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Evaluation of Efficacy of Polyherbal Preparation in Prevention of Target Organ Damage and Intercurrent Infections In Experimentally Induced Diabetes Mellitus In Rats

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ABSTRACT
Aims and objectives:
1 *To evaluate the antidiabetic effect of polyherbal extract in comparison with Gliclazide*
2 *To evaluate the effect of polyherbal extract on serum creartinine, CRP levels and WBC counts in comparison with standard drug Gliclazide in alloxan induced diabetic Rats*

Materials and method: *Rats were randomly divided into 5 groups, each comprising 6 rats. Group 1 - Normal control distilled water, Group 2 – standard drug gliclazide 25mg/kg, Group 3 – Polyherbal extract (T1)– 300mg/kg, group 4 – Polyherbal extract (T2)– 400mg/kg, Group 5 – Polyherbal extract (T3)– 500mg/kg was administered orally for 45 days. The fasting blood glucose, HbA1c values, S.Creatinine, CRP levels and WBC counts were estimated in both normal and alloxan induced diabetic rats.*

Results: *The percentage reduction in FBS after 45days of treatment in T1, T2, T3 and standard groups is 31.9%, 34.4%, 50.7% and 61.03% respectively, and difference between groups is statistically significant, P value <0.0001***. Regarding HbA1c levels, the percentage reduction in T1, T2, T3 & standard groups is 33.3%(P 0.0006***), 40.1%(P<0.0001***), 43.6%(P<0.0001***) & 45%(P<0.0001***) respectively, there is significant statistical difference between different groups before and after treatment.*

*The percentage reduction in S. Creatinine values in T1, T2, T3 and standard groups is 41.5%, 57.4%, 68.32% & 69.5% respectively with statistically significant difference between study groups, P value <0.0001.****

*Similarly in case of WBC counts, the percentage reduction in T1, T2, T3 & standard groups is 25.24%, 33.27%,37.83% & 39.17% respectively during the study period, and there is statistically significant difference between different study groups with P value 0.0007.*** With regard to serum CRP levels, the percentage reduction in T1, T2, T3 & standard groups is 42.7%(P 0.0015**), 48.6%(P 0.0003***), 54.1%(P<0.0001***) and 53.1%(P<0.0001***) respectively with statistical significant difference in each group before and after treatment.*

Conclusion: *The polyherbal extract showed excellent antidiabetic potential and its effect is comparable with Gliclazide in alloxan induced diabetic rats. Extract also showed significant reduction in S.Creatinine, CRP and WBC counts in diabetic rats after treatment.*

Keywords: *Diabetes, Creatinine, CRP, HbA1c, polyherbal extract, Gliclazide*

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I. Introduction

Diabetes Mellitus is a spectrum of common metabolic disorders, arising from a variety of pathogenic mechanisms, all resulting in hyperglycemia. The number of individuals with diabetes is rising rapidly throughout the world. Both genetic and environmental factors contribute to its pathogenesis, which involves insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, and/or abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities. However, the major sources of the morbidity of diabetes are the chronic complications that arise from prolonged hyperglycemia, including retinopathy, neuropathy, nephropathy, and cardiovascular disease.[1]

In spite of the best currently available treatments DM has poor outcome. The long-term complications are still leading causes of death. The development of long term complications in diabetes mellitus has been linked to the extent of hyperglycemia and the duration of the disease. Uncontrolled diabetes mellitus results in oxidative stress and a number of mechanisms or pathways by which hyperglycemia, the major contributing factor of increased reactive oxygen species (ROS) production, causes tissue damage or diabetic complications have been identified. These include hyperglycemia-enhanced polyol pathway, hyperglycemia-enhanced formation of advanced glycation end products (AGEs), hyperglycemia-activated protein kinase C (PKC)

pathway, hyperglycemia-enhanced hexosamine pathway and hyperglycemia-activated Poly-ADP ribose polymerase (PARP) pathway.

EPIDIMEOLOGY:

The two broad categories of DM are designated as type 1 and type 2. Type 1 Diabetes Mellitus (T1DM) is the most severe form. Type 2 Diabetes Mellitus (T2DM) is the predominant form of diabetes worldwide, accounting for 90% of cases globally. Globally, the number of people with diabetes is expected to rise from the current estimate of 382million in 2014 to 592 million by 2035. The average nationwide prevalence of diabetes in India is 9.1%.T2DM has become one of the world's most important public health problems. In the past, it was believed that the overwhelming majority of children with diabetes had type 1diabetes (T1DM), and > 1% - 2% of diabetic children were considered to have T2DM or other rare forms of diabetes[2]. More than 79,000 children developed type 1 diabetes in 2014. Nearly 21 million live births were affected by diabetes during pregnancy in 2014. More than 80% of diabetes deaths occur in low- and middle-income countries. Social and educational status of patient also plays role in understanding diabetes

Diabetes-related mean health expenditure person with diabetes in India (20-79yrs) is 84US$ in 2014. It is total of around 548 billion US$ worldwide in 2014. The average nationwide prevalence of diabetes in India is 9.1%, and is as high as 20% in certain southern cities.

Diabetes is growing alarmingly in India, home to more than 65.1 million people with the disease in 2013, compared to 50.8 million in 2010, rising to 131.2 million by 2035 according to the International Diabetes Federation (IDF).[3] According to the World health Organization (WHO) criteria, the prevalence of known diabetes was 5.6% and 2.7% among urban and rural areas, respectively[4].There is a significantly increasing trend in urban populations while among rural populations the prevalence is increasing at a slower rate. Even in urban India, the prevalence in the Southern part of India was found to be higher at 13.5% in Chennai, 12.4% in Bangalore and 16.6% in Hyderabad, this compares with Eastern India (Kolkata) 11.7%, Northern India (New Delhi) 11.6%, and Western India (Mumbai) 9.3%.

Diabetes is a major cause of mortality, but several studies indicate that diabetes is likely under reported as a cause of death. In the United States, diabetes was listed as the seventh leading cause of death in 2007; a recent estimate suggested that diabetes was the fifth leading cause of death worldwide and was responsible for almost 5.1 million deaths in 2013 (6.8% of deaths were attributed to diabetes worldwide).Every six seconds a person dies from diabetes[5].

Therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. But the currently available drugs are associated with side effects. Hence the search is made for new compounds with multiple targets and without any side effects.

There are numerous traditional medicinal plants reported to have antidiabetic properties.The stem of Tinospora cordifolia is widely used in the therapy of diabetes by regulating the blood glucose. It has been reported to mediate its anti-diabetic potential through mitigating oxidative stress (OS), promoting insulin secretion and also by inhibiting gluconeogenesis and glycogenolysis, thereby regulating blood glucose. Similarly curcuma, neem and amla along with tinospora are used as antidiabetic treatment in Ayurveda practice.

In the present study research was done to evaluate the antidiabetic effect of alcoholic extract of polyherbal preparation which consists of curcuma, amla, neem and tinospora cordifolia in alloxan induced diabetic rats.

II. Aims And Objectives

The present study aims at evaluating the effect of polyherbal preparation in
Alloxan induced diabetic rats.
Main objectives are:

1. To evaluate the effect of polyherbal extract preparation on blood glucose and HbA1c levels in alloxan induced diabetic rats in comparision with standard drug Gliclazide.
2. To evaluate the effect of polyherbal extract preparation on serum creatinine, CRP levels and WBC counts in Alloxan induced diabetic rats in comparison with standard drug Gliclazide.

III. Review Of Literature

HISTORY:

Diabetes Mellitus was described more than 2000 years ago. The history begins with Ebes Papyrus 1500 BC, Madhumeha by Susruta in 4000 BC and the Word Diabetes by Aretans era . Insulin was discovered by Banting and Best in 1921 and were awarded nobel prize.

Milestones Of Diabetes Mellitus:

DATE	SOURCE	OBSERVATIONS
15th Century BC	Ebers papyrus (Egypt)	Clinical description of polyuric condition resembling diabetes.
2nd Century	Galen (Rome) Aretaeus (Cappadocia)	Word diabetes
5th Century	Sushruta & Charaka (India)	Clinical description including sugary urine, obese and thin patients distinguished.
10th Century	Avicenna (Arabic)	Clinical description including sugary urine complications including gangrene and impotence.
19th Century 1810-1869	William Proud (England) Paul Langerhans (Germany)	Diabetic coma described Pancreatic islets identified.
20th Century 1921	Frederick G. Banting Charles H Best James B Collip	Isolation and first clinical use of insulin.
1936	Paul Kimmelstiel & Clifford Wilson (USA)	Described nodular glomerular lesions in the diabetic patient with proteinuria and hypertension.
1955	Frederic Sanger(UK)	Determined sequence of insulin.
1957	Unger	Phenformin
1969	Dorothy Hodgkin.(UK)	3-dimensional structure of insulin.
1971	Pierre Freycheat (USA)	Identified insulin receptors.
1980's		Insulin sensitizers
1983	Frank and Chance	Recombinant human insulin(E.coli)
1996		Insulin analogues(lispro)

From year 2001 onwards further advances regarding insulin include production of long acting preparations for which we are largely indebted to Hagedorn of Denmark and the recent highly purified almost non-antigenic insulin from the Novo Laboratories.

Novel classes of insulin secretogogues, the Meglitinides and Dephenylalanine derivatives are alternatives to short acting sulfonylureas, Tolbutamide. Since 1980's insulin sensitizers Thiazolidinediones have been approved by FDA. They cause increase in glucose utilization in both muscle and liver and reduce insulin resistance by modifying the glucose transporter system especially GLUT1 and GLUT4. Recently incretin mimmetics like GLP1 and GIP are introduced. Other drugs like DPP4 inhibitors and Amylin mimetic drugs are used.

SGLT2 inhibitors are newer antidiabetic drugs that have successfully cleared the phase III clinical trials[6].

Etiologic classification of Diabetes Mellitus:

I) Type-1 diabetes (Insulin dependent diabetic mellitus or IDDM) (□ cell

destruction, usually leading to absolute insulin deficiency).

Rapidly progressive

(i)Auto immune

Slowly progressive

(ii) Idiopathic

II) Type-2 diabetes (Non insulin dependent diabetes mellitus or NIDDM) (Many range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance).

III) Others specific types of diabetes.[7]

A. Genetic defects of B-cell function characterized by mutation in:

1. Hepatocyte nuclear transcription factor (HNF) 4□ (MODY 1).
2. Glucokinase (MODY2).
3. HNF-1□ (MODY3).
4. Insulin promoter factor (IPF) 1 (MODY 4).
5. HNF-1 □ (MODY5).
6. Neuro D1 (MODY 6).
7. Mitochondrial DNA.
8. Proinsulin or insulin conversion.

B. Genetic defects in insulin action.

1. Type A insulin resistance.
2. Leprechaunism.
3. Rabson-Menden hall syndrome.
4. Lipodystrophy syndrome.

C. Diseases of the exocrine pancreas - Pancreatitis, pancreatectomy,

neoplasia, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy.

D. Endocrinopathies - Acromegaly, cushing's syndrome, glucagonoma,

pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma.

E. Drug/chemical induced- Pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, β -adrenergic agonists, thiazides, phenytoin,

α interferon, protease inhibitors, clozapine, β blockers.

F. Infections- Congenital rubella, cytomegalovirus, coxsackie.

G. Uncommon forms of immune mediated diabetes "stiff-man syndrome", and insulin receptor antibodies.

H. Other genetic syndromes sometimes associated with diabetes-Down syndrome, Klinefelter syndrome, Turner syndrome, Wolfram syndrome,

Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome.

IV) Gestational diabetes mellitus (GDM).

Risk factors for type-2 diabetes mellitus:

- Family history of diabetes (i.e., parent or sibling with type-2 diabetes).
- Obesity (BMI 25 kg/m^2).
- Habitual physical inactivity.
- Race/ethnicity (e.g. African American, Hispanic American, Native American, Asian American Pacific Islander).
- Previously identified IFG or IGT.
- History of GDM or delivery of baby >4 Kg (9lb).
- Hypertension (Blood pressure 140/90 mm Hg).
- HDL cholesterol level ≤ 35 mg/dL (0.90 mmol/L) and /or a triglyceride level 250 mg/dl (2.82 mmol/L).
- Polycystic ovary syndrome or acanthosis nigricans.
- History of vascular disease.

Maturity onset diabetes of the young (MODY):

Maturity onset diabetes of the young (MODY) is a subtype of diabetes mellitus characterized by autosomal dominant inheritance, early onset of hyperglycemia, and impairment in insulin secretion. MODY 2 is the result of mutations in the glucokinase gene that leads to mild-to-moderate hyperglycemia MODY 1, MODY 3 and MODY 5 are caused by mutations in the hepatocyte nuclear transcription factors (HNF) 4, HNF-1 and HNF-1.

MODY 4 is a rare variant caused by mutations in the insulin promoter factor-1.

