INVESTIGATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *DICHROSTACHYS CINEREA*

A Dissertation submitted to

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In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

SHRADDHA SHUKLA

261625853

Under the guidance of

Mr. PONNUDURAI, M.Pharm, (PhD)

Professor

DEPARTMENT OF PHARMACOLOGY

CHERRAAN'S COLLEGE OF PHARMACY

COIMBATORE-641039

TAMILNADU.

MAY 2019

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled "**INVESTIGATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *DICHROSTACHYS CINEREA*"** submitted by **SHRADDHA SHUKLA Reg. No:261625853** to The Tamilnadu Dr. M.G.R Medical University, Chennai, in the partial fulfillment for the degree of Master of Pharmacy in Pharmacology is a record of bonafide work carried out by the candidate at the department of Pharmacology, Cherraan's College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2018-2019.

Internal Examiner **External Examiner**

CHERRAAN'S COLLEGE OF PHARMACY

(Affiliated to the Tamilnadu Dr.M.G.R medical university, Chennai)

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All India Council for Technical Education, New Delhi
Recognized by pharmacy council of India, New Delhi

CERTIFICATE

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This work is original and has not been submitted earlier for the award of any other degree or diploma of this or any other university.

K. PONNUDURAI, M.Pharm, (PhD)
Professor
Department of Pharmacology
Cherraan's College of Pharmacy

Place: Coimbatore
Date:

CHERRAAN'S COLLEGE OF PHARMACY

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Prof. (Dr.) K. PRABHU, M.Pharm., Ph.D.,
Principal
Cherraan's College of Pharmacy

Place: Coimbatore
Date:

DECLARATION

The research work embodied in this work "**INVESTIGATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *DICHROSTACHYS CINEREA*"** was carried out by me in the department of Pharmacology, Cherraan's college of Pharmacy, Coimbatore under the direct supervision of **Mr. Ponnudurai, M.Pharm., (Ph.D)** Professor, Department of Pharmacology, Cherraan's College of Pharmacy, Coimbatore-39.

The dissertation submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai, for the award of degree of Master of Pharmacy in Pharmacology during the academic year of 2016-2019.

SHRADDHA SHUKLA
Reg. No: 261625853

Place: Coimbatore

Date:

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SHRADDHA SHUKLA
Reg. No: 261625853

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1. INTRODUCTION

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians[1]. The "Ebers Papyrus", the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 BC. The Chinese *MateriaMedica*, which describes more than 600 medicinal plants, has been well documented with the first record dating from about 1100 BC[2]**.**Documentation of the Ayurvedic system recorded in Susruta and Charaka dates from about 1000 BC[3].The Greeks also contributed substantially to the rational development of the herbal drugs. Dioscorides, the Greek physician (100 A.D.), described in his work "*De MateriaMedica*" more than 600 medicinal plants[1]**.**The World Health Organization estimates that approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care[4]

For centuries, the ethno-botanical literature has described the usage of plant extracts, infusions and powders for diseases now known to be of viral origin. The ethnopharmacology provides an alternative approach for the discovery of antimicrobial agents, namely the study of medicinal plants with a history of traditional use as a potential source of substances with significant pharmacological and biological activities[5]

In recent years, there is a need to study the plants having different values in their medicinal properties. Therefore, several medicinal plants have been evaluated

for possible antimicrobial activity and potential cure from a variety of ailments especially of microbial origin[6].

The traditional methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries too and moreover the use of herbal remedies has increased in the developed countries in the last decades. In this connection, plants continue to be a rich source of therapeutic agents. The remarkable contribution of plants to the drug industry was possible because of the large number of phytochemical and biological studies all over the world.

India- Biodiversity of natural source:

The Indian subcontinent is endowed with rich and diverse local health tradition, which is equally matched with rich and diverse plant genetic source. A detailed investigation and documentation of plants used in local health traditions and ethnopharmacological evaluation to verify their efficacy and safety can lead to the development of invaluable herbal drugs or isolation of compounds of therapeutic value[7].

India is one of the world's leading biodiversity centres with the presence of over 45000 different plant species. India has 15000-18000 species of flowering plants, 2500 algae, 23000 Fungi, 1600 types of lichens, and 1800 varieties of bryophytes and estimated 30 million types of microorganisms. Of these about 15000-20000 plants have good medicinal value. However only traditional communities use about 7000-7500 for their medicinal values. Plant chemistry has been the basis for many synthetic drugs in modern pharmacopoeia[8] .

Importance of Herbal Therapies

The allopathic physician of India is aware of the limitation of modern medicines, and is simultaneously conscious of the strength of traditional medicine certain areas. He may not turn to ayurveda for treatment of acute infection, but tempted to try out therapies for chronic recurrent diseases, metabolic disorder or degenerative diseases where modern medicine has almost nothing to offer. The physician and patient prefer ayurveda or alternative medicine, for promotion of health as the concept of "Positive health" does not have in modern medicine.

Research efforts in ayurveda have increased manifold during the last couple of decades. However most of these efforts have revolved around the intention of discovering new drugs from plants. Many drugs obtained from plant have carved out an important place in modern medicine for themselves.

Ethnopharmacology in Drug Evaluation

The observation, identification, description and experimental investigation of the ingredients and the effects of indigenous drugs is a truly interdisciplinary field of research, the term ethnopharmacology has been used loosely to describe this field.

We have recently defined ethnopharmacology as “the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man”.

The objectives of ethnopharmacology are,

To rescue and document an important cultural heritage before it is lost.

To investigate and evaluate the agents employed.

- It plays an immense role in evaluation of natural products and more particularly the herbal drugs from traditional and folklore resources.

In ethnopharmacological research, it is essential to have proper sampling and analysis methods, and this necessity requires close cooperation by pharmacologist and ethnobotanists on one hand and specialists in chemical analysis on the other.

