linear regression analysis
8. Use Vitamin C as standard reference

Note: IC50 represents the sample concentration required for 50% reduction in DPPH absorbance.

7.4 Experimental Animal

The study employed adult male Wistar rats weighing 150 – 200gm and aged 4- 8 weeks. The rats were kept in our own Innovative College of Pharmacy's main animal facility in Greater Noida , India. They were kept in the animal home under regular laboratory conditions. Rats were housed in polyacrylic cages with a maximum of four animals per cage, air condition , and natural light and dark cycles,with a relative humidity of 50% to 70% and a temperature of 25 degrees Celsius (+/- 2 degrees Celsius). The study protocol was accepted by the

institutional animal ethics committee and complied with the guidelines set out by the Committee for the Control and Supervision of Experiments Animals. rules for the handling and use of research animals in experiment.

7.3.1 Experimental requirement:

Commercial kits for biochemical estimation

1. Glucose kit
2. Total cholesterol kit
3. Triglyceride kit
4. Albumin kit
5. Total Protein
6. Creatinine
7. Blood urea nitrogen kit
8. Nitric oxide

7.3.2 Apparatus required:

1. Semi auto analyzer.
2. Syringe and needle
3. Funnel
4. Centrifuge
5. Oral gavage feeding needle
6. Glass rod
7. Petri dish
8. China dish
9. Analytical balance

10 Weighing machine

11. Rotary evaporator
12. Cellulose syringe filter
13. EDTA tube

7.3.3 Chemical requirement:

1. Alloxan
2. EDTA

3. Ethanol
4. NaCl
5. Diethyl ether
6. Alcohol
7. Heparin

7.4 Drug used:

The standard medication metformin was obtained by the vipra medicose and utilized in the research.

7.5 Albino Wistar rat:

Method of oral administration

The oral administration of drug suspensions to rats was performed using a 1 mL tuberculin syringe fitted with an oral feeding tube and needle. The procedure involved securely holding the rat with one hand while carefully inserting the oral feeding needle directly into the animal's esophagus. The drug suspension was then gently administered by depressing the plunger. To ensure complete delivery of the dose, 0.2-0.3 mL of distilled water was administered immediately following the drug suspension. This method allowed for accurate and controlled delivery of the medication directly into the rat's digestive system.

7.6 Blood Collection:

Blood collection and processing were performed as follows: A retro-orbital puncture was used to obtain the blood sample from the animal. The collected blood was then centrifuged at 3000 rpm for 15 minutes to separate the serum. Once isolated, the serum sample was stored at -80°C for future analysis. This method ensures efficient blood collection, serum separation, and proper preservation of the sample for subsequent use.

7.7 Experimental group

Following successful diabetes induction, the rats were divided into six groups, each consisting of eight animals.

Table 7.7 Experimental group

S.No	Groups	Dose	Route & Duration	Duration (Months)
1.	**Control Group**	Vehicle 0.5ml/day	p.o., daily for 15 days	6
2.	Diabetic group	High fat diet (normal diet) & at the end given a low dose of 35mg/kg/bw STZ	8HFD (3 Week) daily via p.o., & at the end given low dose STZ via i.p	6
3.	Standard group	Metformin 200 mg/kg/b.w		6
4.	Test Group -1	Diabetic rat fed Convolvulus pluricaulis leaves extract 100mg/kg/b.w	p.o., for 15 days	6
5.	Test Group -2	Diabetic rat fed Convolvulus pluricaulis leaves extract 200mg/kg/b.w	p.o., for 15 days	6
6.	Test Group -3	Diabetic rat fed Convolvulus pluricaulis leaves extract 300mg/kg/b.w	p.o., for 15 days	6

7.8 Induction of T2DN by administration of chemical + high fat diet:

The experimental diabetes model was established as follows:

1. Diet modification: A high-fat diet comprising 49.5% of the total dry diet was administered, contributing 72% of the total energy content.
2. STZ administration: After 3-4 weeks of dietary modification, rats received a low dose of streptozotocin (STZ) at 35 mg/kg body weight, intraperitoneally. STZ was dissolved in 0.01M citrate buffer (pH 4.5).
3. Diabetes confirmation: Fasting blood glucose levels were measured, with levels ≥200 mg/dL indicating successful diabetes induction.
4. Observed effects: This protocol initially causes beta-cell failure, leading to insulin resistance and decreased plasma insulin concentration, mimicking human diabetes. Renal damage, evidenced by increased albuminuria and pathological changes, typically manifests 4 weeks post-STZ injection.

7.9 Toxicological evaluation of Convolvulus pluricaulis leaves extract:

7.9.1 Acute toxicity study of Convolvulus pluricaulis leaves extract in Wistar rats

Purpose and rationale

Acute toxicity studies were conducted in accordance with OECD-423 guidelines, utilizing the acute toxic class method. This approach employs a stepwise procedure with three animals of the same sex per stage, typically requiring 2-4 steps to fully assess a substance's acute toxicity. The method is grounded in biometric evaluations using fixed, appropriately spaced doses, allowing for efficient substance classification and risk assessment. By observing mortality and/or morbidity in test animals, researchers can rank and classify the substance according to the Globally Harmonized System (GHS) for acute toxicity. This standardized protocol, which uses predefined dose levels, provides a systematic and ethical means of evaluating a substance's toxicity profile, yielding valuable data for safety assessments and regulatory compliance. The stepwise nature of the process ensures that the minimum number of animals is used while still obtaining reliable toxicological information.

Procedure:

Three female Wistar albino rats were randomly selected for the study. These animals were housed in their cages for a minimum of five days prior to dosing to allow for acclimatization to the laboratory environment. Throughout this period and overnight before dosing, the rats had unrestricted access to water. The experiment began with an initial oral administration of C. pluricaulis leaves extract at a dose of 2000 mg/kg body weight. This protocol ensures that the animals are well-adjusted to their surroundings and in optimal condition before the introduction of the test substance, thereby minimizing stress-related variables that could affect the study results. The animal's general behavior was observed continuously for the first four hours, intermittently for the next six hours, and once again 24 hours and 48 hours after the administration of the drug. For observation, the following parameters were used: depression (sedation, anesthesia, loss of reflex, analgesia), stimulation (hyperactivity, irritability, tremor, convulsion, piloerection), and stimulation (respiration, diarrhea, salivation, and motor activity). Seven days were spent observing the death. The dose was considered toxic if two-thirds or three-quarters of the animals died as a result. However, the same dose was administered once more to confirm the toxic effect if only one rat out of three animals died (OECD, Guideline 423. 2001).

7.10 Body weight change

An electronic weighing balance was used to measure each animal's weight on day 1, day 14th, day 21th, and day 25th.

7.11 Estimation of Blood glucose level:

During the course of the 45-day treatment, the semi-auto analyzer was used to measure the blood glucose level on the first day, the 14th day, and the 25th day.