Gestational diabetes mellitus (GDM):

Glucose intolerance may develop during pregnancy. Insulin resistance related to the metabolic changes of the pregnancy increases insulin requirements and may lead to IGT. GDM occurs in approximately 4% of pregnancies in the United States. Most women revert to normal glucose tolerance post-partum but have a substantial risk (30 to 60%) of developing diabetes mellitus later in life.[8]

Pathophysiology of Type 2 Diabetes Mellitus (T2DM):

The pathogenesis of T2DM is complex and involves the interaction of genetic and environmental factors. A number of environmental factors have been shown to play a critical role in the development of the disease, particularly excessive caloric intake leading to obesity and a sedentary lifestyle. The clinical presentation is also heterogeneous, with a wide range in age at onset, severity of associated hyperglycemia, and degree of obesity. From a pathophysiologic standpoint, persons with T2DM consistently demonstrate three cardinal abnormalities:

- Resistance to the action of insulin in peripheral tissues, particularly muscle, fat and liver.
- Defective insulin secretion, particularly in response to a glucose stimulus.
- Increased glucose production by the liver.

Recently, it has been suggested that the list of cardinal abnormalities in diabetes should be expanded to eight, adding accelerated lipolysis in the fat cell, incretin hormone deficiency and resistance, hyperglucagonemia, increased renal tubular reabsorption, and the role of the central nervous system in metabolic regulation.

Although the precise way in which genetic, environmental, and pathophysiologic factors interact to lead to the clinical onset of T2DM is not known, understanding of these processes has increased substantially. There is an emerging consensus that the common forms of T2DM are polygenic in nature and are caused by a combination of insulin resistance, abnormal insulin secretion, and other factors; with the exception of specific monogenic forms of the disease that might result from defects largely confined to the pathways that regulate insulin action in muscle, liver, and fat or defects in insulin secretory function in the pancreatic beta cell.

From a pathophysiologic standpoint, it is the inability of the pancreatic beta cell to adapt to the reductions in insulin sensitivity that occurs over a lifetime that precipitates the onset of T2DM. The most common factors that place an increased secretory burden on the beta cell are puberty, pregnancy, a sedentary lifestyle, and overeating leading to weight gain. An underlying genetic predisposition appears to be a critical factor in determining the frequency with which beta cell failure occurs[8]

Complications of Diabetes:

The major cause of death in treated patients is due to cardiovascular problems (70%) followed by renal failure (10%) and infections (6%). There is no doubt that the duration and degree of hyperglycemia play a major role in the production of complications. Better diabetic control can reduce the rate of progression of both nephropathy and retinopathy and the DCCT (The Diabetes Control and Complications Trial) showed a 60% reduction in developing complications over 9 years when the HbA1c was kept at around 7% in type 1 diabetes.

Acute Complications of DM:

Diabetic ketoacidosis (DKA) and Hyperglycemic hyperosmolar state (HHS) are acute complications of diabetes. DKA was formerly considered a hallmark of type 1 DM, but it also occurs in Individuals who lack immunologic features of type 1 DM and who can subsequently be treated with oral glucose-lowering agents. HHS is primarily seen in individuals with type 2 DM. Both disorders are associated with absolute or relative insulin deficiency, volume depletion, and acid-base abnormalities. DKA and HHS exist along a continum of hyperglycemia, with or without ketosis.

Chronic Complications of DM:

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications.

1. Vascular complications

a) Microvascular - retinopathy, neuropathy, and nephropathy

b) Macrovascular -coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease.

2. Nonvascular complications

These include problems such as gastroparesis, infections, cataract, glaucoma and skin changes. Long-standing diabetes may be associated with hearing loss. Type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.

The microvascular complications of both type 1 and type 2 DM result from chronic hyperglycemia. Large, randomized clinical trials of individuals with type 1 or type 2 DM have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy, and nephropathy. [8]

Diabetes and kidney :

Generally, the changes produced in the kidneys by diabetes occur very slowly, taking place over years. There are 5 stages in development of diabetic nephropathy.

Stage 1 – enlarged kidney, high blood flow and rate of filtration.

Stage 2 – thickening of basal memebrane, occurs 2-5 yrs of diabetes

Stage 3 – microalbuminuria, rise in B.P, occurs 5-15 yrs of diabetes

Stage 4 – macroalbuminuria, fall in blood flow and filtration, occurs 10-25yrs of diabetes

Stage 5 – occurs in 15-30 yrs of diabetes charecterised by permanenet renal Insufficiency[9]

There are certain factors that determine the development of nephropathy in diabetes. Duration of hyperglycemia i.e uncontrolled blood sugar levels is one of the major determinant of nephropathy. Hereditary factor in which patient had family history of diabetes with renal problem. Patients with associated hypertension are more risk of developing nephropathy[9].Blood parameters which give information about the activity of kidney are urine output, serum Creatinine, creatinine clearance, uric acid, calcium, phosphorous, potassium levels

Diabetes and infections :

Many specific infections are more common in diabetic patients, and some occur almost exclusively in them. Other infections occur with increased severity and are associated with an increased risk of complications in patients with diabetes.

Several aspects of immunity are altered in patients with diabetes. Polymorphonuclear leukocyte function is depressed, particularly when acidosis is also present. Leukocyte adherence, chemotaxis, and phagocytosis may be affected.Antioxidant systems involved in bactericidal activity may also be impaired. Community-acquired pneumonia, foot infections, Acute bacterial cystitis, Emphysematouspyelonephritis, Necrotizing fasciitis, Invasive otitis externa, Rhinocerebral mucormycosis, Emphysematous cholecystitis etc .. are some of the common infections in diabetic patients. And alsoit is noted that there is increased incidence of infections with specific organisms in diabetic patients. Group B streptococci, klebsiella,tuberculosis, salmonella enteritidis, staphylococcus aureus, candidaare some of the common organisms responsible for infections in diabetic patients.[10]

Diabetes mellitus is diagnosed by demonstrating any one of the following.

1) Fasting plasma glucose at/or above 7.0 mmol/L (126 mg/dl).
2) Plasma glucose at/or above 11.1 mmol/L (200 mg/dl), two hours after a 75gm oral glucose load as in glucose tolerance test.
3) Symptoms of hyperglycemia and casual plasma glucose at/or above 11.1 mmol/L (200 mg/dl).
4) HbA1C $\geq$ 6.5. (Glycated haemoglobin concentration represents the integrated values for glucose over preceeding 6 to 8 weeks, it is directly proportional to the concentration of glucose in blood.) This criterion was recommended by the American Diabetes Association in 2010, it has yet to be adopted by the WHO.

1999 WHO diabetes criteria:

Condition	Fasting glucose mmo/L (mg/dl)	2 hr glucose mmo/L (mg/dl)
Normal	< 6.1 (< 110)	< 7.8 (< 140)
Impaired fasting glycemia	□ 6.1 &< 7.0	< 7.8 (< 140)

	(□ 110 &< 126)	
Impaired glucose tolerance	< 7.0 (< 126)	□ 7.8 (□ 140)
Diabetes mellitus	□ 7.0 (□ 126)	□ 11.1 (□ 200)

INVESTIGATIONS:

A. Urine Analysis for:

Glucose: Benedicts test, Fehling's test, clinitest, dipstic method(clinistix)

Proteins: boiling test, albustix, radioimmunoassay, biuret test, salicyl sulphonic acid test, nitric acid test.

Ketones: Gerhard's ferric chloride test, Rothera's nitroprusside test, Ace test, ketostix and ketodiastix test.

B. Determination of Blood Glucose Levels:

1. Glucose oxidase Peroxidase(GOD POD) method
2. Folin –wu method (alkaline copper reduction method)
3. Ortho-toludine method
4. Oral glucose tolerance test.

C. Determination of Glycosylated Hemoglobin(HbA1c) : Estimated by ion exchange resin method.

SCREENING METHODS:

Hypoglycemic activity can be observed in experimental animals by evaluating the changes in the blood sugar levels before and after giving the test drug at regular intervals. The efficacy of the drug can be determined by comparing it with a standard drug. This can be done in either experimentally induced diabetic animals or in normal healthy animals.

Experimental models of diabetes:-

Animal models of diabetes have been widely used in biomedical research, because it provides accurate study in a short period of time. In most cases, the stable incidence of diabetes mellitus in animal colonies provides a powerful and convenient tool for the investigation of the therapeutic modalities for the disease and for its complications. Diabetic animals constitute an excellent biomedical tool for the investigation of carbohydrate metabolism and diabetic complications and for the understanding of the nature of the disease[11].

ANIMAL MODELS FOR DIABETES MELLITUS

IDDM	
1. Chemically induced - Alloxan - Streptozotocin 2. Hormone induced - Dexamethasone 3. Virus induced - Picoma virus - Coxsachie B4 - EMC - Mengo 2T - Rheovirus - LCM	4. Antibody induced - Insulin antibodies 5. Surgically induced - Total pancreatectomy - Partial pancreatectomy 6. Genetic models - NOD mouse - BB rat
NIDD M	
Chemically induced - Streptozotocin - Adrenaline - Chelating agents - EDTA - Diuretics - Diazoxide Anti- OX insulin antibodies Genetic Models	- *Polygenic Models of Obesity & NIDDM* o Newzealand obese mouse o Japanese kk mouse o Nagoya-shibata-yasudamouse o PBB/Ld mouse o OLEFT rats o Goto-kakisaki rat o Chinese hamster

- *Monogenic Models of Obesity & NIDDM* o Yellow mouse o Obese & diabetic mouse o Tubby mouse o Fat mouse o Zukker diabetic fatty rats o Koletsky& JCR:LA-corpulent rats	o Djungarian hamster o South african hamster - *Animal Models of NIDDM with Unknown Hereditary & Environmental Component* o Sand Rat o Spiny mice o Tucotuco - *Polygenic Animal Models Produced by Hybrid Crosses* o BSB (C57BL/6J MUS Spretus o AKR/J SWR/J model o GK FISHER 34 strain o GK Non-diabetic Brown Norway Rat - Transgenic & Knockout Animals

MODELS FOR IDDM:

1. Chemical methods:

Chemically induced type-1 diabetes is the most commonly used animal model of diabetes. Chemical agents which produce diabetes can be classified into three categories:

A. Specifically damage β-cell;
B. Cause temporary inhibition of insulin production and secretion.
C. Diminish the metabolic efficacy of insulin in target tissues.

These agents induce hyperglycemia via four phases:
First phase: Transient hypoglycemia begins within a minute and lasts for 30mins.

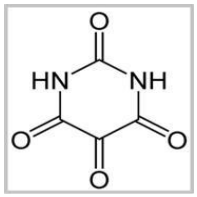
Second phase: Hyperglycemia, Hypoinsulinemia begins after 1hr lasts for 2-4hrs.

Third phase: Hypoglycemia begins 4-8hrs lasts several hours

Fourth phase: beta cell disintegration in next 24-48hrs resulting into hyperglycemia[12]

I. Alloxan-Induced diabetes:

Alloxan, analogue of cyclic urea, was the first agent in this category reported to produce diabetes in animals.
Structure : 2,4,5,6 tetra-oxy-pyrimidine



- An oxygenated pyrimidine derivative. It is also a barbituric acid derivative (5- ketobarbituric acid).
- Hydrophilic compound, present as alloxan mono hydrate in aqueous solution.
- A beta cell toxic glucose analogue with a molecular structure similar to that of glucose.

- Chemically unstable in buffer solutions with a half-life of 1.5min at pH 7.4

and 37^{o}C decomposing to alloxanic acid .

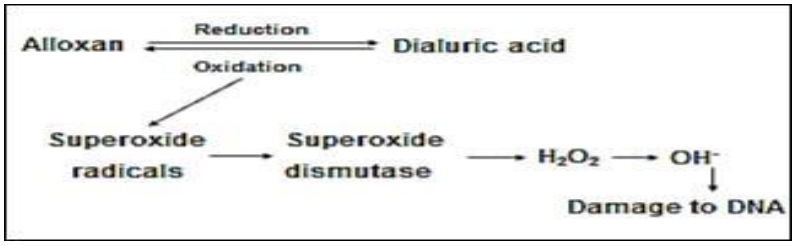
- Stable at acidic pH (0.01M HCl)

- For experimentation, concentrated stock solutions in 0.01M HCl, kept on ice, should be used and added to test medium just prior to the start of the experiment in order to obtain final concentration.

- For injection, stock solution should be diluted with ice cold saline (0.9%

NaCl) immediately prior to injection.