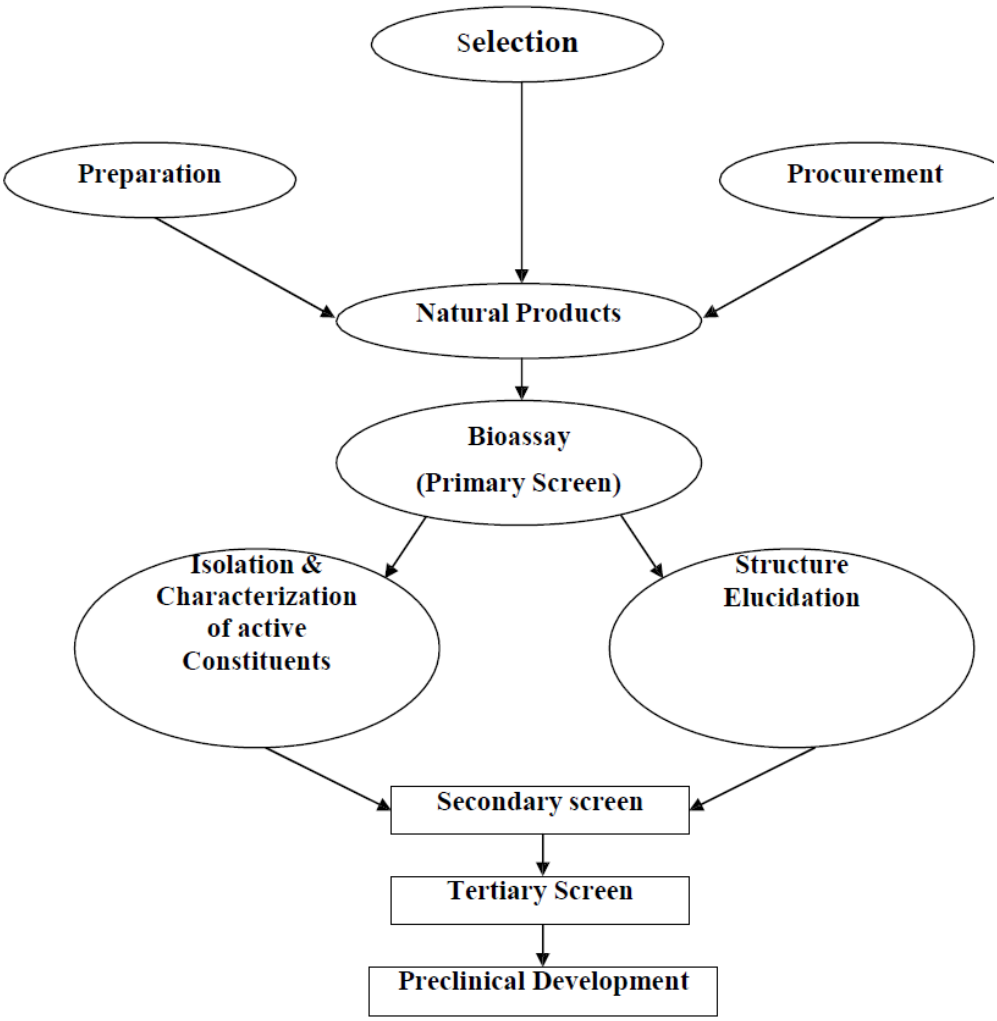
 Most traditional drugs are administered as mixtures of many components, and with today's knowledge of the many possible interaction between drugs, and between food and drugs, ethnopharmacological research must deal with this aspect too.

Impact of Ethnopharmacology in Modern Medicine

Evidently, the ethnopharmacological impulse to modern medicine can lead to many novel useful drugs, but modern and traditional uses may be entirely different. Traditional medicine is a general, powerful source of biological activity. Ethnopharmacology is not just a science of the past using an out model approach. It still constitutes a scientific back bone in the development of active therapeutics based upon traditional medicine of various ethnic groups.

The ultimate aim of Ethnopharmacology is the validation (or invalidation) of these traditional preparations, either through the isolation of active substances or through pharmacological findings.

Fig-1 : Various strategies for drug discovery of drugs from natural sources



Bioassay and Screening:

The experimental approach of setting up bioassays and screening large number of compounds (or extracts) is favoured by many pharmaceutical companies. By choosing specific therapeutic targets and by developing the appropriate assay procedures, each company can have a unique approach to the discovery of lead compounds. Many of these assays are based on isolated cells or enzymes and are automated to allow the throughput of very large numbers of samples. Success depends upon the appropriate choice of assay and the availability of large enough numbers of compounds or extracts.

Need for Phyto-Pharmacological Evaluation:

To demonstrate a pharmacological effect, nothing can replace observation of animal models, but as they are expensive and often difficult to interpret, simpler tests are used. These tests require less effort, and also make possible a better understanding of the mechanisms of action of the substances being tested. Non-animal models are becoming smaller and smaller while still remaining representative of a living organism.

By means of finely multidisciplinary efforts and of a choice of tests that accurately represent future therapeutic applications, research centres have been able to select active substances with some success. Thus the combination of several selective, sensitive and specific tests has made it possible to detect directly up to90% of clinically active compounds. These methods have also helped to eliminate substances that give false /positive results.

Of course most Pharmaco-Chemical research is performed with more limited means, but the scientific literature teems with interesting results. This may categorize into two groups, according to the positive methods of approach. One method is to demonstrate the pharmacological activities or even future clinical applications, from raw materials or natural substances already known.

The second type of approach is the discovery of new natural substances displaying pharmacological or even new therapeutic effects. This is the royal road par excellence that most often leads to patents being taken out.

Need For Good Clinical Research in Herbal Medicine

Compared with the experience of most modern drugs, the human use and approval of most herbal remedies is awesome. The requirement by medical and scientific establishment for research to "Prove" that herbs are effective is not found among the population at large. It is apparent that most ordinary people are relying on their impressions of the world to get it by in it.

However, the public do want to be assured that someone is looking after them. The physician and the regulators are charged with job of making sure that medicines is safe and effective. Knowledge within traditional medicine, however, has generally been in the form of received wisdom moulded to the individually needs and prowess of each practitioner. Their interest in inquiry for its own sake, with secure truths up for constant people refutation, is understandably secondary to their concern to survive in practice.

Better clinical practice can be performed by overcome the limitations:

- The necessary infrastructure is lacking, neither can the costs of under taking research studies easily be justified commercially.
- It is difficult to patent herbs and the size of the market for any individual product is not comparable to that for any patentable conventional drug.
- Being complex of pharmacologically active chemicals, the whole package will have different properties from that of any single constituent acting alone.
- The action of the latter will not predict the effect of former, particularly if the experimental evidence is based on the work done on laboratory animals.

The applications of the herbs and their effect on the body are not always the same as usually understand for conventional medicines[9].

Antidiabetic Activity

Diabetes mellitus is group of metabolic diseases characterized by hyperglycemia, dyslipidemia and protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease is associated with reduced quality of life and increased risk factor for mortality and morbidity. The long term hyperglycemia is an important factor in the development and progression of micro and macro vascular complication, which include neuropathy, nephropathy, cardiovascular and cerebro vascular disease. The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Four major classes of oral hypoglycemic agents have been extensively: insulin secretogogeus, biguanides, thiazolidinediones and α- glucosidase inhibitors[10]. Each drug class works on different mechanism of actions, including stimulation of insulin secretion, reduction of hepatic gluconeogenesis, increase in insulin receptor sensitivity and delay of digestion and absorption of carbohydrate, respectively. Unfortunately, these agents could produce severe hypoglycemia, weight gain and gastro intestinal disturbances. Natural products have been explored for anti diabetic activity[11]

India leads the world with the largest number of the diabetes subject earning the dubious distinction of being termed the "**Diabetic capital of the world**". The most disturbing trend is the shift in the age of onset of diabetes to a younger age in the recent years. This could have long lasting adverse effects on nation's health and economy. As per WHO, India will be the nation with highest number of diabetes in the world by2030 followed by china and then USA. This is an alarming sound as far as the health system of India is concerned.

Prediction of Diabetes in 2030

Ranking	Country	Peoples with Diabetes(in Millions)	
		2000	2030
1	INDIA	31.7	79.4
2	CHINA	20.8	42.3
3	USA	17.7	30.3

In developing countries, the majority of people with diabetes are in the 45 to 64 year age range, similar to finding reported previously. In contrast, the majority of people with diabetes in developed countries are >64 years of age. By 2030, it is estimated that the number of people with diabetes >64 years of age will be >82 millions in developing countries and >48 millions in developed countries. India being one of the fastest developing country, and youth being the driving force for it, it will be hampered at the most is not a good sign. Proper patient education and general awareness about the disease can help reducing the extend of this damage.

All the corners of health system viz: Doctors, Pharmacists, Nurses, Government, NGOs etc. have to realize this fact and plan accordingly to tackle this situation.