7.12 Estimation of serum lipid profile:

A comprehensive lipid profile analysis was conducted on the serum samples. Total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides (TG) were directly measured using a commercial assay kit, providing quantitative values for these key lipid parameters. To complete the lipid profile, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels were calculated using established formulas. LDL was determined by subtracting HDL and one-fifth of the TG value from the total cholesterol

(LDL = TC - HDL - TG/5),

while VLDL was estimated as one-fifth of the triglyceride value (VLDL = TG/5). This combined approach of direct measurement and calculation allowed for a thorough assessment of the subjects' lipid status, providing valuable insights into cardiovascular risk factors and overall metabolic health.

7.13 Estimation of glucose concentration by GOD/POD Method:

Principle:

TThe glucose measurement process utilizes an enzymatic reaction cascade. Initially, glucose oxidase (GOD) catalyzes the oxidation of glucose in the specimen, converting it to gluconic acid while simultaneously producing hydrogen peroxide (H2O2). This reaction occurs in the presence of atmospheric oxygen. Subsequently, the generated H2O2 participates in a second reaction, catalyzed by peroxidase (POD). In this step, H2O2 oxidatively couples 4-aminoantipyrine with phenol, resulting in the formation of a red quinoneimine dye. The intensity of this colored product is directly proportional to the glucose concentration in the original specimen. Quantification is achieved through colorimetric measurement at a wavelength of 540 nm, allowing for precise determination of glucose levels in the sample. The amount of glucose present in the specimen directly correlates with the color's intensity observation, the following parameters were used: depression (sedation, anesthesia, loss of reflex, analgesia), stimulation (hyperactivity, irritability, tremor, convulsion, piloerection), and stimulation (respiration, diarrhea, salivation, and motor activity). Seven days were spent observing the death. The dose was considered toxic if two-thirds or three-quarters of the animals died as a result. However, the same dose was administered once more to confirm the toxic effect if only one rat out of three animals died (OECD, Guideline 423. 2001).

Glucose + O_2 +H_2O Glucose Oxidase(GOD) Gluconic acid + H_2O_2

Phenol +H_2O_2 + 4-aminoantipyrine Peroxidase(POD) Red quinoneimine dye + H_2O

Requirements;

specimen:

Plasma or serum without hemolysis. Because of its ability to antaglycolyze, sodium fluoride was preferred as an anticoagulant.

Glucose Assay Protocol:

Materials:

- Reagents:
    1. Glucose standard (100 mg/dL)
    2. GOD-POD reagent: Enzyme mixture containing glucose oxidase (GOD), peroxidase (POD), 4-aminoantipyrine, phenol, phosphate buffer (pH 7.0), stabilizers, and activators
- Equipment:
    1. Test tubes
    2. Pipettes with disposable tips
    3. Test tube rack
    4. Water bath
    5. Colorimeter

Procedure:

1. Preparation: Label three clean, dry test tubes as Blank (B), Standard (S), and Test (T).
2. Sample Distribution: Pipette reagents and samples into labeled tubes as follows:

Table 7.13 Estimation of glucose concentration

	Blank	**Standard**	**Test**

GOD–POD Reagent	1ml	1ml	1 ml
Distilled water	10micro liter	-	-
Glucose standard	-	10micro liter	-
Sample	-	-	10 micro litre

3. After thoroughly mixing, the mixture was incubated for 10 minutes at 37 degrees Celsius or for 30 minutes at ambient temperature (25 degrees Celsius).

4. Measured the absorbance of the blank and the standard at 540 nm (green filter) throughout a 60-minute period.

Calculation;

Utilize the formula below to determine the specimen's blood glucose concentration.

$$\text{Conc. of glucose in the specimen(mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 100$$

7.14 Estimation total cholesterol

Principle ; Elevated cholesterol levels are associated with various medical conditions, including:

1. Cardiovascular disease (atherosclerosis)
2. Kidney disorders (nephrosis)
3. Endocrine dysfunction (diabetes mellitus and myxoedema)
4. Liver problems (obstructive jaundice)

In hot acidic medium, a cholesterol oxidizes the ferric ion into a brown complex that absorbs at 530 nm and has an intensity that is directly proportional to cholesterol concentration. Hyperthyroidism, some anemias, malabsorption, and wasting syndrome all result in lower levels. Cholesterol from HDL: Heart disease and other atherosclerotic conditions are more likely to occur in people with lower levels.

Table 7.14 Method for total cholesterol

Blank (ml)	**Blank (in ml)**	**STD (in ml)**	**Test (in ml)**
Cholesterol reagent No.1	5.0	5.0	5.0
Distilled water	5.00	_	_
Standard reagent No.2	_	5.00	_
Sample	_	_	5.00

After a thorough 20 seconds of mixing, the contents were immediately submerged in boiling water for precisely 90 seconds before being cooled for five minutes under running tap water. At 530 nm, the solution read. For thirty minutes, the final color remains stable.

Calculation:

$$\textbf{Total-cholesterol(mg/dl)} = \frac{\textbf{O.D of Test}}{\textbf{O.D of STD}} \times 200$$

7.15 Estimation for HDL Cholesterol:

Step 1: Separation

- Mix 0.2 milliliters (mL) of serum with 0.1 mL of HDL reagent.
- Mix the solution thoroughly.
- Wait for ten minutes.
- Centrifuge the mixture at 3,000 revolutions per minute (RPM) to separate the components.
- Collect the clear, upper liquid (supernatant) for further analysis in step 2.

Step 2: Cholesterol Level Measurement

- Prepare a mixture of the separated supernatant and additional reagents (likely specified elsewhere).
- Incubate the mixture for 10 or 15 minutes at either 37°C or 30°C.
- After incubation, measure the absorbance of the solution at 510 nanometers (nm) wavelength (acceptable range is 500-546 nm) or using a green filter. You'll likely need an HDL blank for comparison during this reading.
- The final color developed in this step is stable for up to 30 minutes if kept away from bright light.

Table7.15 Estimation for HDL Cholesterol

Blank (in ml)	**Blank (in ml)**	**STD (in ml)**	**Test (in ml)**
Cholesterol reagent No.- I	5.0	5.0	5.0
HDL Reagent	0.2	0.2	-
Supernatant from STEP I	-	-	0.2
Cholesterol Std (200 mGs/dL)	-	0.02	-