Mechanism of action:
The mechanism by which it produces diabetes is not clear. Alloxan is a highly reactive molecule reduced to dialuric acid which is then autoxidized back to Alloxan resulting in production of free radicals. These free radicals damage the DNA of beta cells and cause cell death. Second mechanism is its ability to react with protein –SH groups, especially the membrane proteins and the beta cells, finally resulting in cell necrosis. However there are major species differences in response to Alloxan.[12]



Routes & Doses of Alloxan:
- Wistar or Sprague Dawley rat – 65mg/kg I.V. (or) 150mg/kg i.p. / s.c.
- Rabbit – 100-150mg/kg I.V. infused via marginal ear vein. About 70% animals become hyperglycemic and uricosuric whereas remaining either die or temporarily hyperglycemic.
- Male Beagle dog – 60mg/kg I.V. ; Monkey – 65 to 200mg/kg I.V.[11]

II. Streptozotocin(STZ):
It is a Glucosamine derivative of Nitrosourea. It is a broad spectrum antibiotic, which is produced from streptomyces achromogens.

Structure: 2-deoxy-2-(3-methyl-3-nitrosurea)1-D-glucopyranose

Mechanism of action:

- Methylation of DNA

Alkylation or breakage of DNA Increases poly-ADP ribose synthase Deplete NAD & ATP in beta cell Leads to cell death.
- Free radical generation

Increased ATP dephosphorylation Supplies substrates to xanthine oxidase formation of superoxides Generation of reactive oxygen species Damage to DNA.
- Nitric oxide production

Nitric oxide + reactive oxygen species Peroxynitrates Damage to DNA

Routes & Doses of STZ: Rat – 50 – 60 mg/kg I.P. or I.V.; Mice - 175-

200 mg/kg I.P. Or I.V.; Dog - 15 mg/kg I.V. for 3 days.

Guinea pigs & rabbits are resistant to STZ.

Multiple low doses of STZ also induces Diabetes by causing immune mediated pancreatic insulitis in rats.

STZ (65mg/kg)+NAD(230mg/kg) in adult rat; STZ (80-100mg/kg) in neonatal rat;

STZ (35mg/kg) + High Fat Diet are the other dosages used.[11]

ANTIDIABETIC DRUGS:
Dietary restrictions, exercise and insulin therapy are the corner stones of management of Type-1 daibetes. There is usually less insulin resistance in type-1 diabetes. However, if resistance develops, glitazones may also be added.[15]

Weight reduction, dietary restrictions and exercise are also proven ways to increase insulin sensitivity in type -2 diabetes and many patients can be effectively treated by such approaches.

However, optimal control of blood glucose levels are not achieved sometimes even when patients adhere to weight reduction program which necessitates drug therapy [15].

Insulin preparations:
Current insulin preparations are generated by recombinant DNA technology and consist of the amino acid sequence of human insulin or variations. Human insulin has been formulated with distinctive pharmacokinetics or genetically modified to more closely mimic physiologic insulin secretion. Insulins can be classified as –

1. Ultra short acting insulins
Examples: insulin lispro, insulin aspart, insulin glulisine.

These have been designed to promote rapid absorption from the subcutaneous tissue. Their action starts within 20-30minutes and lasts for 3-4hours.

2.short acting insulins

Examples: regular insulin, semi lente

These are soluble crystalline zinc – insulins made by recombinant DNA techniques which are identical to human insulin.

3 .Intermediate acting insulins

Example: NPH/isophane insulin, lente insulin

Intermediate acting insulins are not given I.V and are used to control diabetes in a variety of situations except for diabetic ketoacidosis.

4.Longer acting insulins

Examples: ultra lente, insulin glargine, insulin detemir

Insulin glargine (lantus) is a long acting insulin analogue that has two extra amino acids added on to human insulin and one amino acid that is different from human insulin.

Because it is necessary to keep it in solution prior to injection, its pH, has to be maintained acidic (pH4.0).

Insulin detemir has a fatty acid chain which enhances its binding to albumin. Slow dissociation from albumin results in slow and sustained release. Like insulin glargine it should not be mixed with any other preparation.

a. Exubera – prepared using nektar therapeutics proprietary technology.

b. Afrezza – by technospere technology. It carries lower risk of causing hypoglycemia and weight gain. Trials completed in March 2000.[16]

Insulin delivery systems:
The standard mode of insulin treatment is subcutaneous injection using conventional disposable needles and syringes.

1. **Portable pen injectors**
2. **Closed loop system**
3. **Continuous subcutaneous insulin infusion devices**
4. **Inhaled insulin:**

Recently, an inhaled human insulin preparation has been marketed in Europe and the USA. Approved by FDA on 27thJune 2014 in the strength of 4 and 8 units/inhalation. The fine powder is delivered through a nebulizer – absorption is rapid. It is used to control meal time glycaemia. Pulmonary fibrosis and other complications are apprehended on long term use.[17]

5. **Jet injector systems**
6. **Other forms of insulin delivery:**

Intraperitoneal delivery devices, implantable pellets, closed loop artificial pancreas, islet cell and pancreatic transplantation and gene therapy.[18]

ORAL ANTIDIABETIC AGENTS:
1. Insulin secretogogues: Sulfonylureas, Meglitinides, D-phenylalamine derivatives
2. Biguanides
3. Thiazolidinediones
4. Alpha-glucosidase inhibitors

1. Insulin Secretogogues:
The Sulfonylureas and Biguanides have been available the longest and are traditional initial treatment of choice for Type 2 DM. Novel classes of rapidly acting insulin secretogogues, the Meglitinides and D-phenylalamine

derivatives are alternatives to the short acting sulfonylureas, tolbutamide.

A. Sulfonylureas Mechanism of action:

The major action of sulfonylureas is to increase insulin release from the pancreas. Two additional mechanisms are

a.***Insulin release from pancreatic beta cells***

Binding of a sulfonylurea inhibits the efflux of potassium ions through a chemical and results in depolarization. Depolarization opens a voltage gated calcium channel and results in calcium influx and the release of preformed insulin.

b.Reduction of serum glucagon concentration

Long term administration of sulfonylureas to type 2 diabetics reduces serum glucagon levels (increases blood glucose levels)which may contribute to the hypoglycemic effect of the drugs.

Sulfonylureas are divided into two generations:

Ist Generation sulfonylureas: Chlorpropamide, Tolbutamide, Tolazamide,

Acetohexamide

IInd Generation sulfonylureas: Glibenclamide, Glipizide, Gliclazide, Glimipiride

B. Meglitinides analogues:

These are recently developed quick and short acting insulin releasers. **Repaglinide:** is a meglitinide analogue, designed to normalize meal time glucose excursions.It is administered before each major meal to control

postprandial hyperglycemia; the dose should be omitted if a meal is missed. Because of short acting action it may have a lower risk of serious hypoglycemia. It should be avoided in liver disease.

C.Nataglinide: This D-phenylalamine derivative principally stimulates the first phase insulin secretion result in rapid onset and shorter duration of hypoglycemic action than Repaglinide.

2 .Biguanides :

These agents are termed as "euglycemic agents". Their blood glucose lowering action does not depend on functioning pancreatic beta cells. PHENFORMIN – old Biguanides – discontinued because of lactic acidosis; and METFORMIN are members of this group. Mechanism of action was described in metformin section.

3. Thiazolidinediones:

Thiazolidinediones/glitazones were developed following chance observation that a clofibrate analogue, ciglitazone, which was being screened for effects on lipids, unexpectedly lowered blood glucose. Thiazolidinediones show :

- Reduced hepatic glucose output
- Increased glucose uptake into muscles, by enhancing the effectiveness of endogenous insulin.

These are considered as " euglycemics".

Ex:- PIOGLITAZONE – has PPAR- , PPAR- activity ROSIGLITAZONE – risk of heart failure, macular edema

TROGLITAZONE – withdrawn because of hepatotoxicity.

4.Alpha glucosidase inhibitors:

This group of drugs competitively inhibits intestinal alpha glucosidase (sucrases, maltases, glucoamylase) and reduces the post-prandial digestion and absorption of starch, dextrin and disaccharides.

Ex:- ACARBOSE (glucobay, Acar): this is an oligosaccharide of microbial origin.

Mechanism of action: it binds completely to carbohydrate binding regions of alpha glucosidase enzymes in the brush border of the enterocytes in the jejunum.

Meglitol is similar to Acarbose but is claimed to be less hepatotoxic.

5.Other newer drugs are :

a. GLP-1 receptor agonists:

There are currently two GLP-1 receptor agonists that have been approved for treatment of diabetic patients in the US and several others in advanced phases of development.

Exendin-4 is a naturally occurring reptilian peptide of 39 amino acids with considerable homology to GLP-1.

Exenatide is a synthetic exendin- 4 is approved for use as monotherapy and as adjunctive therapy for type-2 diabetes patients not achieving glycemic targets with metformin, sulfonylureas, the combination of metformin and sulfonylurea or thiazolidinediones.

Liraglutide is a second GLP-1 recptor agonist.

b. **DPP-IV inhibitors:** Sitagliptin, Vildagliptin and Saxagliptin are orally active DPP-IV inhibitors.
Mechanism of action:

These drugs inhibits the enzyme DPP-IV which is responsible for the inactivation of incretins such as glucogan-like-peptide-1.

c .Amylin mimetic drugs: Pramlintide (Symlin):

Amylin helps to control post-prandial hyperglycemia by

o Suppressing endogenous glucan production, especially post-prandially;

o Reducing the gastric emptying rate ; and

O Inducing generally mediated satiety, by opposing the action of Ghrelin.

d. Sodium – glucose cotransporters-2 (SGLT-2) inhibitors:

SGLT-2 inhibitors are newer antidiabetic drugs that have successfully cleared phase-III clinical trials.

Ex: Dapaglifozin, Serglifozin and Remoglifozin[18]

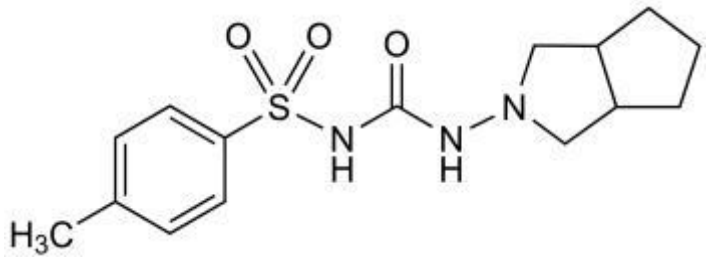
Some drugs that cause hyperglycemia or hypoglycemia:

Hyperglycemia producing drugs include glucocorticoids, antipsychotics, protease inhibitors, beta adrenergic agonists, thiazide and loop diuretics, phenytoin, opioids (fentanyl, morphine), diazoxide, nicotinic acid, pentamidine, interferons, amphotericin-B, asparginase, acomprosate, balisiximab, thyroid hormones[19]

Hypoglycemia producing drugs include Beta adrenergic antagonists, Ethanol, salicylates, Non-steroidal anti-inflammatory drugs, Pentamidine, ACE inhibitors, Lithium chloride, Theophylline, Bromocriptine, Mebendazole [20]

DRUGS USED IN PRESENT STUDY
GLICLAZIDE:
Gliclazide is an oral anti hyperglycemic agent used for the treatment of non-insulin dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin

Chemistry :



N-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-ylcarbamoyl)-4-methylbenzenesulfonamide

Pharmacokinetics:

Absorption : Rapidly and well absorbed

Gliclazide has low volume of distribution (13 to24L) due to its high protein binding affinity (85 to 97%) .

Half life

10.4 hours. Duration of action is 10-24 hours

Metabolism

Extensively metabolized in the liver. Less than 1% of theorally administered dose appears unchanged in the urine.Metabolites include oxidized and

hydroxylated derivates, as well as glucuronic acid conjugates Metabolites and conjugates are eliminated primarily by the kidneys (60-70%) and also in the faeces (10-20%).

Dosage and Administration

Dosage should be initiated at 40mg (1/2 tablet) daily and may be increased if necessary up to 320 mg daily (4 tablets). Dosesup to 160mg daily may be taken in a single dose, preferably at thesame time each morning. Doses in excess of 160mg should be taken in divided doses in the morning and the evening.[21]

Adverse Effects and Drug Interactions :

Hypoglycaemia

Weight gain

Nausea, vomiting

Jaundice

Agranulocytosis

Aplastic and haemolytic anemias

Hypersensitivity

Thiazide diuretics are known to aggravate the diabetic state so caution should be taken Blood sugar control may also be adversely affected where interaction between gliclazide and barbiturates, glucocorticoids or oestrogens occurs. Acute alcohol intoxication potentiates the hypoglycaemic action of sulphonylurea agents. Disulfiram-like reactions with characteristic flushing of the face, throbbing headache, giddiness, tachypnoea, tachycardia or angina pectoris may also occur. The hypoglycaemic effect of gliclazide may be potentiated by insulin, biguanides, sulphonamides, salicylates, coumarin derivatives, chloramphenicol, monoamine oxidase inhibitors, β-blockers, oxyphenbutazone, phenylbutazone, clofibrate, cimetidine and ethanol.[22]

Therapeutic uses :
Adult onset diabetes mellitus (type 2) that cannot be controlled by diet alone

Contraindications :
Gliclazide should not be used in cases where diabetes is complicated by acidosis, ketosis or coma. Gliclazide is contraindicated in severe hepatic or renal insufficiency

DESCRIPTION OF PLANTS

1. Tinospora Cordifolia

Tinospora cordifolia, which is known by the common names *Heart-leaved* Moonseed, Guduchi and Giloy, is an herbaceous vine of the

family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka**.** In ayurvedic practice this is one of the components of the polyherbal preparation used to treat Diabetes.