CLASSIFICATION OF DIABETES MELLITUS

There are different types of diabetes mellitus,

1. TYPE- I (Beta cells destruction, usually leading to absolute insulin deficiency)

- Autoimmune
- Idiopathic

2. TYPE-II (Ranges from predominantly insulin resistant, with relative insulin deficiency, to predominantly insulin secretary defect, with/without insulin resistance)

3. Other specific types of Diabetes mellitus

- Maturity Onset Diabetes Mellitus (MODY) 1 (HNF4); rare
- MODY 2 (Glucokinase); less rare
- MODY 3 (HNF 1); accounts for 2/3 of all MODY
- MODY 4 (IPF-1); very rare
- MODY 5 (HNF-1); very rare
- MODY 6 (neuroD1); very rare
- Mitochondrial DNA

4. Genetic defects in insulin action

- Type A insulin resistance
- Leprechaunism
- Rabson-Mendenhall Syndrome
- Lipoatrophic diabetes

5. Disease of the exocrine pancreas

6. Endocrinopathies

7. Drug or Chemical induced diabetes

8. Other genetic syndromes (Down's, Klinefelter´s, Turner's and others) associated with diabetes[12]

TYPE- 1

- Type 1 diabetes was also called insulin dependent diabetes mellitus (IDDM), or juvenile onset diabetes mellitus. In type 1 diabetes, the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making insulin. Abnormal antibodies have been found in the majority of patients with type 1 diabetes. Antibodies are proteins in the blood that are part of the body's

immune system. The patient with type 1 diabetes must rely on insulin medication for survival. Type 1diabetes tends to occur in young, lean individuals, usually before 30 years of age, however, older patients do present with this form of diabetes on occasion. This subgroup is referred to as latent autoimmune diabetes in adults (LADA). LADA is a slow, progressive form of type 1 diabetes.

TYPE -2

- Type 2 diabetes was also referred to as non-insulin dependent diabetes mellitus (NIDDM), or Adult onset diabetes mellitus (AODM). In type 2 diabetes, patients can still produce insulin, but do so relatively inadequately for their body's needs, A major feature of type 2 diabetes is a lack of sensitivity to insulin by the cells of the body (particularly fat and muscle cells). In addition to the problems with an increase in insulin resistance, the release of insulin by the pancreas may also be defective and suboptimal. In fact, there is a known steady decline in beta cell production of insulin in type 2 diabetes that contributes to worsening glucose control. Finally, the liver in these patients continues to produce glucose through a process called gluconeogenesis despite elevated glucose levels[13]

Gestational diabetes:

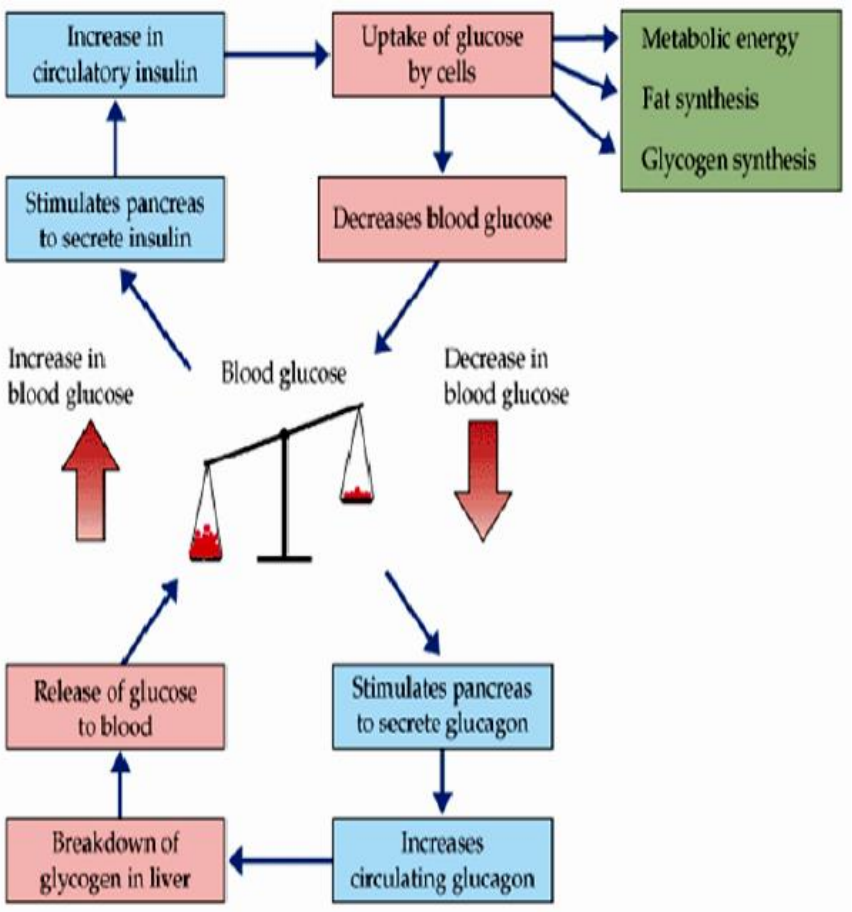
- Diabetes can occur temporarily during pregnancy. Significant hormonal changes during pregnancy can lead to blood sugar elevation in genetically predisposed individuals. Blood sugar elevation during pregnancy is called gestational diabetes. Gestational diabetes usually resolves once the baby is born. However, 25%-50% of women with gestational diabetes will eventually develop type 2 diabetes later in life, especially in those who require insulin during pregnancy

and those who remain overweight after their delivery. Patients with gestational diabetes are usually asked to undergo an oral glucose tolerance test about six weeks after giving birth to determine if their diabetes has persisted beyond the pregnancy, or if any evidence (such as impaired glucose tolerance) is present that may be a clue to the patient's future risk for developing diabetes[14]**.** Approximately 10% of the patients have Type 1 diabetes and the remaining 90% have Type 2 diabetes. Individuals who are at high risk of developing Type II diabetes mellitus include people who:

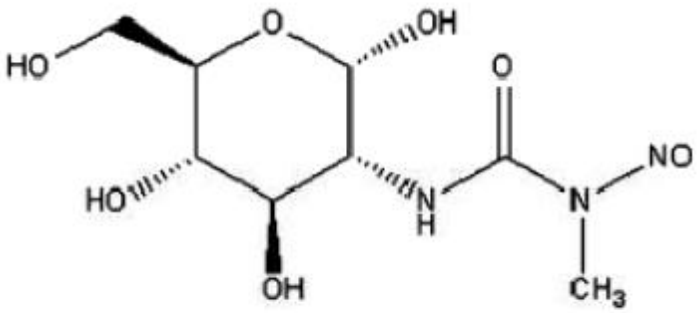
- Are obese (more than 20% above their ideal body weight)
- Have a relative with diabetes mellitus
- Have been diagnosed with gestational diabetes or have delivered a baby weighing more than 9 lbs (4 kg)
- Have high blood pressure (140/90 mmHg or above)
- Have a high density lipoprotein cholesterol level less than or equal to 35 mg/dL and/or a triglyceride level greater than or equal to 250 mg/dL

Fig-2

Glucose Regulation in Human Body



Streptozotocin Induced Diabetic Model:



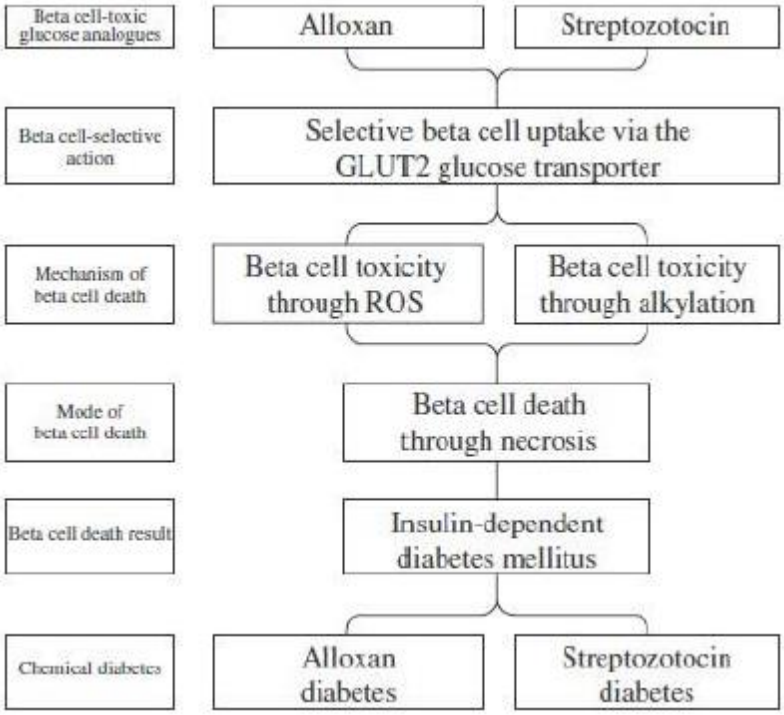
Streptozotocin

Streptozotocin : Mechanism of action:

Streptozotocininhibits insulin secretion and causes a state of insulin dependent diabetes mellitus. Both effects can be attributed to its specific chemical properties, namely its alkylating potency. As with alloxan, its beta cell specificity is mainly the result of selective cellular uptake and accumulation.

Fig-3

Mechanism of Streptozotocin Producing Diabetes Mellitus



Beta cell toxicity of streptozotocin :

It is generally assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnimethylnitrosourea moiety . The transfer of the methyl group from *streptozotocin*to the DNA molecule causes damage, which along a defined chain of events[15] results in the fragmentation of the DNA**[16].** Protein glycosylation may be an additional damaging factor **[17].** In the attempt to repair DNA, poly (ADP-ribose) polymerase (PARP) is over stimulated. This diminishes cellular

NAD+, and subsequently ATP, stores[18]**.**The depletion of the cellular energy stores ultimately results in beta cell necrosis. Although *Streptozotocin*also ethylated proteins[19].DNA methylation is ultimately responsible for beta cell death, but it is likely that protein methylation contributes to the functional defects of the beta cells after exposure to *streptozotocin*. Inhibitors of poly ADP-ribosylation suppress the process of DNA methylation. Thus, injection of nicotinamide and other PARP inhibitors in parallel with, or prior to the administration of *streptozotocin*is well known to protect beta cells against the toxic action of *streptozotocin*and to prevent the development of a diabetic state[20].Also, mice deficient in PARP are resistant to beta cell death mediated by *streptozotocin*, in spite of DNA fragmentation. The absence of PARP prevents the depletion of the cofactor NAD+ and the subsequent loss of ATP **[21].** and thus cell death. The role of alkylation in beta cell damage has also been examined by the use of ethylating agents, which are less toxic than their methylating counterparts, on account of O6- ethyl guanine being less toxic than O6-methylguanine[22]. The fact that N-ethyl-N-nitrosourea and ethyl methanesulphonate are significantly less toxic to insulin-producing cells than MNU and methyl methanesulphonate has been taken as support for the notion that in insulin producing cells, as in other cell types, the mechanism of toxic action is due to alkylation, with methylation of DNA bases being more toxic than ethylation**[23].**

An alternative hypothesis proposes that part of the diabetogenic effect of *streptozotocin*may relate not to its alkylating ability but to its potential to act as an intracellular nitric oxide (NO) donor[24]**.** Both *streptozotocin*and MNU contain a nitroso group and can liberate NO. In fact, *streptozotocin*has been shown to increase the activity of guanylyl cyclase and the formation of cGMP, which are characteristic effects of NO. However, the alkylating agent methyl methane sulphonate is the most

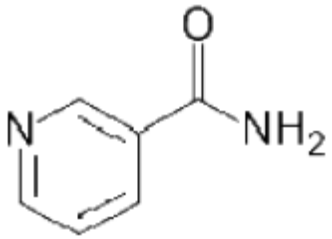
toxic compound, though unlike MNU, it is not a NO donor indicating that NO is not an indispensable prerequisite for the toxic action of the family of alkylating agents that *streptozotocin*belongs to. Finally, some minor generation of ROS, including superoxide and hydroxyl radicals originating from hydrogen peroxide dismutation during hypoxanthine metabolism[25], may accompany the effect of *streptozotocin*and accelerate the process of beta cell destruction but ROS do not play a crucial role[23].

Inhibition of insulin secretion by Streptozotocin :

The effects of *streptozotocin*on glucose and insulin homeostasis reflect the toxin-induced abnormalities in beta cell function. Initially, insulin biosynthesis, glucose-induced insulin Secretion and glucose metabolism (both glucose oxidation and oxygen consumption) are all affected [26]. On the other hand, *streptozotocin*has no immediate, direct inhibitory Effect upon glucose transport[27] or upon glucose phosphorylation by glucokinase**.** However, at later stages of functional beta cell impairment, deficiencies in terms of gene expression and protein production lead to the deterioration of both glucose transport and metabolism[28]. Even before the negative effects of mitochondrial DNA and protein alkylation and glycosylation become evident, *streptozotocin*-induced depletion of NAD+ may result in the inhibition of insulin biosynthesis and secretion[16,18]**.** Later, inhibition of glucose-induced and amino acid induced insulin secretion[29]**,** as a result of mitochondrial enzyme dysfunction[30] and damage to the mitochondrial genome become apparent. This impairment is more marked for nutrient- than for non-nutrient-induced insulin secretion. This interpretation has been confirmed through studies which have shown that pre-treatment of isolated pancreatic islets with the poly (ADP-ribose) polymerase (PARP) inhibitor nicotinamide prevents early inhibition of beta cell

function during the first day after *Streptozotocin*exposure, while long-term inhibition of insulin secretion 6 days after *streptozotocin*exposure was not counteracted by nicotinamide[31].

Nicotinamide



Nicotinamide, also known as niacinamide and nicotinic acid amide, is the amide of nicotinic acid (vitamin B3 / niacin). Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. Nicotinic acid, also known as niacin, is converted to nicotinamide in vivo, and though the two are identical in their vitamin functions, nicotinamide does not have the same pharmacologic and toxic effects of niacin. Nicotinamide is presently tested for its potential benefit in preventing Type 1(insulin- dependent) diabetes. (Wikipedia.com)

Addition of *nicotinamide*to *Streptozotocin*treated beta cells could thus prevent their rapid necrosis through preservation of the cellular NAD and ATP levels, but the surviving cells might be suppressed both in their DNA repair mechanisms and in their mitochondrial functions and could therefore become more susceptible to apoptosis.

2. AIM AND OBJECTIVE

Medicinal plants have been used in India for centuries as an important therapeutic source for treating variety of ailments and have been found to be of immense global importance.

The family "Fabaceae" consists of the leaf of *DICHROSTACHYS CINEREA*(L.) with wide range of pharmacological, biological activities and having interesting phytochemical constituents. The plant has been used for a wide range of diseases as reflected from collected literature.

The selection of the leaf of *DICHROSTACHYS CINEREA* (L.) was view of its

Easy availability

Therapeutic value

Degree of research work which can be done.