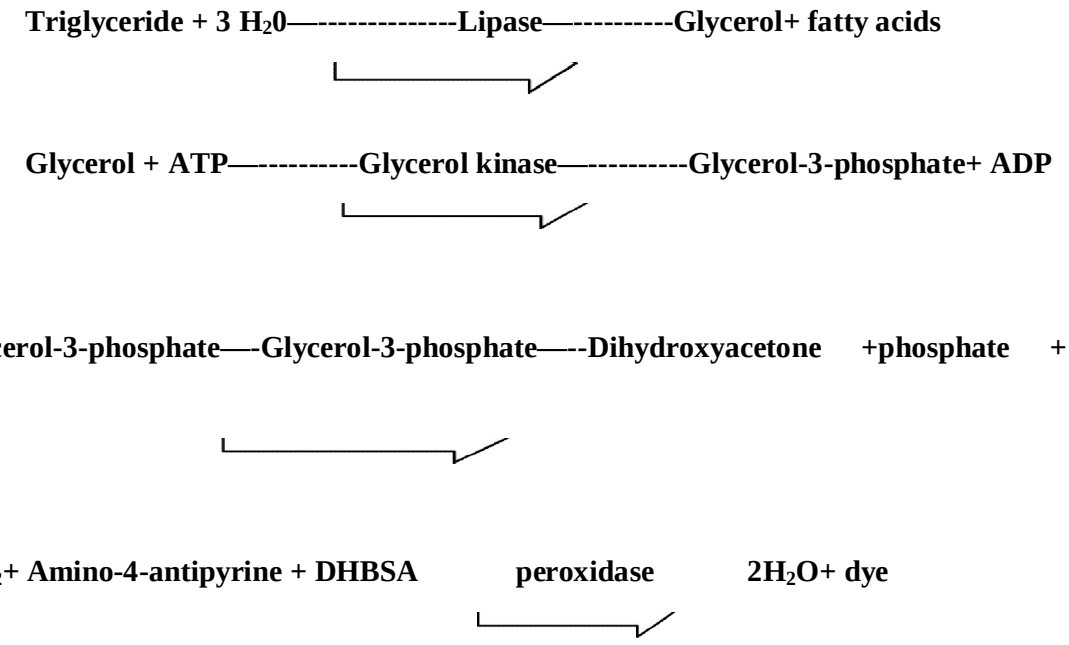
Calculation;

HDL cholesterol(mg/dl) = $\frac{\text{O.D. of Test HDL – O.D. of HDL Blank}}{\text{O.D. of STD HDL – O.D. of HDL Blank}}$ x 60

7.16 Estimation triglyceride

Principal; Lipoprotein lipase breaks down plasma triglycerides into glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate using ATP and glycerol kinase. This compound is then oxidized by glycerol phosphate oxidase, resulting in the production of

hydrogen peroxide. When DHBSA and amino-4-antipyrine are oxidized in the presence of peroxidase and hydrogen peroxide, a rose-colored dye with a wavelength of 500 nm is formed. The intensity of this color is directly proportional to the concentration of triglycerides in the sample.



Procedure Using a Triglyceride solution kit, plasma total Triglyceride levels were measured in the samples.

1. poured into three test tubes.

2. Blended well. for ten minutes at 370 C in the incubator.

3. Against a blank, read at 500 nM. Added 2 ml refined water for 3 ml optical cuvette. When kept away from bright light, the final color stays the same for at least 30 minutes.

Table 7.16 Estimation triglyceride

Blank (ml)	STD (ml)	Test (ml)

0.05	-	-
-	0.05	-
-	-	0.05
1.00	1.00	1.00

Calculation;

The plasma concentration of triglyceride will be determined as follows

Triglyceride (mg/dl) = **OD of the STD/ OD of the test × 200**

O.D of Test = Absorbance of sample solution

O.D. of STD = Absorbance of standard solution

7.17 Estimation of albumin

Principle ; Egg whites is responding with BCG reagent containing bromocresol green, succinate cushion keeping up with the pH of 4.2 and Brij 35 which increment the awareness of the response. The color blue-green is produced by the reaction mixture. The amount of albumin in the solution determines the intensity of the color.

Reagent

1.BCG Reagent:

Brij 35

Bromocresol Green

Succinate Buffer PH 4.2

2.Standard (Albumin)

Procedure ; Using an albumin solution ready-to-use reagent, the levels of albumin in the samples will be analyzed.

Table 7.17 Method for determination of albumin

Sr. no.	Reagents	Blank (in ml)	Standard (in ml)	Test (in ml)
1	Reagent 2	3.0	3.0	3.0
2	Standard Reagent 4	-	0.02	-
3	Serum sample	-	-	0.02

For five minutes, the solutions were incubated at 37°C. Working reagent solution was used as a blank for the absorbance measurement at 620 nm. The amounts of albumin in the samples were calculated using the formula below:

Cu = [Au / As] x Cs

Cu = Conc. of albumin in serum sample Au = Absorbance of sample

As = Absorbance of standard

7.18 Estimation of total protein

Principal; The proteins gave off a violet color when alkaline cupric sulfate was present, and their absorption was highest at 550 nm. Color intensity is inversely proportional to protein content. Hypoalbuminemia and malnutrition are linked to lower levels. Hyper globulinaemia and dehydration result in elevated levels.

Cs = Conc. of standard of sample

Reagent Supplied:

1. Biuret Reagent	100ml
Sodium and Potassium tartarate	21mMols/L
Cupric Sulphate	6mMols/L
Potassium iodide	6mMols/L
Sodium hydroxide	750mMols/L
2. Standard (Albumin)	2.5ml
Value of albumin standard	8.0gm/dl

Procedure The amount of total protein in the samples was determined using the ready-to-use reagent total protein solution.

7.19 METHOD FOR DETERMINATION OF TOTAL PROTEIN

1. 3 Test tubes with a pipette

2. Blend well. at RT, incubate for five minutes.

3. Read through a GREEN or Blank filter at 550 nM (550+20).

4. The final color lasts for 30 min

Table 7.19 Determination of total protein

	Blank (in ml)	STD (in ml)	Test (in ml)
Reagent No.-2	3.00	3.00	3.00
Standard Reagent No. -3	--	0.1	--
Sample	--	--	0.1

Calculation:

Total protein: OD of std/OD of test ×C

C = Conc. of standard of sample

7.20 Estimation of creatinine

Principle: Alkaline picrate and creatinine combine in a protein-free solution to generate a red complex that may be measured colorimetrically.

Procedure: Diagnostics kit provided the reagents kit that was used to measure plasma creatinine in the samples. The working reagent was made as directed by the manufacturer. The procedure consisted of two steps:

The material was deproteinized using Step A. For precisely one minute, 0.5 milliliters of plasma, 0.5 milliliters of filtered water, and 3 milliliters of picric acid were placed in a boiling water bath. centrifuged, then quickly let cool using tap water.

Step B- For color development

Table 7.20 Measurement of creatinine

	Blank (ml)	STD (ml)	Test (ml)
Purified water	0.5	-	-
Working Standard	-	0.5	-
Filtrate (From step-A)	-	-	2.0
Reagent 1: Picric cid	1.5	1.5	-
Reagent 2: NaOH, 0.75 N	0.5	0.5	0.5

Mixing all reagents thoroughly, the mixture was kept at room temp. for 20 minutes. At 520 nm, the absorbance was measured.

Calculation: Concentration of the samples was measured using following formula-

Plasma Creatinine(mg/dl)= O.D of test / OD of std × 3

O.D. of Test = Absorbance of sample solution

O.D. of STD = Absorbance of standard solution

7.21 Estimation of urea nitrogen

Principle; A pink-colored compound is produced when diacetylmonoxime and urea react in a warm acidic medium with ferric ions. Urea concentration is inversely correlated with this color's intensity. This reaction is catalyzed by thiosemicarbazide, which helps prevent plasma deproteinization.