The stem of *Tinospora cordifolia* is widely used in the therapy of diabetes by regulating the blood glucose in traditional folk medicine of India[23]. It has been reported to mediate its anti-diabetic potential through mitigating oxidative stress (OS), promoting insulin secretion and also by inhibiting gluconeogenesis and glycogenolysis, thereby regulating blood glucose.Alkaloids, tannins, cardiac glycosides, flavonoids, saponins, and steroids as the major phytoconstituents of*Tinospora cordifolia* have been reported to play an anti-diabetic role[24]. The methanol extracts of *Tinospora*

cordifolia have been reported to have potential against microbial infections. In mice models, TCE has been reported to function in bacterial clearance and improved phagocytic and intracellular bactericidal capacities of neutrophils. [25] TCE has been reported of immunostimulant properties on macrophages.

Names :
Sanskrit: Amrita, Hindi: English : Bengali : Telugu : Tamil : Marathi : Gujarati : Kannada :

Amritavalli , Guduchi, Madhuparni,

Chinnaruha, Vatsadaani, Tantrika Kundalini

geloy

Tinospora Gulancha / Indian tinospora

Gulancha

Tippaatiga (Telugu)

Shindilakodi

Shindilakodi

Galo

Amrita balli

Scientific classification:

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

Order: Ranunculales

Family: Menispermaceae

Genus: *Tinospora*

Species: ***T. cordifolia***

Habitat :
Tinospora cordifolia prefers wide range of soil, acid to alkaline and it needs moderate level of soil moisture. Found throughout tropical India ascending to an altitude of 1000 feet and in South Asia, Indonesia, Philippines, Thailand, Myanmar, China and in Srilanka.

Bark : is creamy white to grey, deeply left spirally and stem contains rosette like lenticels.

Leaves : are membranous and cordate in shape.

Flowers : are in axillary position, 2-9 cm long raceme on leaflet branches,

unisexual, small and yellow in color. Male flowers are clustered and female are

usually solitary.

Seeds are curved.

Fruits : are fleshy and single seeded.

Flowers : grow during the summer and fruits during the winter.[26]

Therapeutic uses of plant :
Tinospora cordifolia extracts are extensively used in various herbal preparations for the treatment of different ailments for its anti-periodic, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic properties.[27]

Chemical constituents :
Alkaloids : Berberine , Palmatine, Tembetarine , Magnoflorine ,Choline, Tinosporin, Isocolumbin, Palmatine, Tetrahydropalmatine, Magnoflorine Glycosides : 18-norclerodane glucoside Furanoid diterpene glucoside

Tinocordiside, Tinocordifolioside Cordioside, Cordifolioside A, Cordifolioside B Syringin , Syringin-apiosylglycoside, Palmatosides C, Palmatosides P, Cordifoliside A , Cordiofoliside B, Cordifoliside C, Cordifoliside D , Cordifolisid E Diterpenoid Lactones : Furanolactone Clerodane derivatives,and Tinosporon, Tinosporides and, Jateorine, Columbin Steroids :β-sitosterol ,

δ-sitosterol, 20β- hydroxy ecdysone, Ecdysterone , Makisterone A , Giloinsterol.

Aliphatic compound : Octacosanol , Heptacosanol , Nonacosan. Miscellaneous : 3,4 tetrahydrofuran, Jatrorrhizine, Tinosporidine, Cordifol, Cordifelone, N-trans-feruloyl tyramine as diacetate, Giloin, Giloinin, Tinosporic

Acid.[28]

2. CURCUMA LONGA (TURMERIC)

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family ,Zingiberaceae. It is native to southwest India It needs temperatures between 20 and 30 °C (68 and 86 °F). The bright yellow color of turmeric comes mainly from fat-soluble, polyphenolic pigments known as curcuminoids. Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent. Other curcuminoids found in turmeric include demethoxycurcumin and bisdemethoxycurcumin. In addition to its use as a spice and pigment, turmeric has been used in India for medicinal purposes for centuries. More recently, evidence that curcumin may have anti-inflammatory and anticancer activities has renewed scientific interest in its potential to prevent and treat the disease.

Names :
English – Hindi – Sanskrit – Telugu – Kannada – Gujarati – Marathi –
Turmeric

Haldi

Haridra

Pasupu

Arisina

Halad

Halad

Scientific classification :

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Monocots

(unranked): Commelinids

Order: Zingiberales

Family: Zingiberaceae

Genus: Curcuma
Species: C. longa

Turmeric probably originated in India, However, it is now common throughout Southeast Asia, China and southern Australia and in fact it has been naturalized in all wet tropical regions of the world.Turmeric belongs to the family of Zingiberacea the ginger family. Turmeric is an upright, relatively short and stout plant that rarely grows more than about 1 meter in height. Leaves : are elongated, dark green, and pointed, often curling slightly along the margins.
Rhizomes : grow to about 5-8 cm x 1.5 - 2.5cm, appears scaly due to the remaining rings of previous leaves. Its outer skin is brownish, but its flesh is deep orange-yellow inside.
Flowers : yellow-reddish flowers are arranged spirally along the cylindrical spike, which may be partially protected by a leaf sheath.[29]

Therapeutic uses :
Antioxidant :Curcumin protects against free radical damage because it is strong antioxidant.

Anti inflammatory : its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states.

Hepatoprotective : Turmeric's hepatoprotective effect is mainly a result of its antioxidant properties, as well as its ability to decrease the formation of proinflammatory cytokine.
Anti carcinogenic property : Animal research demonstrates inhibition atall three stages of carcinogenesis-initiation, promotion, and progression.
Anti diabetic : turmeric ethanolic extract containing both curcuminoids and sesquiterpenoids is more strongly hypoglycemic than either curcuminoids or sesquiterpenoids.

Anti microbial : Turmeric extract and the essential oil of Curcuma longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi Cardiovascular diseases : Turmeric's protective properties on the cardiovascular system include lowering cholesterol and triglyceride levels, decreasing susceptibility of low density lipoprotein (LDL) to lipid peroxidation and inhibiting platelet aggregation.

Gastrointestinal disorders : Helicobacter pylori infection, peptic ulcer, Irritable bowel syndrome, Crohn's disease, and ulcerative colitis.[30]

Chemical constituents :
The active constituents of turmeric are the flavonoid Curcuminoids which is a mixture of curcumin (diferuloylmethane), monodexmethoxycurcumin and bisdesmethoxycurcumin Curcumin makes up approximately 90% of the curcuminoid content in turmeric. Other constituents include sugars, proteins, and resins. The best- researched active constituent is curcumin, which comprises0.3-5.4% of raw turmeric.

3. EMBLICA OFFICIANALIS (AMLA)

Emblica officinalis Gaertn. or Phyllanthus emblica Linn, commonly known as Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Various parts of the plant are used to treat a range of diseases, but the most important is the fruit. The fruit is used either alone or in combination with other plants to treat many ailments such as common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory. Amla, due to its high vitamin C content, is effective in controlling diabetes by stimulating the pancreas and enable is to secrete insulin, thus reducing the blood sugar in the diabetes.[31]

The tree is small to medium in size, reaching 8–18 m (26–59 ft) in height, with a crooked trunk and spreading branches.

Branches : are glabrous or finely pubescent, 10–20 cm (3.9–7.9 in) long, usually deciduous;

Leaves : are simple, subsessile and closely set along branchlets, light green, resembling pinnate leaves.

Flowers : are greenish-yellow. The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with six vertical stripes or

furrows. Found in India, Pakistan, Uzbekistan, Srilanka, South East Asia,

China and Malaysia. Fruits ripen from November to February

Common names :
Sanskrit : amalika

Hindi : amla

Gujrati : amla

Telugu : rasi usiri

Tamil : Nellikaai

Bengali : amloki

Oriya : anlaa

Punjabi :Aula

Scientific classification

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Rosids

Order: Malpighiales

Family: Phyllanthaceae

Tribe: Phyllantheae

Subtribe: Flueggeinae

Genus: Phyllanthus

Species: P. emblica

Therapeutic uses:

- Inhibits the aging process
- Prevents common cold
- Nutritional powerhouse and hence used to boost immunity and restore body's vitality.
- Provides energy to vital organs and hence used in chronic illness recovery.
- Helps in regulating blood glucose levels in diabetic patients.
- Facilitates absorption of iron in the body and improves hemoglobin level.
- Possess potent anti-inflammatory action and hence used in various gastrointestinal tract inflammation such as gastritis.
- Contains natural digestive enzymes and hence used in indigestion.
- Reduces serum cholesterol and reduces high blood pressure.[32]

Chemical constituents :
The fruits contain a high concentration of ascorbic acid, which degrades with heating or cooking. In addition, they contain phenols, including ellagic acid, gallic acid, quercetin, kaempferol, corilagin, geraniin, furosin, gallotanins, emblicanins, flavonoids, glycosides, and proanthocyanidins. The roots contain glycosides and tannins. [33]

Most of the properties assigned to emblica are attributed to its strong antioxidant action. The ascorbic acid content of the fruit has been assayed as approximately 900 mg per 100 gm of fresh pressed juice and accounts for 45% to 70% of the antioxidant activity.[34]

4. NEEM (AZADIRACHTA INDICA)

Neem (Azadirachta indica), is native of India and naturalized in most of tropical and subtropical countries are of great medicinal value and distributed widespread in the world. The chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, lavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones, biologically most active compound is azadirachtin A-G and

azadirachtin E is more effective.Aqueous extract ofneem significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia. Recently, hypoglycaemic effect of neem was observed within normal as well as alloxan-induced diabetic rabbits.

Common names :

English	- Margosa, Neem Tree
Hindi	- Neem
Marathi	- Kadunimba
Tamil	- Veppai , Sengumaru
Malayalam	- Ariyaveppu
Telugu	- Vepa
Kannada	- Turakabevu
Punjabi	- Nimm
Urdu	- Neem
Gujarati	- Dhanujhada , Limba
Sanskrit	- Pakvakrita, nimbaka

Scientific classification

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Rosids

Order: Sapindales

Family: Meliaceae

Genus: Azadirachta

Species: A. indica

Therapeutic uses :
Immunostimulant, Hypoglycaemic activity, Antiulcer effect, Antifertility effect.

Antimalarial activity, Antifungal activity, Antibacterial activity, Antiviral activity,

Anticancer activity, Antioxidant activity, Effect on central nervous system [35]

Chemical constituents :

The most common active compounds found in the neem tree are *Azadirachtin:* Provides repellant, anti-hormonal and anti-feedant properties *Nimbin:* Provides anti-inflammatory, anti-pyretic, antihistamine and antifungal properties

Nimbidin: Provides antibacterial, anti-ulcer, analgesic, anti-arrhythmic and antifungal properties

Nimbidol: Provides anti-tubercular, anti-protozoan and antipyretic properties

Sodium nimbinate: Provides diuretic, spermicidal and anti-arthritic properties

Gedunin: Provides vasodilator, anti-malaria and antifungal properties

Salannin: Provides repellant properties

Quercetin: Provides anti-protozoal, antioxidant, anti-inflammatory and antibacterial properties

Neem seed oil, leaves and bark contains the concentrations of these active compounds along with many fatty acids like oleic acid, stearic acid, palmitic acid, linoleic acid, etc.[36]

IV. Materials And Methods

The study was conducted at Department of Pharmacology, Gandhi Medical College. Ethical clearance was obtained from Institutional Animal Ethics Committee meeting conducted on 29-03-2014 in Gandhi Medical College, Secunderabad before conducting the experiment.

MATERIALS

A. Chemicals: Alloxan monohydrate (NH-CO-NH-CO-CO.H2O) – Lobal chemical laboratory, distilled water, 0.9% Normal saline, 25% dextrose, xylol, surgical spirit.

B. DRUGS: Tab Gliclazide 25mg/kg

C. Plant material: alcoholic extract of polyherbal preparation

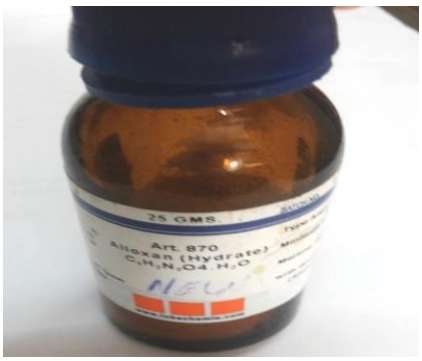
D. Equipment : Tuberculin syringe, syringes(2ml, 5ml, 10ml), Spirit, surgical gloves, cotton, Weighing Balance, Markers, Cages, Oral feeding tube, Beakers, glucometer, HbA1c kits, capillary tubes, glass slides, sample collection bottles.