3. LITERATURE REVIEW

1. A. Doss ,et al., (2009)[32] Compounds of pharmacological interest (tannins) were isolated from the plant species, *Solanum trilobatum* Linn and assayed against the bacteria, *Staphylococcus aureus, Streptococcus pyrogens, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli* using agar diffusion method. Tannins exhibited antibacterial activities against all the tested microorganisms. *S.aureus* was the most resistant to tannins isolated from the plant material followed by *Streptococcus pyrogens, Salmonella typhi*, *Escherichia coli*, *Proteus vulgari* and *Pseudomonas aeruginosa*. Minimum inhibitory concentration of the tannins ranged between 1.0 and 2.0 mg/ml while the minimum bactericidal concentration ranged between 1.5 and 2.0 mg/ml.

2. Tim Smith, et al., (2005)[33] Indehiscent fruits of six tree species, common in Matabeleland were examined in *in vitro* and in vivo trials. The results for two of them, Acacia nilotica and Dichrostachys cinerea are presented here. Acacia nilotica contained more total phenolics than D. cinerea, but less nitrogen (N) and fibre (ADF and NDF). After 48h incubation, in vitro OMD of both species was increased by PEG and NaOH, except or wood ash were used in combination with PEG with D. cinerea fruits DM intake, DMD were lowest and N-retention negative in goats fed A. nilotica as supplement. Fed a supplement of D. cinerea, untreated or treated with PEG or NaOH, digestibility and N-retention were highest, and similar to a goat meal, with the untreated fruit. Deaths of twin-born kids were lowest in the supplemented. Supplementation with D. cinerea fruit resulted in improved goat performance. The only treatment applied of practical significance, wood ash, is currently being tested in an in vivo study.

3. S. Jayakumari, et al., (2011)[34] Dichrostachys cinerea (L.) Wight & Arn belongs to Mimosaceae, is commonly known as "Vidathalai' in Tamil. Earlier folklore claims reveals that the plant is used in diarrhoea and dysentery. The whole plant has been used for antidiarrhoeal activity in Indian traditional medicine. So the leaf, stem,bark and root of the plant were screened separately for their antidiarrhoeal activity by castor oil induced model and small intestinal transit model. In the resent study vaccum dried ethanolic extract of leaf, bark and root of the plant were used at two dose level (200 mg / kg and 400 mg / kg p.o). All the extracts showed significant antidiarrhoeal activity by both the tested models. Hence the present study supports the traditional claim of Dichrostachyscinerea (L.) Wight & Arn as an antidiarrhoeal drug in the Indian System of Medicine.

4.J. Fotie1, et al., (2009)[35] The antibacterial activities of ethyl acetate, methanol and aqueous extracts of the stem bark of *Dichrostachys cinerea* and the roots of *Parkia bicolor* have been evaluated. Ethylacetate extracts have been investigated, studies that led to a series of known compounds, amongst which many are reported here for the very first time from both the species.

5.Victor Mlambo, et al., (2004)[36] This study investigated the potential of Dichrostachys cinerea fruits as a protein supplement in semi-arid areas of Zimbabwe. The tanniniferous fruits were treated with aqueous solutions of polyethylene glycol (PEG) or sodium hydroxide (NaOH). Both treatments increased the soluble fraction, rate of degradation and effective degradability (ED) of nitrogen (N) in sacco. The PEG effects were higher than the NaOH effects (e.g. a 25% vs. 6% increase in effective N degradabilities, respectively). Five treatments were evaluated in a N-balance trial using Matebele goats: ground, PEG- or NaOH-treated D. cinerea

fruits, a commercial protein supplement (CPS) and no supplement. Animals offered ground fruits or CPS retained most N (3.7 or 4.1 g N/day, respectively), while those offered NaOH- or PEG-treated fruits retained significantly less N (2.7 or 1.0 g/day, respectively). Unsupplemented animals were in negative N balance (−2.4 g/day). PEG treatment resulted in excessive protein degradation in the rumen leading to high urine N loss. It is concluded that the D. cinerea fruits were beneficial for goat N-nutrition and that the tannins did not require inactivation. D. cinerea fruits can, therefore, replace the expensive commercial protein supplement.

6.Reham T. El-Sharawy,et al., (2004)[37] The spectroscopic techniques to the aqueous alcoholic leaves extract of Dichrostachys cinerea (L.) Wight et Arn. (Mimosaceae) led to the isolation and identification of ten phenolic compounds. Among them, apigenin-7-O-apiosyl (1→2) glucoside, chrysoeriol-7-O-apiosyl (1→2) glucoside and clovamide were isolated for the first time from the plant. The rest of the compounds are: flavonol glycosides (quercetin-3-O-rhamnopyranoside, quercetin-3-O-glucopyranoside, and myricetin-3-O-glucopyranoside), and three aglycones. The crude extract of D. cinerea showed a significant antitrypanosomal and antiviral effects. Clovamide as a major constituent was investigated for its antiviral and antitrypanosomal activities. It showed a significant antiviral effect against H5N1 influenza A virus with inhibition rate (74%) and a momentous trypanocidal effect against Trypanosoma evansi with IC50 value of 3.27 μg/ml, compared with the standard drug; diminazene aceturate (IC50=0.72μg/ml). Therefore, clovamide is playing an important role in antitrypanosomal and antiviral activities for D. cinerea extract and it can be considered a new candidate for the treatment of these two infections.

7. A Banso, et al., (2007)[38] Compounds of pharmacological interest (tannins) were isolated from Dichrostachys cinerea and assayed against Staphylococcus aureus, Shigella boydii, Shigella flexneri, Escherichia coli and Pseudomonas aeruginosa using the agar diffusion method. Tannins exhibited antibacterial activities against all the test microorganisms. Sh. flexneri was the most resistant to tannins isolated from the plant material followed by Sh. boydii, E. coli, Staph. aureus and P. aeruginosa respectively. Minimum inhibitory concentration of the tannins ranged between 4.0 and 5.5 mg/ml while the minimum bactericidal concentration ranged between 4.5 and 6.0 mg/ml.

8. Steenkamp, et al., (2007)[39] Crude methanol and water extracts of 32 plant species, used for the treatment of infectious diseases in Venda, were screened for in vitro activity against Candida albicans standard strain (ATCC 10231) and five clinical isolates. Water extracts of 16 plant species and methanol extracts of 11 plant species inhibited candidiasis growth. Inhibition at b1 mg/ml, against the C. albicans strains tested, was observed for the methanol extracts of Combretum molle (root), Piper capense (bark), Solanum aculeastrum (fruits), Syzygium cordatum (bark) and Solanum aculeastrum (fruits), Syzygium cordatum (bark) and Zanthoxylum davyi (bark) as well as the aqueous bark extract of Afzelia quanzensis and root extract of Tabernaemontana elegans. These results implicate that the extracts contain compounds with therapeutic potential against C. albicans.

9. V. Steenkamp,et al., (2007)[39] Crude methanol and water extracts of 32 plant species, used for the treatment of infectious diseases in Venda, were screened for in vitro activity against Candida albicans standard strain (ATCC 10231) and five clinical isolates. Water extracts of 16 plant species and methanol extracts of 11 plant

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10.A.A. Ajao, et al.,(2018)[40] The aim of this paper is therefore to present a review of aphrodisiac medicinal plants used for the treatment of sexual dysfunction in order to identity existing gaps regarding the indigenous knowledge, ethnopharmacology, and toxicology of the implicated plants in the region. This review presents a total of 209 plant species belonging to 73 families used in sub-Saharan Africa to treat sexual dysfunction. With the roots being the most used plant parts, and decoctions the most preferred method of preparation. Forty-eight plant species including Gloriosa superba, Mucuna pruriens, Sphenocentrum jollyanum, and Morella serrata have previously been evaluated scientifically, thus lending credence to the folkloric usage of the plants. Regarding the toxicity, the review reveals that only 77 plant species have been evaluated, of which 25 exhibited varying degrees of toxicity in test animals. Overall, this review demonstrates the need for further studies including ethnopharmacology and toxicology of the documented plants, so that they can be properly utilized for traditional treatment.