Procedure ; The following was done with the reagent kit for estimating the plasma urea nitrogen level:

Table 7.21 Estimation of urea nitrogen

	Blank (ml)	STD (ml)	Test (ml)
Std reagent	-	0.02	-
Sample-	-	-	0.02
Reagent No.- 1	1.50	1.50	1.50
Reagent No.- 2	1.50	1.50	1.50

After thoroughly combining all reagents, the mixture was immediately immersed for ten minutes in boiling water., cooled for five minutes in running water. At 540 nm, absorbance was measured.

Calculation: Concentration of samples was measured using following formula-

$$\text{Urea Nitrogen (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of STD}} \times 18.64$$

O.D. of Test = Absorbance of sample solution

O.D. of STD = Absorbance of standard solution

7.22 Estimation of albumin excretion rate(AER)

Purpose and rationale Because albumin concentration on its own is not reliable, a ratio is calculated: 2.5 indicates normal, 2.5-30 indicates microalbuminuria, and a ratio greater than 30 indicates proteinuria.

Principle; A blue-purple complex is formed when protein, pyragallol red, and molydbate combine in an acidic medium. The intensity of color generated is closely correlated with the amount of protein present in the sample.

Protein + Pyrogallol Red + Molydbate Acidic Medium blue purple colored

Procedure Pipette contents into a test tube that is dry and clean and labeled "Blank," "Standard," and "Test":

Table 7.22 Estimation of albumin excretion rate(AER)

Addition sequences	B (in ml)	S(in ml)	T(in ml)
Micro Protein Reagent-	1.0	1.0	1.0
Distilled Water-	0.01	-	-
Micro Protein Standard (S)-	-	0.01	-
Sample-	-	-	0.01

Mix well and let sit at room temperature for five minutes. Measure the absorbance of the standard and test sample at 600 nm (Hg 623 nm)/Red in 30 minutes, comparing it to the blank..

calculation;

Micro Protein in mg/dl = Abs.T / Abs.S ×1000

For determination of AER per day the measurement of microalbuminuria (mg/L) was done and calculated as per following formula-

AER (mg/day) = 24 h Urine vol. (ml) × Conc. of microalbuminuria / 1000 ml

UAER (µg/24h) = urinary albumin (µg/mL) × 24-h urine volume (mL).

7.3 EVALUATION OF OXIDATIVE STRESS IN RAT KIDNEY

Preparation of tissue homogenate:

The kidneys were rapidly removed, washed in cold saline, and weighed. A 10% w/v homogenate was prepared using 0.15 M KCl by centrifuging at 800 g for ten minutes at 4°C. The supernatant obtained from this homogenate was used to estimate catalase and lipid peroxidation (MDA) values. Additionally, SOD and glutathione levels were assessed from the supernatant after centrifugation at 1000 g for 20 minutes at 4°C. Another kidney from each animal was sent to the AFMC histopathology center in Pune for further processing in a 10% formaldehyde solution. Antioxidant parameters were determined based on protein examination in renal tissues.

7.3.1 Estimation of tissue protein

Principle: The Lowry protein assay is a biochemical method used to determine the total protein content in a solution. Colorimetric techniques can measure protein concentration by detecting color changes proportional to protein levels. Named after biochemist Oliver H. Lowry, this method relies on the reactivity of peptide nitrogen with copper ions under alkaline conditions. The subsequent reduction of Folin-Ciocalteu's phosphomolybdic-phosphotungstic acid to heteropolymolybdenum blue occurs through copper-catalyzed oxidation of aromatic acids. The assay's accuracy depends on maintaining a pH between 10 and 10.5. It is sensitive to low protein concentrations, typically using 0.005 to 0.10 mg of protein per milliliter, with a range from 0.10 to 2 mg per milliliter. However, its drawback lies in the limited pH range for accuracy. Substances like drugs, lipids, sugars, salts, nucleic acids, and amino acid derivatives can interfere with the Lowry method. Thiol compounds,

zwitterionic buffers, nonionic buffers, and ammonium ions may also disrupt the reaction. Prior to using Lowry assays, it's essential to remove or dilute these interfering substances.

Reagents:

A - 2% Na2CO3 in 0.1 N NaOH

B - 1% NaK Tartrate in H_2O

C - 0.5% CuSO4.5 H_2O in H_2O Reagents A, B and C may be stored indefinitely]

D - 48 ml of A, 1 ml of B, 1 ml C

E - Phenol Reagent - 1 part Folin-Phenol [2 N] : 1 part water

[BSA Standard - 1 mg/ ml; Bovine Serum Albumin: 5 mg in 5 ml of water [1 µg / µl]. Freeze 1 ml aliquots.

Procedure:

1. A rack with eleven sets of three 16 x 150 mm test tubes was prepared.
2. Bovine serum albumin (BSA) was added to these tubes in varying amounts (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µl).
3. Two milliliters of solution D were added to each test tube, followed by incubation for ten minutes at room temperature.
4. Each tube contained 0.2 milliliters of a diluted Folin-phenol solution.
5. Vortexing was performed immediately after adding the Folin-phenol solution.
6. The samples were incubated for 30 minutes at room temperature.
7. Absorbance at 600 nm was measured for each sample.
8. A standard curve was generated by plotting absorbance against protein concentration (mg protein).

Calculation: Each renal sample's protein content was estimated using a trend line equation from the graph's plot.

7.4 Body and kidney weight change

The body weight of each person was measured using a digital balance on the first, 14th, 21st, and 25th days, and the changes in body weight were calculated. After the animals were sacrificed, one kidney was quickly weighed following decapsulation, blotting on

filter paper, and rinsing in chilled saline. To standardize the total kidney weight, the kidney/body-weight ratio was used.

Relative kidney weight (%) = [Absolute kidney weight/Body weight at sacrifice] × 100

7.5 Histopathological Examination

At the conclusion of the trial , all the rats were put to death using excessive anesthetic, and the kidneys were removed from each group and cleaned with ice and saline. As soon as possible, the kidney tissues were preserved in10% buffered neutral formalin solution and prepared for histological analysis.

Result

8.1 Initial phytochemical analysis of the leaves

The leaves of Convolvulus pluricaulis were collected and analyzed to assess various normalization parameters. The preliminary phytochemical analysis of the drug revealed the presence or absence of specific phytochemicals through tests conducted with various extracts. The results of the phytochemical testing showed whether alkaloids, saponins, glycosides, flavonoids, steroids ,polysaccharides, and tannins were present or not as detailed in Table 8.1.