E. Animals: adult male albino wistar rats

1.Chemicals & Drugs:

a.Alloxan monohydrate:-5% Alloxan Monohydrate dissolved in 0.9%

NaCl was used for induction of diabetes in the present study.



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b. Gliclazide :

Selection of the Dose :
The dose was selected using Animal equivalent dose (AED) calculation for gliclazide. Based on the respective body weight doses were administered.

Absolute Human dose = 60mg/70kg= 0.857

AED[37]= Human Dose in mg/kg ÷ (Animal weight in kg/human weight in kg)$^{0.33}$

C.Polyherbal Extract :

PREPARATION OF EXTRACT: Fine powder of the four plants(Turmeric - root, Amla-fruit, Neem-bark and Tinospora-stem) were purchased from local market. These four ingredients were taken in equal proportions. This mixture was packed into thimble of filter paper and put in Soxhlet extractor in 5 batches of 200gm each and subjected to continuous extraction with 99.99% ethanol for about 48 hours at 600C till solvent in the siphon tube becomes colorless and it

took around 8-10 cycles/200 gram powder. Small porcelain pieces were added to the flask to avoid bumping of solvent. The solvent so obtained was distilled off and was heated evaporated using water bath /magnetic heart stirrer to get concentrated thick extract. This extract is prepared at department of Botany Osmania University, College for women, Koti, Hydreabad.

2.Animals

Healthy adult male albino rats weighing 150 to 250 g were used in the experiment. Rats were obtained from the central animal house of Gandhi Medical College, secunderabad with CPSCEA registration no.428/01C, date 20/6/2001.They were housed in appropriately labelled cages in a room maintained at 12 hour light-dark cycles and at a constant temperature of 24±2°C.They are kept under observation for one week before experiment. The animals were fed with standard pellet diet, water ad libitum.

3.Equipments:

a) **Tuberculin syringe:** used for intra peritoneal injection of alloxan.

b) **Feeding tube:** used for the administration of the standard drug and test compounds.

c) **Glucometer:** used for measuring blood glucose levels.

d) **HbA1c kit:** used for Glycated Hemoglobin estimation by ion exchange resin method.

METHODS:

Grouping:

Thirty male Albino Rats were randomised into 5 groups. All Rats were allowed a one-week acclimatization period to become accustomed to the laboratory conditions. Rats were randomly divided into five groups, each comprising six Rats.

GROUP I : Normal control ,distilled water.

GROUP II : Standard control (Gliclazide)

GROUP III : (T 1) – administered 300 mg/kg dose of polyherbal preparation.

GROUP IV : (T 2) – administered 400 mg/kg dose of polyherbal preparation. GROUP V : (T 3) – administered 500 mg/kg dose of polyherbal preparation through oral route for a period of 45 days.

Duration of the Study:

The duration of the study was for 60 days and included 15 days of diabetic induction period and next 45 days of treatment period. The study duration did not include days for acclimatization.

Induction of diabetes in rats :

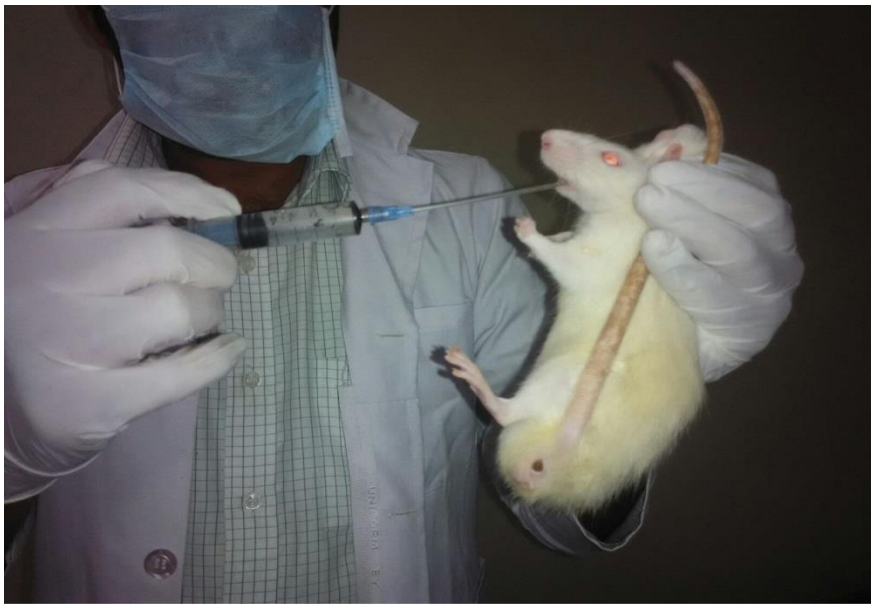
Freshly prepared 5%(dissolved in 0.9% W/V normal saline) solution of Alloxan monohydrate (130mg/kg) was injected intraperitoneally to overnight fasted rats to induce diabetes.

Fasting blood glucose level was estimated at the time of induction of diabetes and regularly thereafter till stable hyperglycemia was established. Rats were kept for the next 24 hrs on oral 10% glucose to prevent

hypoglycemia. After 72 hrs of induction, the fasting blood glucose levels in rats increased and animal developed stable hyperglycemia after 4 days. Rats with fasting blood sugar greater than 200mg/dl were selected for study.

1.Normal control group :
This group of 6 non diabetic animals were taken as control group for study. Animals were fed with 0.5ml distilled water for 45 days. Blood samples were taken on 0, 7,14, 28, and 42 days for fasting blood sugar measurement. On 0 and 45 days HbA1c, S.Creatinine, CRP and WBC counts are taken.

2. Standard Group :
This group consisting of 6 animals were rendered diabetic by injecting alloxan monohydrate 5% in 0.9% normal saline intraperitoneally (130mg/kg). The animals were carefully observed for any untoward effects like hypoglycemia and ketosis.. Later animals were given Gliclazide 25mg/kg orally through intrgastric tube daily once in morning for 45 days. . Blood samples were taken on 0, 7, 14, 28, and 42 days for fasting blood sugar measurement. On 0 and 45 days HbA1c, S.Creatinine, CRP and WBC counts are taken.



3. Test Groups :

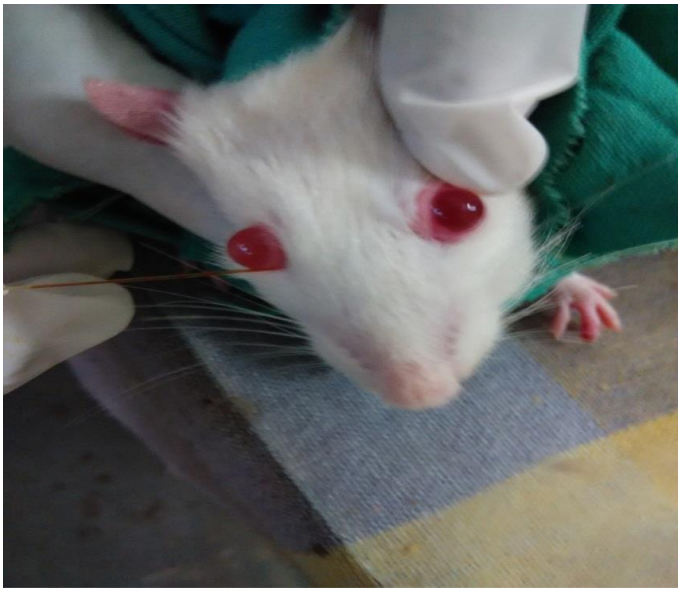
This group of animals were rendered diabetic by injecting alloxan monohydrate 5% in 0.9% normal saline intraperitoneally (130mg/kg). The animals were carefully observed for any untoward effects like hypoglycemia,and ketosis..These animals were divided into 3 groups with 6 animals in each group :

GROUP 3 : (T 1) - administered 300 mg/kg dose of polyherbal preparation.

Group 4 : (T 2) - administered 400 mg/kg dose of polyherbal preparation.

Group 5 : (T 3) – administered 500 mg/kg dose of polyherbal preparation

The alcoholic ployherbal extract to each rat in different groups is given as 0.5ml of 2% gum acacia solution, through oral route for a period of 45 days. Blood samples were collected as in standard group schedule.



The various biochemical parameters in this study are estimated by following methods. Blood glucose by glucose oxidase method. The estimation of HbA1c levels by HbA1c kit which follows ion exchange resin method. The estimation of total white blood cells was done by visual method using New Improved Neubauer counting chamber.[38] Determination of serum creatinine levels done by Creatininase and creatinase method.[39] after centrifugation of blood to separate serum. Estimation of CRP levels is done by turbidometric immunoassay.[40]

V. Statistical Analysis:

Results were expressed as mean ± standard deviation (SD) of six values (n=6) for each group. Statistical differences between the controls and the treatment groups were evaluated by using one way ANOVA (Analysis of Variance) followed by Post-hoc Tukey's test and within group analysis by student paired t test.

Values were considered		
1.	Not significant	P > 0.05
2.	Significant	P <0.05*
3.	Highly significant	P <0.01 **
4.	Very highly significant	P <0.001***

Calculation of Percentage Change in serum parameters:

Percentage change from initial values calculated by the formula

Percent Change (%) on day 45 = ———————————————

VI. Results

Effect of polyherbal extract preparation in alloxan induced Diabetic rats in comparision with standard drug (Gliclazide) is observed as follows

GROUP I : Normal control ,distilled water.

GROUP II : Standard control (Gliclazide 25mg/kg)

GROUP III : (T 1) – administered 300 mg/kg dose of polyherbal preparation.

GROUP IV : (T 2) – administered 400 mg/kg dose of polyherbal preparation. GROUP V : (T 3) – administered 500 mg/kg dose of polyherbal preparation through oral route for a period of 45 days.

Blood glucose Analysis:

Table 1: statistical analysis (ANOVA) showing comparision of blood glucose levels (mg/dl) between different groups on different days (0,7,14,28,42).

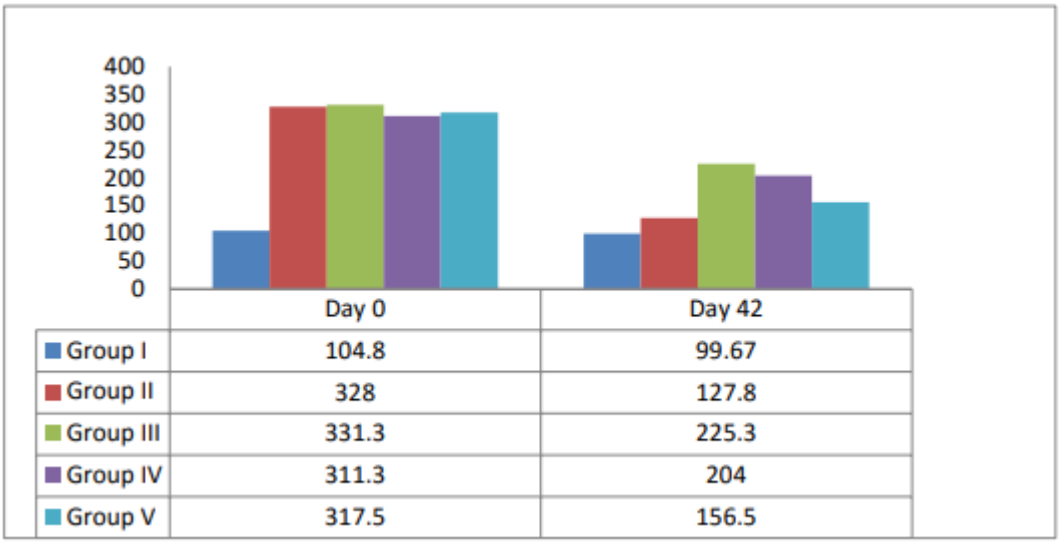
Group	Treatment	Day0 (Mean± SD)	Day7 (Mean± SD)	Day14 (Mean± SD)	Day28 (Mean± SD)	Day42 (Mean± SD)
I	Normal control	104.8±10.28	106.8± 8.49	95.17±7.75	96.0 ±7.34	99.67± 9.04
II	Standard control (Gliclazide 25mg/kg	328.0± 17.15	241.5± 7.635	197± 12.20	157.5±11.10	127.8± 10.05
III(T1)	Extract 300mg/kg	331.3± 20.02	260.5± 15.29	243.2± 13.29	228.8±14.27	225.3± 8.14
IV(T2)	Extract 400mg/kg	311.3± 17.34	244.0± 8.53	229.7± 6.12	205.8± 10.01	204.0± 7.01
V(T3)	Extract 500mg/kg	317.5± 16.12	242.8± 9.02	218.3± 7.23	169.8± 6.01	156.5± 5.57
	F value	209.4	231.5	221.3	175.2	284.5
	P value	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***