11. Eisa MM, et al; (2000) [41] The antibacterial activities of the chloroform, methanol and aqueous extracts of Dichrostachys cinerea fruits and leaves are reported.

12. Deborah K B Runyoro, et al; (2006)[42] *Candida albicans* has become resistant to the already limited, toxi*c* and expensive anti-*Candida* agents available in the market. These factors necessitate the search for new anti-fungal agents.

13. Johnstone neondo, et al.,(2012)[43]. To provide scientific rationale to the traditional use of Dichrostachys cinerea as medicinal plant in Kenya, phytochemical analysis, antimicrobial screening and evaluation of toxic concentration levels of D. cinerea extracts were done. Qualitative assessment of phytochemicals, in vitro antimicrobial (selected bacteria and fungus) and brine shrimp toxicity assays were done. Explants (leaves, bark of stems and roots) were collected from D. cinerea trees growing in Jomo Kenyatta University of Agriculture and Technology (JKUAT) fallow land behind Botany laboratory. They were washed and then air dried under light exposure (27°C − 30°C for 14 days). A portion of each extracts was used for phytochemical screening. Sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using disc diffusion assay. Brine shrimps lethality test (BST) was used to predict the presence of bioactive compounds in the extracts. Methanol extracts contained all the tested phytochemicals while hot water extracts lacked steroids. Methanol and hot water extracts had no significant difference in terms of antibacterial screening. The LC 50 value was found to be 2000ppm (parts per million). The results suggest that extracts of D. cinerea contain potential antibacterial and antifungal agents.

14. M.Vijayalakshmi,K.Periyanayagam, et al., (2013)[44].The leaves of *Dichrostachys cinerea* are used as laxative, diuretic, painkiller.

15. Paul Mungai Kiman, et al.,(2018)[45]. This study investigated the antiproliferative activity of methanolic and water extracts from four plant species

namely *Aloe secundiflora, Maytenus obscura, Vernonia zanzibarensis* and *Dichrostachys cinerea* using prostate cancer cells (DU145 and 22Rv1), cervical cancer cells (HeLa) and African green monkey cells (Vero) cell lines using the MTT assay. All extracts suppressed the growth of the cancer cells in a dose-dependent manner at concentrations of 1.37 µg/ml to 1000 µg/ml. The methanol extract of *D. cinerea* stem bark had the highest anti proliferative activity among the plant extracts studied. The study indicates that the methanol extract of *D. cinerea* stem bark has potential anti-proliferative activity with low cytotoxicity to normal cells. Our results validate the ethnomedicinal use of these plants for management of cancer. The active elements in the extracts studied here need to be isolated and purified to investigate the synergy and additive pharmacological effect in killing cancer cells.

4. PLANT PROFILE

PLANT INTRODUCTION:

DICHROSTACHYS CINEREA (L.) Wight &Arn[46,47]

SYNONYMS :

Caillieadichrostachysguill.etPerrot.DichrostachysglomerataChiov.Dichrostachysnutans (Pers.) Benth. DichrostachysnyassanaTaub Mimosa cinerea L.

Family : Fabaceae

Genus : Dichrostachys

Species : Cinerea flower

Fig. 4 Whole plant

DICHROSTACHYS CINEREA, known as sicklebush, Bell mimosa, Chinese lantern treeorKalahari Christmas tree (in SouthAfrica) is a legume of genus Dichrostachys belonging to Fabaceae family.

ETYMOLOGY

The generic name Dichrostachysmeans 'two-colored spike', referring to its two-colored in fluorescence, from Ancient Greek δί- (di-, 'twice'), χροός(khroos, 'color'), and στάχυς (stakhus, 'ear of grain'). The specific name cinerea refers to the greyish hairs of the typical subspecies, from the latincinereus.

DISTRIBUTION

It is native to Africa but has been introduced to India, the Caribbean and parts of Southeast Asia. In Ethiopia, the species is common in the Nechisar National Park. The tree was brought to the Caribbean in the 19th century. In Cuba, where it is known as El Marabú or Marabou weed, it has become a serious invasive species problem, occupying close to five million acres (20,000 km²) of agricultural land. Plans are underway to exploit it as a source of biomass for renewable power generation.

DESCRIPTION AND ECOLOGY

Dichrostachys cinerea is a semi-deciduous tree characterized by barkon young branches, dark grey-brown fissures on older branches and stems and smooth on the spines. They typically grow up to 7m (23 ft) in height and have strong alternate thorns, generally up to 8 cm (3.1 in) long.

Flower and Pods

"Flowers of the *Dichrostachys cinerea* is characteristically in bi-colored cylindrical spikes that resemble Chinese lanterns and are 6–8 cm long and fragrant. Upper flowers of a hanging spike are sterile and are of a lilac or pale purple. Pods are usually mustard brown and are generally twisted or spiraled and may be up to

100 × 15 mm. The species has can be sub-categorized with two slight variations that have been recognised: D. cinerea sp. africana and D. cinerea ssp. nyassana, the latter which is typically larger and less hairy in its foliage.

Seeds

Seeds bi-convex, elliptical to sub-circular 4mm long, 2-4mm wide, pale tan, glossy, pleurogram elliptic.

CULTIVATION AND COLLECTION

The species tends to grow in rainforest zones that are clearly defined and in altitude up to 2000m. It often occurs in areas with a storage seasonal climate with a wide ranging mean annual temperature and with a mean annual rainfall ranging from200-400 mm. It occurs in brushwood, thickets, hedges, teak forest and grassland and generally takes to poor quality clay soils or deep and sandy soils with a wide pH scale range.

Uses

Fruit and seeds that grow on *Dichrostachys cinerea* are edible. Cattle, camels and game such as giraffe, buffalo, kudu, hartebeest, nyala, red forest duikerfeed on the infusions are used for leprosy, syphilis, coughs, as an anthelmintic, purgative and strong diuretic, leaves are used for epilepsy and also as a diuretic and laxative and a powdered form is massaged on limbs with bone fractures. The roots are also sometimes used for bites or stings.

Juicy pods that fall to the ground. Such animals also feed on the immature twigs and leaves of the tree which are rich in protein (11–15%) and minerals. The flowers can be a valuable source of honey. The wood is of a dense nature and burns

slowly with no toxicity, so it is often used for fuel wood. The species yields a medium to heavy, durable hardwood and is often used in smaller domestic items as walking sticks, handles, spears and tool handles, particularly in central Africa.

In traditional medicine, the bark is used for headache, toothache, dysentery, elephantiasis, root, in Siddha medicine of the Tamils in southern India, *Dichrostachys cinerea* is called vidathther and used for Gonerrhoea, syphilis and eczema. The actual medical preparations can be seen in the book on Siddha medicines.

As they are rich in nutrients, the plants are often used as fertilizer, particularly in the Sahel region of Africa along riverbanks. The plant is widely used for soil conservation, particularly in India, for shallow soils and in arid western and sub humid alluvial plains.