Table 8.1 phytochemical analysis for different solvent extract of leaves of Convolvulus Pluricaulis

S. No	Natural products	Tests performed	Result

1.	Alkaloids	Dragendorff's test	+ve
2.	Amino acids	Ninhydrin	+ve
3.	Flavonoids	Shinoda test	+ve
4.	phytosterols/Steroid	Liebermann - Burchard reagent/ Salkowski test	+ve
5.	Reducing Sugar/ carbohydrates	Fehling's solutions / Benedict's reagent	+ve
6.	Gums	Molisch's test	+ve
7.	Tannins	Lead acetate	+ve
8.	Saponins	Foam test	+ve

8.2 An Initiating Phytochemical Screen:-

Alkaloids, phenolic compounds, flavonoids, tannins, terpenoids & saponins were among the several phytoconstituents found in the methanolic C. pluricaulis extract, according to the results of the phytochemical screening.

8.3 Result for Radical Scavenging Activity methods:

Natural antioxidants found in plants help mitigate damage from oxidative stress, leading to growing interest in researching their antioxidant properties. This study assessed the antioxidant activity of methanolic extract of Convolvulus pluricaulis by comparing it with a standard drug using DPPH, hydrogen peroxide, and hydroxyl radical scavenging assays, and discussed the observed effects.

Table 8.3 Concentration-dependent percent inhibition of DPPH radical by methanolic extract of *C. pluricaulis* and ascorbic acid in in-vitro studies

C. pluricaulis	**Percentage inhibition of** DPPH **radical** **Quantity of extract/ ascorbic acid in micrograms (µg/ml)**				
	20	**40**	**80**	**100**	**150**
Methanolic extract	12.21±0.64	33.7±0.47	57.4±0.43	68.3±1.54	81.1±0.62
Ascorbic acid	34.17±0.41	61.72±0.62	78.42±1.03	86.4±0.77	96.1±0.42

8.3 Urine and Blood glucose levels are affected by methanolic C. pluricaulis extract:

When contrasted with the ordinary benchmark group, the diabetes gathering's fasting blood glucose levels were essentially higher after STZ infusion. Moreover, rodents treated with STZ had significantly higher pee glucose levels. When contrasted with diabetic control, the utilization of methanolic concentrate of C. pluricaulis at different portions alone or related to glibenclamide brought about a moderate and huge reduction observed in fasting blood glucose & urine glucose levels . The findings suggest that methanolic extract of C. Pluricaulis has the potential to gradually lower urine and blood glucose levels.

Table 8.3.1: Effect of Methanolic C. pluricaulis extract on Urine Glucose Level compared with STD drug.

Week	**Control group**	**Diabetic group**	**Metformin 200 mg/ kg/ b.w**	**C.pluricaulis 100 mg/ kg/ b.w**	**C.pluricaulis 200 mg/ kg/ b.w**	**C. pluricaulis 300 mg/ kg/ b.w**
0 Week	1.73 ± 0.34	1.85 ± 0.37	2.21 ± 0.18	1.64 ± 0.17	1.84 ± 0.36	2.27 ± 0.24
Week 1	1.88 ± 0.27	53.21 ± 1.45*	49.56 ± 1.24**	58.37 ± 2.64**	54.13 ± 0.88**	44.64 ± 3.54**
Week 2	2.03 ± 0.38	69.41 ± 1.42*	46.41 ± 0.54**	51.28 ± 0.87**	46.31 ± 0.46**	38.13 ± 0.44**
Week 3	2.15 ± 0.35	71.46 ± 1.24*	42.23 ± 0.42**	47.66 ± 0.33**	40.22 ± 0.32**	31.94 ± 0.38**
Week 4	1.86 ± 0.40	78.64 ± 1.25*	35.31 ± 0.44**	43.25 ± 0.18**	32.81 ± 0.27**	25.67 ± 0.28*

All the attributes are expressed as mean ±SD. * $P < 0.05$ for comparison with normal control, ** $P < 0.05$ for comparison with diabetes control.

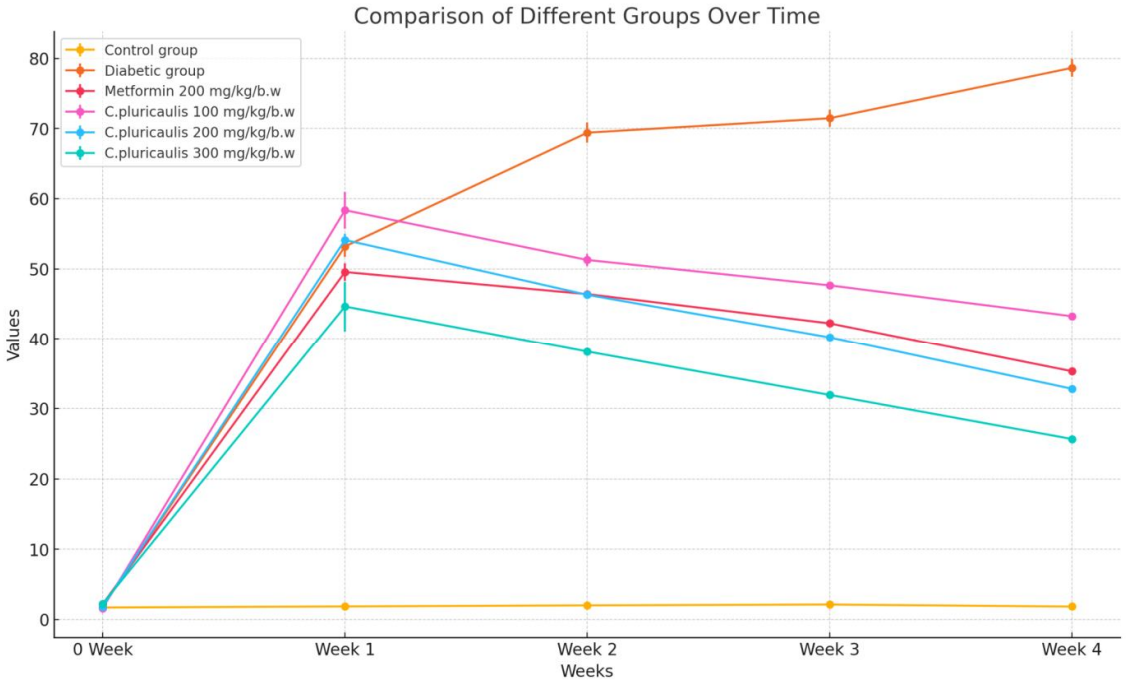


Fig 8.3.1: Effect of Methanolic C. pluricaulis extract on Urine Glucose Level compared with STD drug.

All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control

Table 8.3.2: Effect of Methanolic C. pluricaulis extract on Blood Glucose Level compared with STD drug.