Post-hoc tukey's multiple comparison test

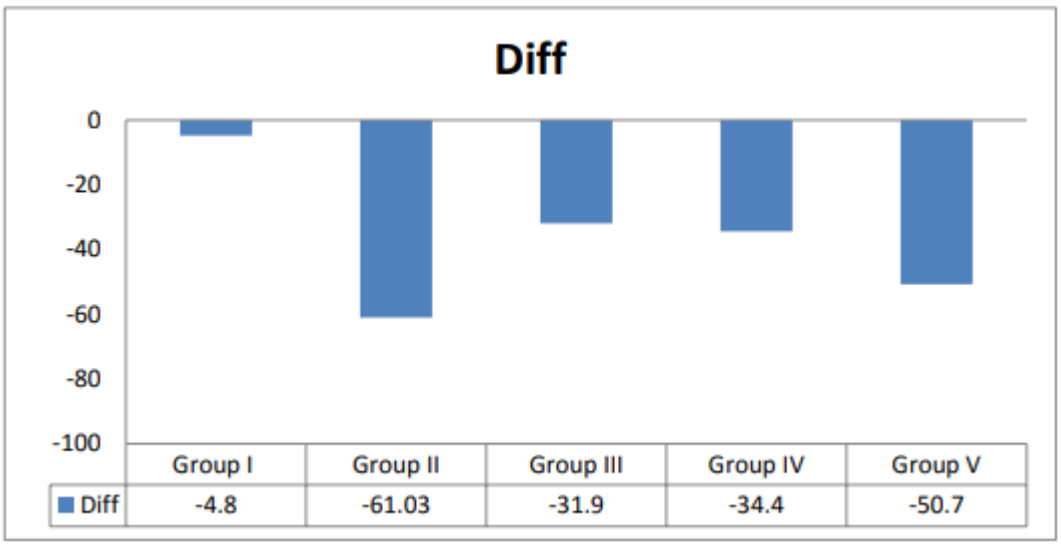
Groups compared	Day 0	Day 42
Standard vs Group III (T1)	0.49	31.57***
Standard vs Group IV (T2)	2.47	24.66***
Standard vs Group V (T3)	1.55	9.28***

Group III (T1) vs Group IV (T2)	2.96	6.90**
Group III (T1) vs Group V (T3)	2.05	22.29***
Group IV (T2) vs Group V (T3)	0.91	15.38***

Bar diagram 1 : showing day wise variation in blood glucose levels in various study groups. Y-axis represents blood glucose levels in mg/dl



Bar diagram 2: showing percentage reductions of mean blood glucose levels In 42 days for Normal Control, Standard, Test 1, Test 2 and Test 3 groups



HbA1c Analysis:

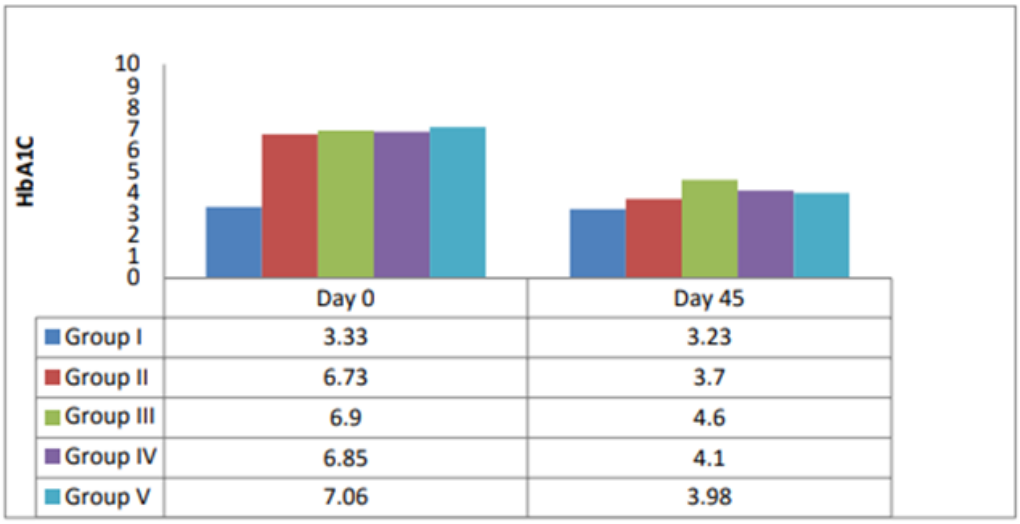
Table2: statistical analysis (ANOVA) showing comparison of HbA1c Levels between different groups on day 0 (Before treatment) and day 45(After treatment) followed by post-hoc tukey`s multiple comparision test.

Group	Treatment	Day 0(Mean± SD)	Day45(Mean± SD)
I	Normal control	8.33± 0.57	8.23± 0.27
II	Standard control (Gliclazide 25mg/kg)	6.73± 0.42	8.7± 0.38
III (T1)	Extract 300mg/kg	6.90± 0.40	4.60± 0.54
IV (T 2)	Extract 400mg/kg	6.85± 0.41	4.10± 0.28
V (T3)	Extract 500mg/kg	7.06± 0.42	8.98± 0.26
	F value	73.93	11.30
	P value	<0.0001***	<0.001***

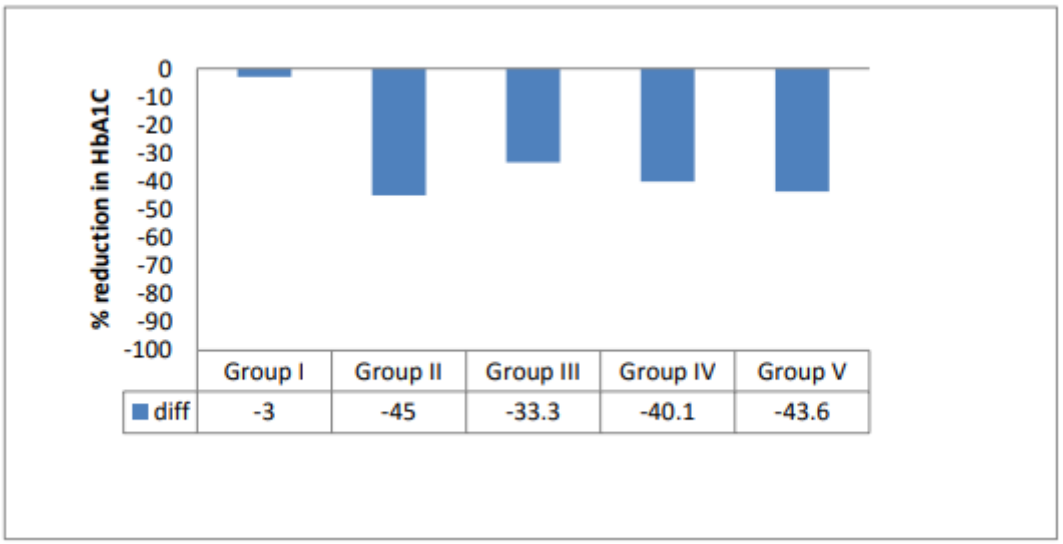
Post-hoc tukey's multiple comparison test

Groups compared	Day 0	Day 45
Standard vs Group III (T1)	0.89	5.99**
Standard vs Group IV (T2)	0.62	2.66
Standard vs Group V (T3)	1.79	1.88
Group III (T1) vs Group IV (T2)	0.26	3.33
Group III (T1) vs Group V (T3)	0.89	4.10
Group IV (T2) vs Group V (T3)	1.16	0.77

Bar diagram3: showing before and after treatment variation in HbA1c levels in various groups. Y-axis represents HbA1c levels in %.



Bar diagram 4: showing percentage reductions of mean HbA1c levels in 45 days for Normal Control, Standard, Test 1, Test 2 and Test 3 groups.



Serum creatinine analysis :

TABLE 3 : statistical analysis (ANOVA) showing comparison of creatinine levels (mg/dl) between different groups on day 0 (Before treatment) and day 45(After treatment) followed by post-hoc tukey`s multiple comparision test.

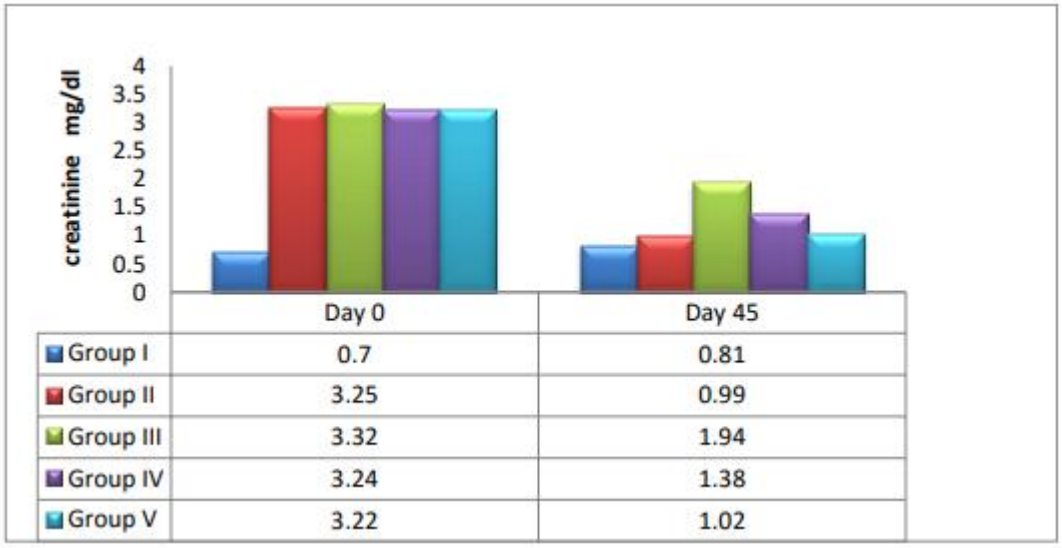
Group	Treatment	Day 0(Mean± SD)	Day45(Mean± SD)
I	Normal control	0.70± 0.06	0.81± 0.05
II	Standard control (Gliclazide 25mg/kg)	3.25± 0.36	0.99± 0.1
III (T1)	Extract 300mg/kg	3.32± 0.37	1.94± 0.23

IV (T 2)	Extract 400mg/kg	3.24± 0.4	1.38± 0.18
V (T3)	Extract 500mg/kg	3.22± 0.29	1.02± 0.21
	F value	66.89	51.63
	P value	<0.0001***	<0.0001***

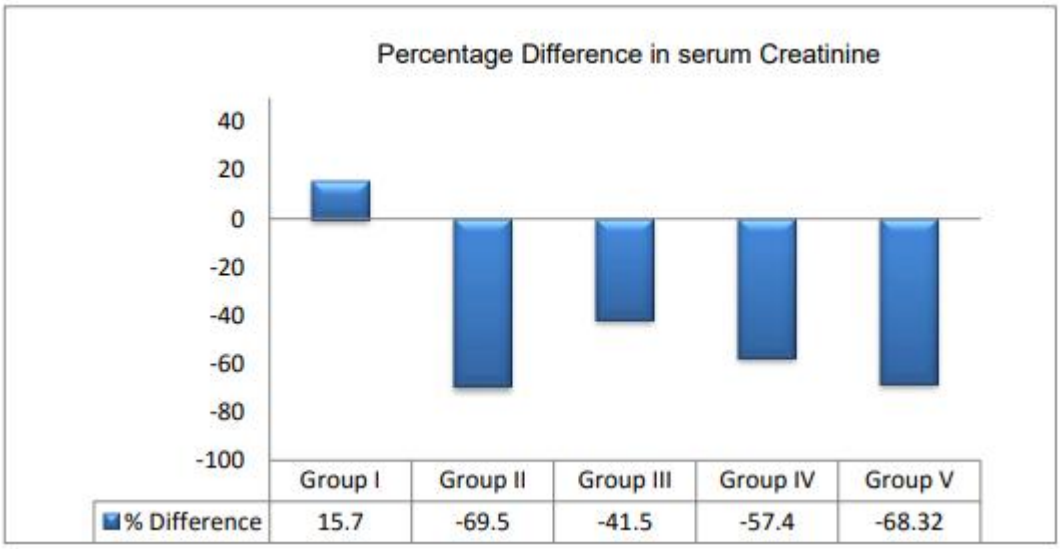
Post-hoc tukey's multiple comparison test

Groups compared	Day 0	Day 45
Standard vs Group III (T1)	0.511	15.24***
Standard vs Group IV (T2)	0.107	6.225**
Standard vs Group V (T3)	0.190	0.509
Group III (T1) vs Group IV (T2)	0.618	9.015***
Group III (T1) vs Group V (T3)	0.701	14.73***
Group IV (T2) vs Group V (T3)	0.083	5.715**

Bar diagram 5: showing day wise variation in serum creatinine levels in various study groups. Y-axis represents serum creatinine levels in mg/dl



Bar diagram 6: showing percentage reductions of mean serum creatinine levels in 45 days for Normal Control, Standard, Test 1, Test 2 Test 3 groups



WBC Count Analysis :

Table 4: statistical analysis (ANOVA) showing comparison WBC count (/ml) between different groups on day 0 (Before treatment) and day 45(After treatment) followed by post-hoc multiple comparision test.