5. PLAN OF WORK

- Phytochemical Anti-Diabetic Investigation
- Extraction
- *Streptozotocin*
- Phytochemical Analysis
- TLC Method
- UV Spectroscopy
- IR Spectroscopy
- HPTLC

6. MATERIALS AND METHODS

Collection of specimen:

The species for the proposed study, that is leaves of ***DICHROSTACHYS CINEREA*** collected carefully from Bhopal, Madhyapradesh, India

Preliminary Phytochemical Studies

Plant may be considered as biosynthetic laboratories, in which various kinds of organic compounds are such as carbohydrates, amino acid, proteins, alkaloids, glycosides, flavonoids, fixed oils, tannins, phytosterols, and saponins etc., which exert a physiologic effect and are utilized as biologically active components by men since time immemorial. The medicinal value of any plant drug however depends on the nature of chemical constituent's presents in it and is referred to as active principle.

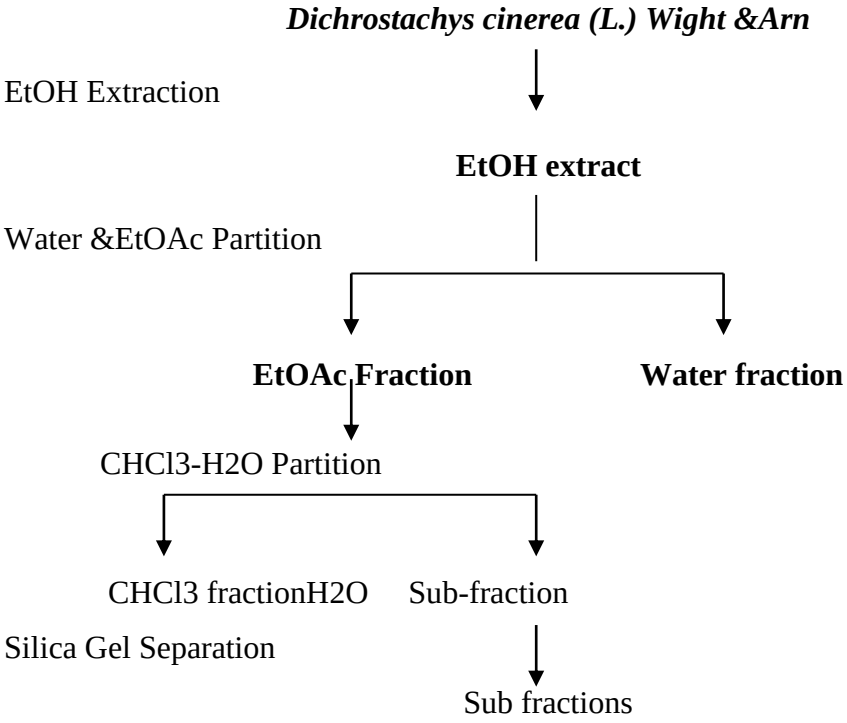
For our present study, we have taken *Dichrostachys cinerea* (L.) Wight &Arn powdered leaf extract to evaluate the compounds and the phytochemical constituents present them.

For the extracts, here we used chromatographic techniques by developing various solvent**.** systems and confirm the active compounds present them **.**

A. Preparation of extracts:

Air-dried & coarsely powdered (300gm) of *Dichrostachys cinerea* (L.) Wight &Arn leaf was extracted successively using soxhlet extractor using petroleum ether and ethanol. The extract was carried out until the solvent found to be colourless. Then the solvent was filtered and distilled off. Final traces of ethanol were removed under pressure by using rotary vacuum flask evaporator and they were preserved in desiccators. The percentage yield was 30% w/w. The crude leaf extracts were further

fractionated with different fractions as illustrated below. The filtrate was concentrated by evaporator and wash with hot water. The water extract was further partitioned with ethyl acetate and divided into ethyl acetate and water fractions through liquid-liquid partition. The ethyl acetate fraction (Yield 3.2%) was concentrated by evaporation and further partitioned with equal volume of Chloroform and water. This water sub fraction was also concentrated by evaporations for further chromatographic purifications[9].



B. Qualitative Phytochemical Analysis:

Qualitative chemical tests were conducted for the extract of leaf of *Dichrostachys cinerea* (L.) Wight & Arn to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded**.**[48,49]

Table- 1

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF DICHROSTACHYS CINEREA (L.) WIGHT & ARN

TEST	ETHANOL EXTRACT
CARBOHYDRATES	+
PROTEINS & AMINO ACIDS	–
GLYCOSIDES	–
ALKALOIDS	–
PHYTO STEROIDS	+
FLAVONOIDS	+
SAPONIN	+
TANNINS & PHENOLIC COMPOUNDS	+
FIXED OILS & FATS	+

+ = indicates presence - = indicates absence

Column chromatography

Principle:

Each compound in a mixture will have a particular solubility in the solvent and a Particular tendency to be absorbed by the solid adsorbent. Mostly no two compounds behave exactly alike in these respects. This principle is utilized in column chromatography.

Details of column chromatography:

Adsorbent	: Silica gel (for column chromatography 60 -120)
Eluent	: Chloroform & Methanol
Length of the column	: 100 cm
Diameter of the column	: 3.5 cm
Extract used	: 3 gm
Length of column packed	: 70 cm
Rate of Elution	: 30 drops per minute.
Fraction collected	: 50 ml.

Procedure:

The column with cotton plug is filled with the sufficient silica gel (60- 120) upto 70 cm and 3.5 cm width. The column was carefully packed and uniformly filled with silica gel, by tapping the side of the column. Then the aqueous fraction extract of powdered leaf of *Dichrostachys cinerea* (L.) Wight & Arn was charged on column and eluted with different solvent ranging from noncc-polar to polar at the time of 30 drops per minute. Each fraction was collected in the volume of 50 ml with different solvent ratio as given in table no. 2.

Table-2

Isolation of Compound

Extract	Solvent system	No of Spots	Rf Values
Aqueous fraction	CHCl3- CH3OH (90: 10) Ratio	1	0.56

Description of the Isolated Compounds

Aqueous fraction of *Dichrostachys cinerea* (L.) Wight &Arn

Colour and Nature of the compound : Greenish- Yellow colour substance

TLC Eluting Solvent System : Chloroform: Methanol (90:10)

TLC Pattern : Single spot

Rf value : 0.56

Phytochemical Test : Terpenoids

Purification : Purified by Column chromatography on Silica Gel (100-120 mesh) with Chloroform

solubility : Methanol

Melting point : 290-299^{0}C

C. Thin Layer Chromatography (TLC)

Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different components. Hence, when we spot a mixture of components on a TLC plates, the compounds, which are readily soluble but not strongly adsorbed moves up along with the solvent and those not so soluble but more strongly adsorbed move up less readily leading to separation of compounds.

Preparation of TLC

- Silica gel G was weighed in required quantity
- Homogeneous slurry was made with sufficient quantity of distilled water.
- The coated plates were allowed to dry in air and activated by heating in hot Then the slurry was poured into TLC glass plates by spreading technique and the uniform silica gel layer was adjusted to 0.25 mm thickness.
- Hot air oven at 100-105°C for 1 hour and then used for TLC.
- The extract were prepared with the respective solvents like ethanol and made up to 10 ml in different test tube.
- Then with the help of capillary tube an extract was spotted on TLC plate, which was developed in TLC chamber, previously saturated with different solvent system.

By trial and error method, methanol extract showed resolution of spot with following solvent system Chloroform-Methanol (90:10). The compound eluted as 1. The RF values were 0.56 respectively.