Time	**Control group**	**Diabetic group**	**Metformin 200 mg/kg/b.w**	**C.pluricaulis 100 mg/kg/b.w**	**C.pluricaulis 200 mg/kg/b.w**	**C.pluricaulis 300 mg/kg/b.w**
0 Week	93.7 ± 2.71	95.5 ± 3.21	96.1 ± 2.22	99.1 ± 2.52	95.2 ± 3.63	93.1 ± 2.73
48 Hours	94.2 ± 2.73	532.6 ± 3.52*	551.2 ± 2.81**	556.7 ± 2.64**	557.9 ± 4.21**	532.6 ± 10.64**

Week 1	95.7 ± 1.73	541.9 ± 2.55*	504.1 ± 1.83**	524.6 ± 1.83**	498.6 ± 4.62**	478.1 ± 8.25**
Week 2	94.2 ± 2.23	548.3 ± 3.11*	457.2 ± 2.53**	491.3 ± 3.71**	481.3 ± 3.73**	414.8 ± 4.81**
Week 3	94.3 ± 1.34	552.7 ± 3.14*	413.3 ± 1.92**	475.8 ± 3.81**	432.9 ± 3.94**	354.7 ± 7.54**
Week 4	92.8 ± 1.17	563.2 ± 4.73*	361.8 ± 1.54**	421.6 ± 1.73**	382.1 ± 4.24**	288.5 ± 4.54**

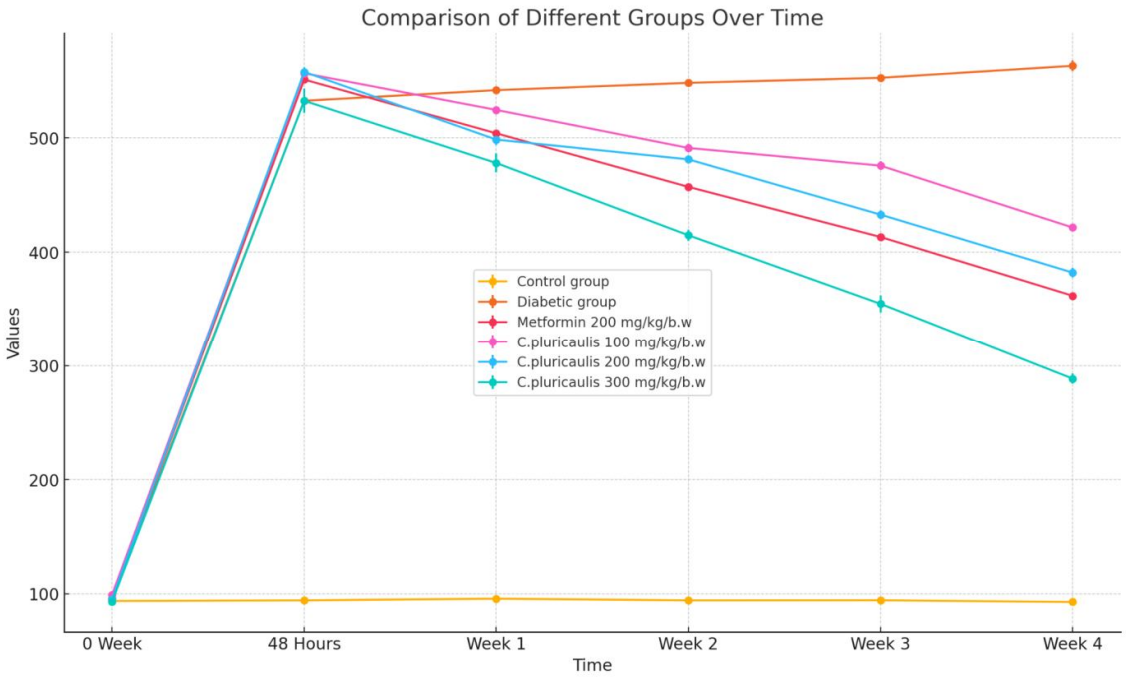


Fig 8.3.2 : Effect of Methanolic C. pluricaulis extract on blood Glucose Level compared with STD drug.

All the attributes are expressed as mean ±SD. * $P < 0.05$ for comparison with normal control, ** $P < 0.05$ for comparison with diabetes control.

8.4 Changes in body weight

Differences in body weight of normal and streptozotocin induced rats (standard drug, plant extract- 100, 200, 300 mg/kg/b.w) were studied.

Table 8.4 : Effect of C. pluricaulis, Plant extract doses on body weight study 0th day

Groups	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ± SD**
Normal Control (N.C)	204	202	201	206	197	201	202.5 ± 2.87
Disease Control (D.C)	205	198	205	207	207	205	204.5 ± 2.31
Glibenclamide (STD)	196	201	210	209	212	214	205.5 ± 5.86
C. pluricaulis 100 mg/kg	213	211	210	221	226	215	217.5 ± 6.88
C. pluricaulis 200 mg/kg	204	205	207	213	210	208	206.5 ± 4.66
C. pluricaulis 300 mg/kg	205	195	208	210	199	204	201.5 ± 5.01

T**Table 8.4 : Effect of C. pluricaulis, Plant extract doses on body weight study 7th day**

Groups	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ± SD**
Normal Control (N.C)	211	204	207	214	201	201	207.3 ± 5.21
Disease Control (D.C)	172	165	175	175	174	175	171.0 ± 4.34
Glibenclamide (STD)	197	201	201	197	195	201	204.0 ± 4.54
C. pluricaulis 100 mg/kg	201	196	194	207	211	201	201.0 ± 3.18
C. pluricaulis 200 mg/kg	202	198	204	207	211	203	203.1 ± 4.26

C. pluricaulis 300 mg/kg	195	187	201	197	193	195	195.3 ± 4.37

Table 8.4 : Effect of C. pluricaulis, Plant extract doses on body weight study 14th day

Groups	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ± SD**
Normal Control (N.C)	216	210	211	208	201	208	211.3 ± 5.24
Disease Control (D.C)	162	159	165	171	165	168	166.0 ± 3.93
Glibenclamide (STD)	181	181	184	189	185	190	184.0 ± 4.5
C. pluricaulis 100 mg/kg	172	172	171	177	183	182	176.0 ± 3.38
C. pluricaulis 200 mg/kg	185	181	181	185	191	192	187.2 ± 4.1
C. pluricaulis 300 mg/kg	182	174	188	186	178	181	181.0 ± 4.34

Table 8.4 : Effect of C. pluricaulis, Plant extract doses on body weight study 21st day

Groups	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ± SD**
Normal Control (N.C)	217	210	210	215	197	216	211.8 ± 7.35
Disease Control (D.C)	157	150	162	167	160	162	157.6 ± 5.75
Glibenclamide (STD)	186	188	191	191	193	201	193.0 ± 4.6

C. pluricaulis 100 mg/kg	187	185	182	191	194	185	186.0 ± 4.89
C. pluricaulis 200 mg/kg	192	188	194	197	202	193	195.3 ± 4.45
C. pluricaulis 300 mg/kg	193	187	198	198	191	192	191.3 ± 4.8