Group	Treatment	Day 0(Mean± SD)	Day45(Mean± SD)
I	Normal control	9.817±0.926	10.233±1.334
II	Standard control (Gliclazide 25mg/kg)	20.467±1.926	12.450 ±1.823
III (T1)	Extract 300mg/kg	19.933±1.638	14.900±1.183
IV (T 2)	Extract 400mg/kg	20.333±2.370	13.500±2.019
V (T3)	Extract 500mg/kg	21.100 ±2.119	13.117±1.513
	F value	39.45	6.821
	P value	<0.0001***	0.0007***

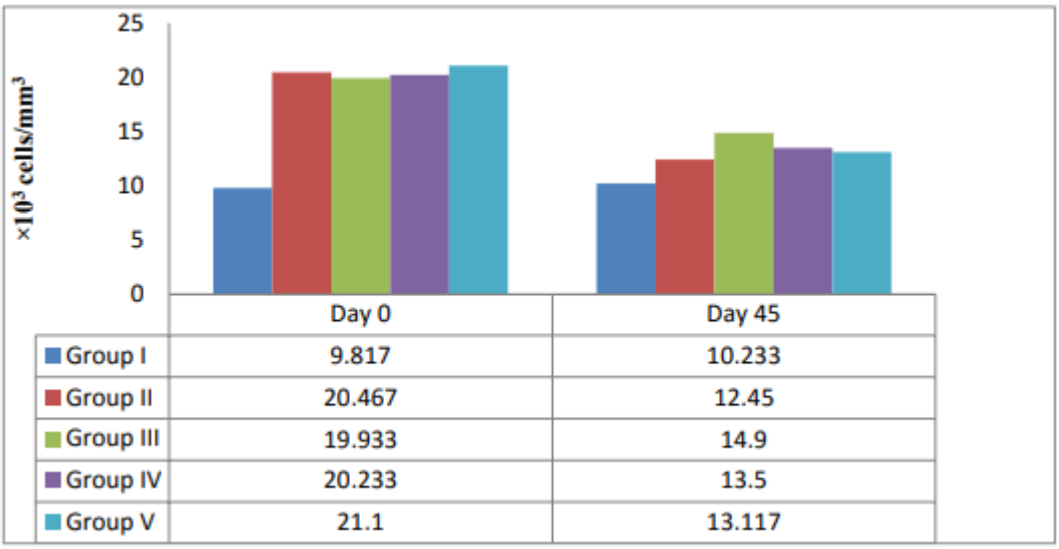
Counts are expressed in × 10^3 cells/mm^3

Post-hoc tukey's multiple comparison test

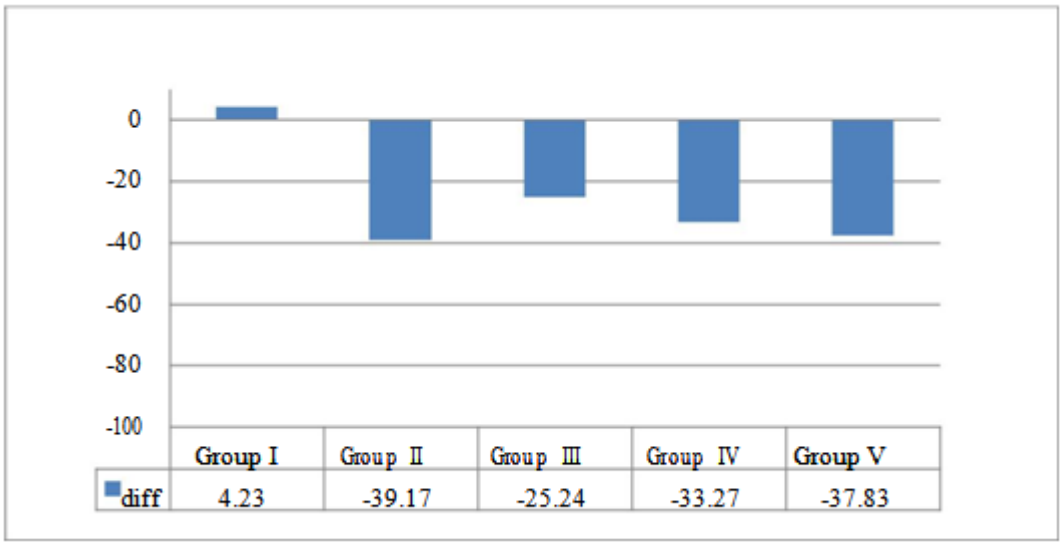
Groups compared	Day 0	Day 45
Standard vs Group III (T1)	0.701	3.741
Standard vs Group IV (T2)	0.175	1.603
Standard vs Group V (T3)	0.832	1.018

Group III (T1) vs Group IV (T2)	0.525	2.138
Group III (T1) vs Group V (T3)	1.534	2.723
Group IV (T2) vs Group V (T3)	1.008	0.585

Bar diagram 7: showing day wise variation in WBC counts in various study groups. Y-axis represents WBC counts in × 10^3 cells/mm^3.



Bar diagram 8: showing percentage reductions of mean WBC counts in 45 days for Normal Control, Standard, Test 1, Test 2 Test 3 groups



C-Reactive protein Analysis :

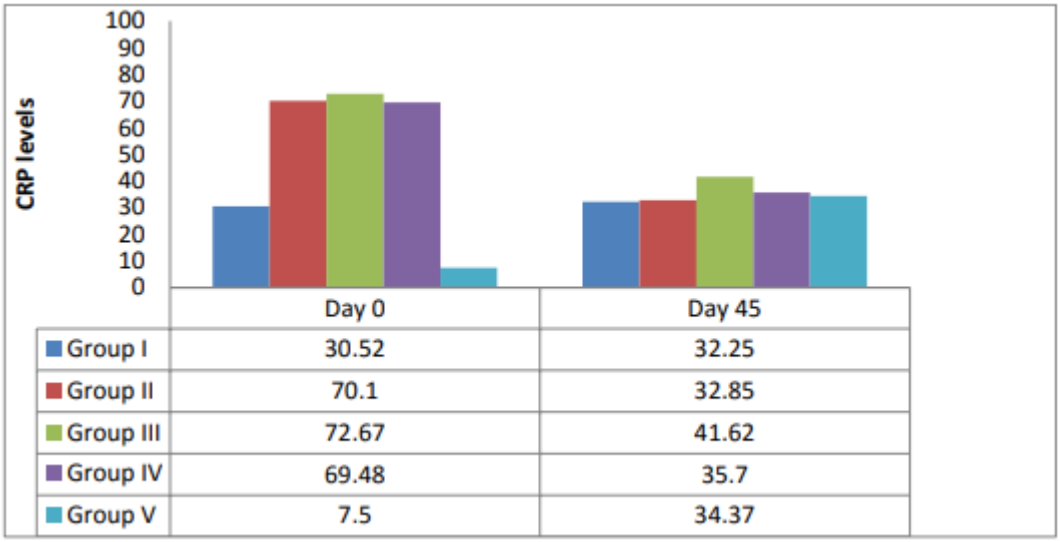
Table 5: statistical analysis (ANOVA) showing comparison of CRP (mg/dL) between different groups on day 0 (Before treatment) and day 45(After treatment) followed by post-hoc multiple comparision test

Group	Treatment	Day 0	Day 45
I	Normal control	30.52± 4.89	32.25± 1.77
II	Standard control (Gliclazide 25mg/kg)	70.10± 4.40	32.85± 2.45
III (T1)	Extract 300mg/kg	72.67 ±12.95	41.62 ±13.11
IV (T 2)	Extract 400mg/kg	69.48 ±10.63	35.70± 4.97
V (T3)	Extract 500mg/kg	75.0 ±6.81	34.37±4.24
	F value	28.01	1.884
	P value	<0.0001***	0.144

Post-hoc tukey's multiple comparison test

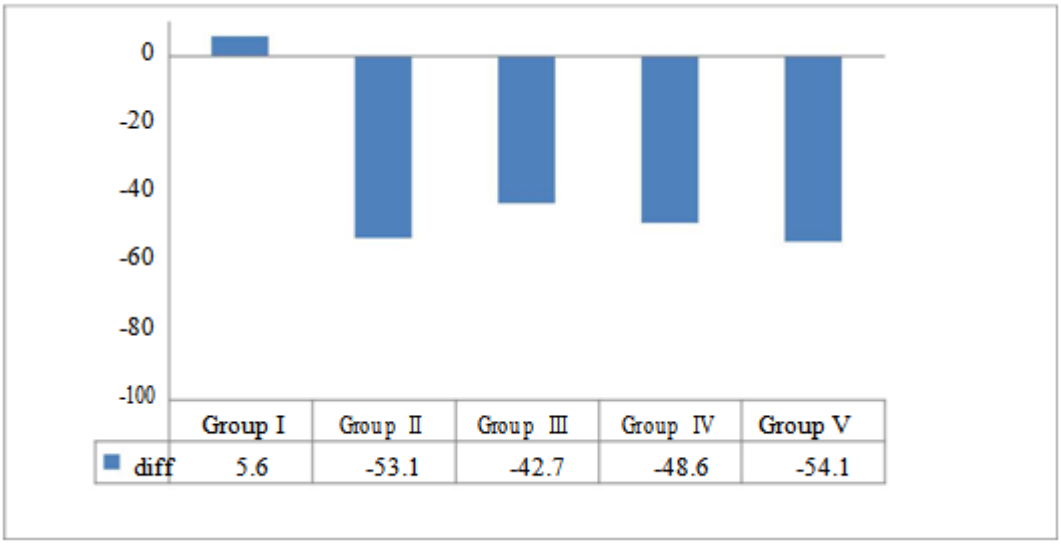
Groups compared	Day 0	Day 45
Standard vs Group III (T1)	0.730	3.210
Standard vs Group IV (T2)	0.175	1.043
Standard vs Group V (T3)	1.394	0.555
Group III (T1) vs Group IV (T2)	0.905	2.166
Group III (T1) vs Group V (T3)	0.664	2.654
Group IV (T2) vs Group V (T3)	1.570	0.4882

Bar diagram 9: showing day wise variation in serum CRP levels in various study groups. Y-axis represents serum CRP levels in mg/dl



	Day 0	Day 45
Group I	30.52	32.25
Group II	70.1	32.85
Group III	72.67	41.62
Group IV	69.48	35.7
Group V	7.5	34.37

Bar diagram 10: showing percentage reductions of mean serum CRP levels in 45 days for Normal Control, Standard, Test 1, Test 2 and Test 3 groups



	Group I	Group II	Group III	Group IV	Group V
diff	5.6	-53.1	-42.7	-48.6	-54.1

VII. Discussion

The present study evaluated the antidiabetic effect of polyherbal preparation in comparison with Gliclazide in "Alloxan induced diabetic rat model".

GROUP I : Normal control ,distilled water.

GROUP II : Standard control

GROUP III : (T 1) –administered 300 mg/kg dose of polyherbal preparation.

GROUP IV : (T 2) – administered 400 mg/kg dose of polyherbal preparation. GROUP V : (T 3) – administered 500 mg/kg dose of polyherbal preparation through oral route for a period of 45 days.

Analysis of blood glucose levels:
Table1-shows before(day 0) and After(day 42) treatment variations in mean blood glucose levels in each group of rats and ANOVA analysis followed by Post-hoc Tukeys test .

- Mean blood glucose levels in Normal Control (Group I) varied from104.8±10.28 mg/dl to 99.67± 9.04mg/dl without much variation during

the study.

- Mean blood glucose levels in Standard (Group II) varied from 328.0± 17.15mg/dl to 127.5±10.05mg/dl which was statistically *very highly*

significant (p<0.0001***) and mean percentage reduction was 61.03%.

- Mean blood glucose levels in Test-1 (Group III) varied from 331.3± 20.02 mg/dl to 225.3± 8.14mg/dl which was statistically significant(p=<0.0001***)

and mean percentage reduction was 31.9%.

- Mean blood glucose levels in Test-2 (Group IV) varied from 311.3±17.34mg/dl to 204±7.01mg/dl which was statistically highly

significant (p=<0.0001***) and mean percentage reduction was 34.4%.