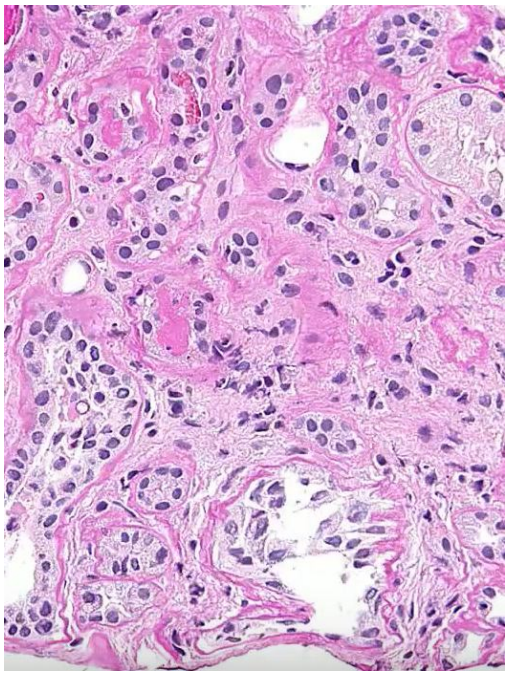
Table 8.4 : Effect of C. pluricaulis, Plant extract doses on body weight study 28th day

Groups	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ± SD**
Normal Control (N.C)	221	214	214	217	213	220	217.5 ± 4.41
Disease Control (D.C)	144	137	149	154	147	149	144.6 ± 5.7
Glibenclamide (STD)	192	194	195	196	198	206	197.5 ± 5.04
C. pluricaulis 100 mg/kg	194	192	187	196	201	194	195.0 ± 4.79
C. pluricaulis 200 mg/kg	195	192	193	198	206	198	194.5 ± 5
C. pluricaulis 300 mg/kg	205	198	205	204	204	203	202.5 ± 2.42

8.5 Histopathology:

The histopathological analysis of the kidney from the control group (Figure 8.5.1) reveals normal renal structures, with intact glomeruli and tubules, serving as a baseline for comparison. The standard group (Figure 8.5.2), treated with a known therapeutic agent, shows mild improvements in renal architecture, demonstrating the efficacy of the standard treatment. In stark contrast, the diabetic group (Figure 8.5.3) exhibits severe diabetic

nephropathy with glomerular hypertrophy, basement membrane thickening, and tubulointerstitial fibrosis, indicative of advanced diabetic kidney damage. Remarkably, the test groups treated with plant extract show dose-dependent improvements. Test group 1, treated with 100 mg/kg/b.w extract, displays moderate amelioration of nephropathic features, with reduced glomerular and tubular damage. Test group 2, receiving 200 mg/kg/b.w extract, shows further improvement, with significant reduction in glomerular hypertrophy and interstitial fibrosis. Test group 3, administered 300 mg/kg/b.w extract, demonstrates near-normal histological features, with marked preservation of renal architecture, suggesting a potent therapeutic effect of the plant extract in mitigating diabetic nephropathy.



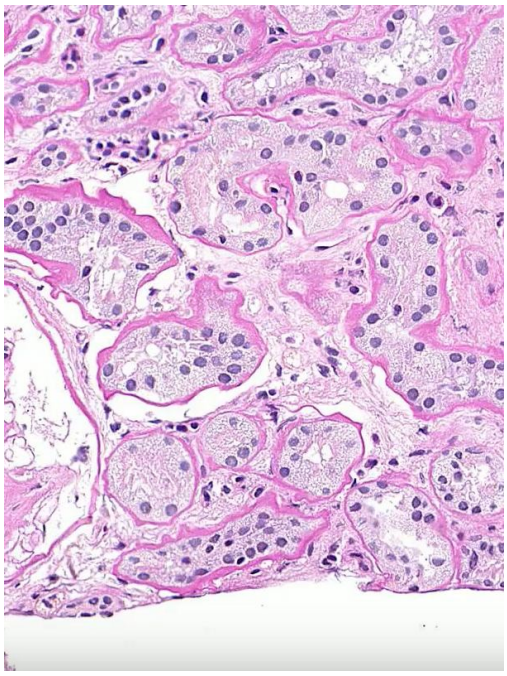
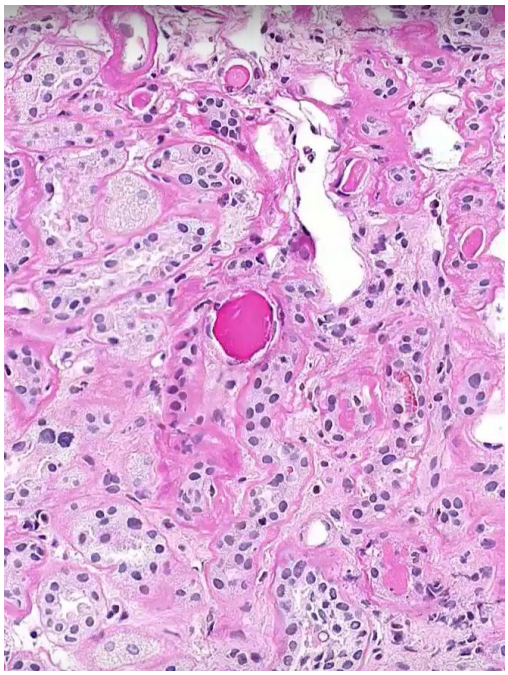


Fig 8.5.1 : Histopathology of kidney of control group

Fig 8.5.2 : Histopathology of kidney of Standard group



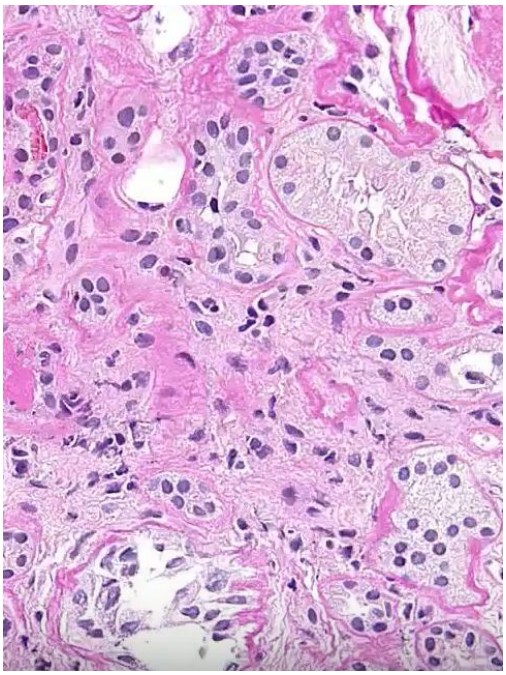
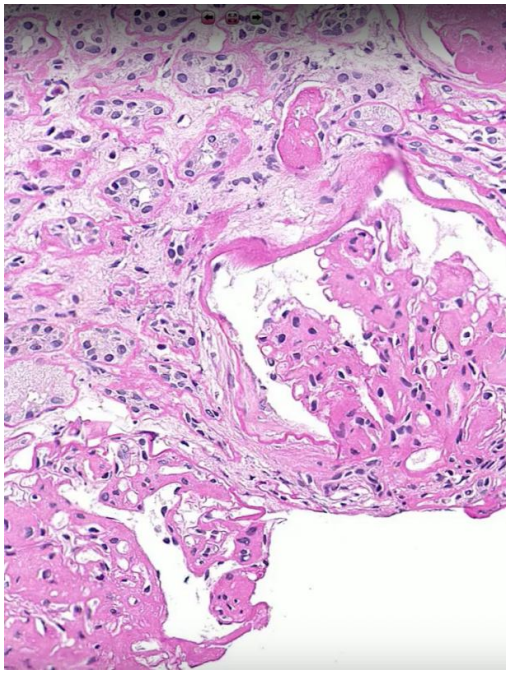