- Mean blood glucose levels in in Test-3 (Group V) varied from 317.5±16.12 mg/dl to 156.5± 5.57 mg/dl which was statistically *very highly significant* (p

<0.0001***) and mean percentage reduction was 50.70%

- **On Day 0 (Before treatment):** one-way ANOVA analysis showed that there was *highly significant* mean difference (p<0.0001**) between normal

control and other groups. Post-hoc tukey's multiple comparison test showed *highly significant* mean difference between Normal Control group and other four groups whereas no significance between standard, Test1, Test2 and Test 3 groups.

- **On Day 42 (After treatment):**one-way ANOVA analysis showed that there was significant mean difference between overall groups. Post-hoc tukey's

multiple comparison tests showed significance mean difference between standard and test groups(T1, T2, T3). In T1 & T2 groups there is reduction blood glucose levels, but not to the normal levels. In case of T3 group at the end, the blood glucose level is comparable to that of standard group.

Analysis of HbA1c levels:
Table2 – shows before(day 0) and After(day 45) treatment variations mean

HbA1c levels in each group of rats and ANOVA analysis followed by Post-hoc

Tukeys test.

- Mean HbA1c levels in Normal Control group varied from 3.33± 0.57% to 3.23±0.27% without significant variation during the study period.

- Mean HbA1c levels in Standard group varied from 6.73± 0.42% to 3.7±0.38% which was statistically *very highly significant* (p< 0.0001***) and

mean percentage reduction was 45%.

- Mean HbA1c levels in Test-1 group varied from 6.90± 0.40% to 4.60± 0.54% which was statistically highly significant(p=0.0006***) and mean

percentage reduction was 33.3%.

- Mean HbA1c levels in Test-2 group varied from 6.85 ±0.41% to 4.10± 0.28% which was statistically highly significant (p<0.0001***) and mean

percentage reduction was 40.1%.

- Mean HbA1c levels in Test 3 group varied from7.06 0.42% to 3.98 0.26% which was statistically *very highly significant* (p<0.0001***) and mean

percentage reduction was 43.6%.

- **On Day 0 (Before treatment):** one-way ANOVA analysis showed that there was very highly significant mean difference(p<0.0001***) between

normal control and other groups. Post-hoc tukey's multiple comparison test showed *very highly significant* mean difference between Normal Control group and other four groups whereas no significanct difference between standard, Test1, Test2 and Test 3 groups.

- **On Day 45 (After treatment):**one-way ANOVA analysis showed that there was *very highly significant* mean difference (p<0.0001***) between normal

control and other groups. Post-hoc tukey's multiple comparison test showed *significant* mean difference between Normal Control group and test groups (T1, T2, T3); *no significant* mean difference between standard and T2, T3 groups and also in between test groups (T1, T2, T3). But there is significant mean difference between standard and T1 group.

Guruprasad Rao, Surekha Bhat et al in 2013 studied the effect of Nishamalaki powder which contains Curcuma longa and Emblica officianalis in diabetic rats which showed that there is significant decrease in blood glucose levels and HbA1c levels in diabetic rats which is comparable to standard drug.[41]

In another study by Nagaraja Puranik et al showed that Tinospora cordifolia effectively reduces the fasting blood sugar levels in rats. This study also shows that rats treated with Tinospora showed increased activity of liver glycogen synthase which could be responsible for decreased blood sugars in peripheral circulation.[42]

Ashok Purohit And V. P. Dixit studied the anti diabetic effect of neem bark and flower in diabetic mice which showed that there is decreased blood sugar upto 54.39% by neem bark extract..[43]The percentage reduction of blood sugar in present study is 50.70% which is comparable to above study values.

Chandrashekar singh et al showed the anti diabetic effect of tinospora cordifolia, which showed that in rats treated with tinospora showed increased hepatic glycogen synthase and decreased glycogen phosphorylase activity leading to decresed blood glucose in diabetes.[44

Mai A Elobeid and Elham A Ahmed studied the antidiabetic effect of amla fruit extract in STZ induced diabetic rats. This study shows that bloos glucose reduced from 306mg/dl to 146mg/dl with amla which is comparable to results in the present study.[45]

Serum Creatinine Analysis :

Table3 – shows before(day 0) and After(day 45) treatment variations mean serum creatinine levels in each group of rats and ANOVA analysis followed by Post-hoc Tukeys test.

- Mean creatinine levels in Normal Control group varied from 0.7 ±0.06mg/dl to 0.81± 0.05mg/dl without much variation during the study period.

- Mean creatinine levels in Standard group varied from 3.25 ±0.36 to 0.99± 0.1 which was statistically *very highly significant* (p=< 0.0001***) and mean

percentage reduction was 69.5%.

- Mean creatinine levels levels in Test-1 group varied from 3.32±0.37mg/dl to 1.94± 0.23mg/dl which was statistically significant($p < 0.0001$***) and

mean percentage reduction was 41.5%.

- Mean creatinine levels in Test-2 group varied from 3.24 ±0.4 to 1.38 ±0.18 which was statistically highly significant ($p < 0.0001$***) and mean

percentage reduction was 57.4%.

- Mean creatinine levels in Test 3 group varied from 3.22± 0.29 to 1.02 ±0.21 which was statistically *very highly significant* ($p<0.0001$***) and mean

percentage reduction was 68.32%

- **On Day 0 (Before treatment):** one-way ANOVA analysis showed that there was very highly significant mean difference($p=0.0001$***) between

normal and other groups. Post-hoc tukey's multiple comparison test showed *very highly significant* mean difference between Normal Control group and other four groups whereas no significanct difference between standard, Test1, Test2 and Test 3 groups.

- **On Day 45 (After treatment):**one-way ANOVA analysis showed that there was *very highly significant* mean difference ($p=0.0001$***) between overall

groups. Post-hoc tukey's multiple comparison test showed *significant* mean difference between Normal Control group and test groups (T1, T2, T3); and also inbetween Standard & T1; Standard & T2; T1 & T2; T1 & T3 groups where as *no significant* mean difference between standard & T3 groups, which indicates that the effect of T3 dose on creatinine levels is comparable to that of standard drug.

Snehal S Patel, Rajendra S Shah & Ramesh K Goyal studied effect of Dihar which contains amla, neem, curcuma, tinospora and four other plant products(syzygium cumini, Momordica charantia, Gymnema sylvestre, Enicostemma) showed significant decrease in blood glucose, LDL, Triglyceride, urea and creatinine levels in animals treated with Dihar for a period of 6 weeks.[47]In present study the polyherbal extract reduces blood glucose and creatinine levels comparable to the standard drug.

Shravan Kumar Dholi, Ramakrishna Raparla et al studied antidiabetic activity of neem in alloxan induced rats, which showed decreased serum concentration in glucose, total cholesterol, urea and creatinine in groups treated with neem.[48]

Murugan and Pari studied the effect of tetrahydrocurcumin on Hepatic and Renal functional markers in diabetic rats. Increased levels of urea, creatinine, uric acid in diabetes reversed to normal after treatment with curcuma.[49]

Total WBC count Analysis :
Table4 – shows before(day 0) and After(day 45) treatment variations mean leucocyte count in each group of rats and ANOVA analysis followed by Post-hoc Tukeys test.

- Mean leucocyte counts in Normal Control group varied from 9.81±0.92 to 0.81±10.23 1.33 $\times 10^3$ cells/mm^3 without significant difference during the

study period

- Mean leucocyte counts in Standard group varied from 20.46±1.9 to 12.45±1.82 $\times 10^3$ cells/mm^3 which was statistically *very highly significant*

(p=< 0.0001***) and mean percentage reduction was 39.17%.

- Mean leucocyte counts in Test-1 group varied from 19.93 ±1.63 to 14.9± 1.18 $\times 10^3$ cells/mm^3 which was statistically significant(p< 0.0001***) and

mean percentage reduction was 25.24%.

- Mean leucocyte counts in Test-2 group varied from 20.33±2.37 to 13.5± 2.09 $\times 10^3$ cells/mm^3 which was statistically highly significant (p<

0.0001***) and mean percentage reduction was 33.27%.

- Mean leucocyte counts in Test 3 group varied from 21.1±2.1 to 13.11±1.51 $\times 10^3$ cells/mm^3 which was statistically *very highly significant* (p<0.0001***)

and mean percentage reduction was 37.83%.

- **On Day 0 (Before treatment):** one-way ANOVA analysis showed that there was very highly significant mean difference(p=0.0001***) between

normal and other groups. Post-hoc tukey's multiple comparison test showed *very highly significant* mean difference between Normal Control group and other four groups whereas no significanct difference between standard, Test1, Test2 and Test 3 groups.

- **On Day 45 (After treatment):**one-way ANOVA analysis showed that there was *very highly significant* mean difference (p<0.0001***) between normal and other groups. Post-hoc tukey's multiple comparison test showed *significant* mean difference between Normal Control group and test groups (T1, T2, T3); *no significant* mean difference between standard and Test groups(T1,T2, T3 groups) and also in between test groups (T1, T2, T3).

Itemobong S. Ekaidem et al studied the effect of azadirachta indica on blood glucose levels and hematological parameters in diabetic wistar rats, which showed decreased WBC counts in treated group when compared to diabetic control.[51] In present study there is there is decreased blood glucose and leucocyte count in groups treated with polyherbal extract similar to above study.

Analysis of serum CRP levels :

Table5 – shows before(day 0) and After(day 45) treatment variations mean serum CRP levels in each group of rats and ANOVA analysis followed by Post-hoc Tukeys test.

- Mean serum CRP levels in Normal Control group varied from 30.52± 4.89mg/dl to 32.25±1.77mg/dl without much variation during the study period.

- Mean CRP levels in Standard group varied from 70.10 ±4.40mg/dl to 32.82 ±2.45mg/dl which was statistically *highly significant* (p< 0.0001***) and mean percentage reduction was 53.1%.

- Mean CRP levels levels in Test-1 group varied from 72.67± 12.95mg/dl to 41.62± 13.11mg/dl which was statistically significant(p< 0.0015**) and mean percentage reduction was 42.7%.

- Mean CRP levels in Test-2 group varied from 69.48±10.63mg/dl to 35.70 ±4.97mg/dl which was statistically significant (p< 0.0003***) and mean percentage reduction was 48.6%.

- Mean CRP levels in Test 3 group varied from 75 ±6.81mg/dl to 34.37± 4.24mg/dl which was statistically *highly significant* (p<0.0001***) and mean percentage reduction was 54.1%.

After 45 days, there is significant difference in CRP levels before and after treatment in each group. There is significant fall in CRP levels in T3 group($P<0.0001^{***}$) which is comparable to the standard group,($P<0.0001^{***}$) with percentage reduction being 53.1% and 54.1% respectively.

Kushu Roy et al showed that tinospora cordifolia siginificantly reduced the CRP levels in diabetic patients in comparision to other group patients.[53]
B Antony, M Benny and T N B Kaimal has done study in which patients supplemented with Emblica officianalis showed significant decrease in blood glucose and also fall in serum CRP levels upto 40% during the study period, which can be comparable to the present study[54]

VIII. Conclusion

The present study evaluated the effect of polyherbal extract in alloxan induced diabetic rats in comparision with standard drug Gliclazide.

Group I : Normal control ,distilled water; Group II : Standard control (Gliclazide

25mg/kg); Group III : (T 1) – administered 300 mg/kg dose of extract; Group IV : (T 2) – administered 400 mg/kg dose of extract; Group V : (T 3) – administered 500 mg/kg dose of extract. In this study

1.Polyherbal extract is effective in reducing blood sugar and HbA1c levels in diabetic rats, and the efficacy is comparable to the standard drug

2. Polyherbal extract is effcective in reducing the S..Creatinine, WBC counts and S. CRP levels significantly in alloxan induced diabetic rats.

The polyherbal preparation is promising agent for the treatment of Diabetes mellitus and can be used either as alternative or in combination with standard drugs.

IX. Summary

- In present study the effect of polyherbal preparation in diabetic rats is evaluated.

- The parameters measured were:1.blood glucose 2.HbA1c levels 3.S. Cretinine 4.WBC counts 5. S. CRP levels. The results obtained were

statistically analyzed by calculating mean values, standard deviation, one-way ANOVA followed by Post- hoc Tukey's multiple comparison test.

There is decrease in blood glucose and HbA1c levels with all the three doses of test groups, but more efficacy is seen in T3 group, but less than that of standard group. In regard to HbA1c levels there is no significant difference between test and standard groups.

Regarding creatinine levels, T3 group showed maximum activity which is comparable to the standard group. There is no significant difference in creatinine values of T3 and standard group at 45 days
With regard to WBC count, percentage reduction of T1, T2 and T3 groups is 25.24%, 33.27% and 37.83% respectively which is comparable to the standard group values i.e 39.17% reduction.

With regard to CRP values, at the end of 45 days there is no significant difference between the test groups and standard group values. The percentage reduction in CRP levels in T3 group is 54.1% which is slightly higher than the standard group. (53.1%)
To obtain the final assessment of activity of polyherbal preparation in diabetes, further studies are necessary.

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