Fig 8.5.3 : Histopathology of kidney of diabetic group

Fig 8.5.4 : Histopathology of kidney of test group 1 (extract 100mg/kg/b.w)



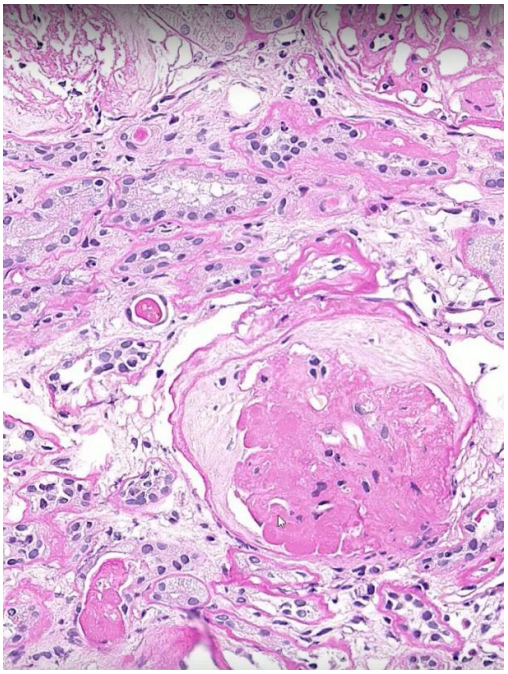


Fig 8.5.5 : Histopathology of kidney of test group 2 (extract 200mg/kg/b.w)

Fig 8.5.6 : Histopathology of kidney of test group 3 (extract 300mg/kg/b.w)

Discussion

End-stage renal failure is commonly caused by diabetes mellitus (DM), which is recognized as a major complication. Diabetic nephropathy (DN) affects nearly a quarter of individuals with diabetes. Interest in using herbal medications for DN treatment has increased significantly in recent decades. The potential of Convolvulus pluricaulis methanolic extract in experimental DN is the main topic of this work.

Nephropathy in diabetes is primarily due to high blood and urine glucose levels, which impair blood filtration by the kidneys. In this work, streptozotocin (STZ) was used to experimentally develop diabetes in rats. The injection of C. pluricaulis methanolic extract lowered STZ-induced hyperglycemia and its development to DN, confirming the extract's previously documented antihyperglycemic activity. Chronic hyperglycemia is known to diminish antioxidative defenses and increase oxidative stress. Elevated renal TBARS content and decreased SOD and CAT levels in diabetic mice indicated increased oxidative stress in the kidneys. Treatment with methanolic extract of C. pluricaulis increased SOD and CAT levels and decreased TBARS levels, indicating a significant improvement in renal function. This suggests that C. pluricaulis extract can inhibit oxidative stress and protect against kidney damage caused by DN.

It is well established that prolonged hyperglycemia in DN induces oxidative stress and inflammation. Increased reactive oxygen species (ROS) levels elevate IL-1, TNF-α, TGF-β, and other inflammatory mediators. Therefore, hyperglycemia significantly impacts kidney damage in diabetes. Oxidative stress and inflammation are biologically interconnected. When oxidative stress produces pro-inflammatory cytokines, it further generates reactive oxygen species (ROS), which exacerbates the condition and damages serum and kidney tissue in diabetic animals. Methanolic extract of C. pluricaulis significantly reduced inflammation in diabetic rodents by lowering the expression of renal pro-inflammatory cytokines.

The results highlight the strong anti-inflammatory and antioxidant properties of methanolic extract of C. pluricaulis in experimental DN. There have been few studies on the renoprotective and antidiabetic effects of C. pluricaulis extract. This study provides evidence that methanolic extract of C. pluricaulis reduces hyperglycemia, inflammation, oxidative stress, and kidney damage in Streptozotocin -induced diabetic rodents, paving the way for further research in this area.

Summary & Conclusion

The majority of patients with DN continue to experience increasing renal impairment despite the availability of current treatments, such as antioxidant, anti-diabetic, and hypertensive medications. Current treatments have not changed the primary pathogenic mechanism that causes DN to develop. DM-related nephropathic consequences have emerged, and the current investigation has been carried out.

Diabetes mellitus (DM) is frequently the cause of end-stage renal failure, and approximately 25% of diabetics suffer from diabetic nephropathy (DN). The use of herbal medicines to treat DN has recently garnered more and more attention. This study focuses on the potential of Convolvulus pluricaulis methanolic extract in experimental DN. Streptozotocin (STZ) was used in the study to induce diabetes in rats, which eventually led to DN. Supporting the extract's antihyperglycemic properties, the administration of C. pluricaulis methanolic extract effectively reduced STZ-induced hyperglycemia and its progression to DN. Antioxidative defenses are known to be compromised and oxidative stress elevated by chronic hyperglycemia. Diabetic rodents showed expanded renal Ski lifts content and diminished Turf and Feline levels, demonstrative of uplifted oxidative pressure. By increasing SOD and CAT levels and decreasing TBARS levels, C. pluricaulis methanolic extract improved renal function, demonstrating its antioxidative efficacy.

Oxidative stress and inflammation are known to be triggered by prolonged hyperglycemia in DN. Inflammatory mediators like IL-1, TNF-, and TGF- are more likely to be expressed when ROS levels are elevated. The exchange between oxidative pressure and irritation fuels kidney harm in diabetic creatures. The methanolic concentrate of C. pluricaulis was found to lessen irritation by diminishing the outflow of renal supportive of fiery cytokines in diabetic rodents essentially. C. pluricaulis methanolic extract's strong antioxidant and anti-inflammatory properties in experimental DN are demonstrated by this study. Methanolic

extract of Convolvulus pluricaulis demonstrated potent renoprotective, anti-diabetic, antioxidant, and anti-inflammatory effects in rodents with streptozotocin-induced diabetic nephropathy. C. pluricaulis methanolic extract emerges as a promising treatment option for DN by reducing hyperglycemia, inflammation, oxidative stress, and the ensuing damage to the kidney. Notwithstanding restricted research on the renoprotective and antidiabetic impacts of C. pluricaulis separate, this study gives indisputable proof to its adequacy. These outcomes make ready for additional examinations concerning the helpful capability of C. pluricaulis, planning to foster powerful natural medicines for diabetic nephropathy.

CERTIFICATE

Certified that **Naman Gautam (enrollment** no. 2202270986003) has carried out the research work presented in this thesis entitled **"The Protective effect of convolvulus Pluricaulis leaves on straptozocin and high fat diet induced diabetic Nephropathy in Wistar Albino rats"** for the award of **Master of Pharmacy** from Dr. APJ Abdul Kalam Technical University, Lucknow under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this University or any other university/Institution.

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