Fig-5

TLC OF AQUEOUS FRACTION OF

DICHROSTACHYS CINEREA (L.) Wight & Arn

D. High Performance Thin Layer Chromatography (HPTLC)

High performance thin layer chromatography is a modern powerful technique with separation power, performance and reproducibility superior to classic TLC. Based on the use of High Performance TLC plates with small particle size (3- 4µ m) and precise instruments for each step of the chromatographic process (sample application, chromatogram development, chromatogram evaluation). HPTLC provides the means not only for flexible screening procedure and qualitative analysis but also for demanding quantitative determination[9].

Steps involved in HPTLC:

1. Sample application

2. Chromatogram development

3. Densitometry chromatogram evaluation

4. Post chromatographic derivatization`

Preparation of Test solution

The given extract 100mg was dissolved in 5ml Methanol, Centrifuged and collected the supernatant liquid. This solution was used as test solution for HPTLC analysis.

Samples:

5µ l of the above test solutions were loaded as 8mm band length in the 5 x 10Silica gel 60F254, TLC plate using Hamilton syringe and CAMAG LINOMAT 5 applicator.

Spot Development:

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm.

Photo-documentation:

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.

Derivatization:

The developed plate was sprayed with respective spray reagent and dried at 120 C in Hot air oven. The plate was photo-documented at UV 366nm and Daylight using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning:

Finally, the plate was fixed in scanner stage and scanning was done at 500nm. The Peak table, Peak display and Peak densitogram were noted.

ANALYSIS DETAILS: Mobile phase:

Toluene: Acetone: Formic acid [11: 6: 1 v/v]

Software:

CATS software, Version 1.4.1

Scanning Speed:

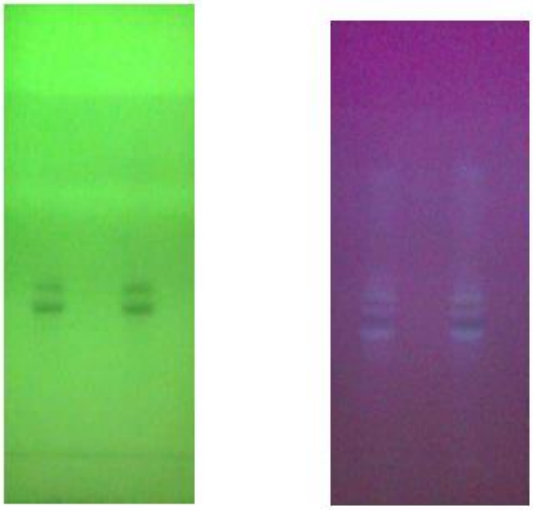
100mm/s

Detection:

Blue colored fluorescence shows in both tracks observed in the chromatogram after derivatization, which confirmed the presence of Terpenoids in the fraction.

Fig-6

HPTLC studies of Aqueous Fraction



UV 254nm **UV 366nm**

UV- SPECTRASCOPY:

The measurement of absorption of ultraviolet and visible radiation provides a Convenient means fir the analysis of numerous inorganic and organic species. The wavelength in UV region are usually expressed in nm that is 200 -400 nm.

Instrument used : Shimadzu

Solvent : Methanol

Wavelength : 200 – 400 nm

Speed : Medium.

The UV spectrum of isolated fraction sample solution was prepared in Methanol and the same Methanol was used as blank. UV scan was done between 200 – 400 nm and the speed of instrument Scanning was set as medium. The peak absorbance maximum absorbance at 349 nm and absorbance in 0.9833. The peak absorbance obtain for fraction are as follows.

Fig-7

UV Spectroscopy of Standard Compound –Wedelolactone

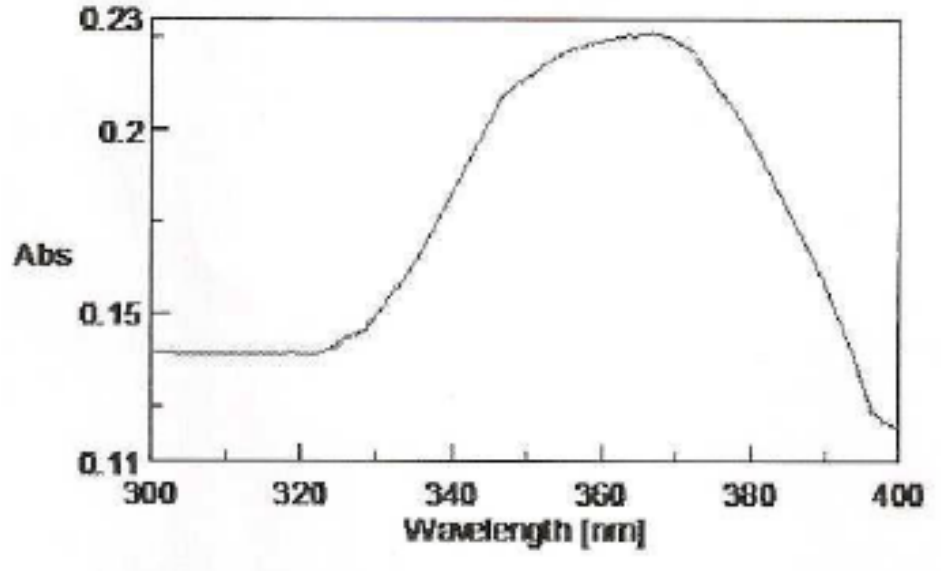
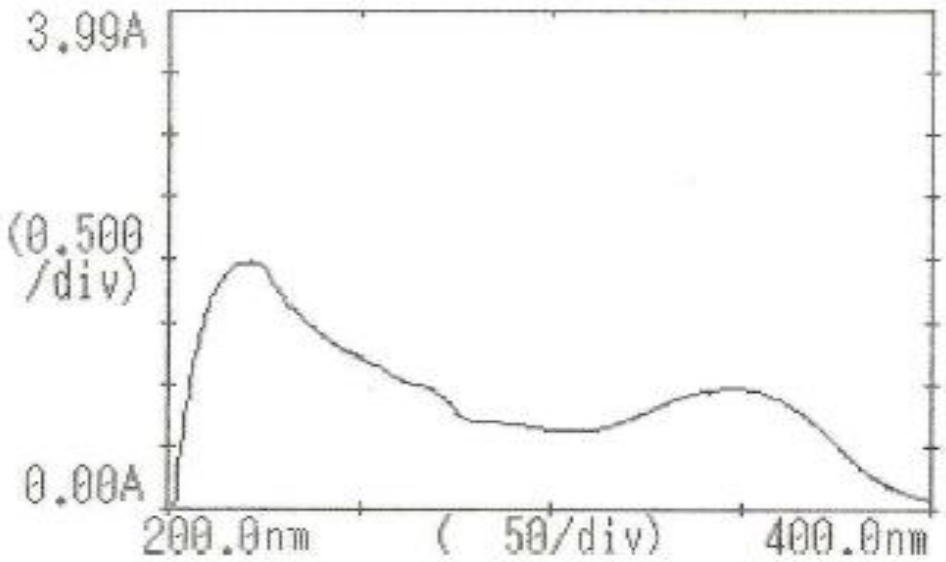


Fig-8

UV Spectroscopy of Isolated compound



2. IR- SPECTROSCOPY:

IR Spectroscopic studies are an important tool to detect the functional group present in the compound.

Instrument used : Shimadzu FTIR-8400S

Method : KBr pellet method

Wave number : 4000- 450 cm-1

Software : IR Solutions

Fig-9

IR Spectroscopy of Standard compound- Wedelolactone

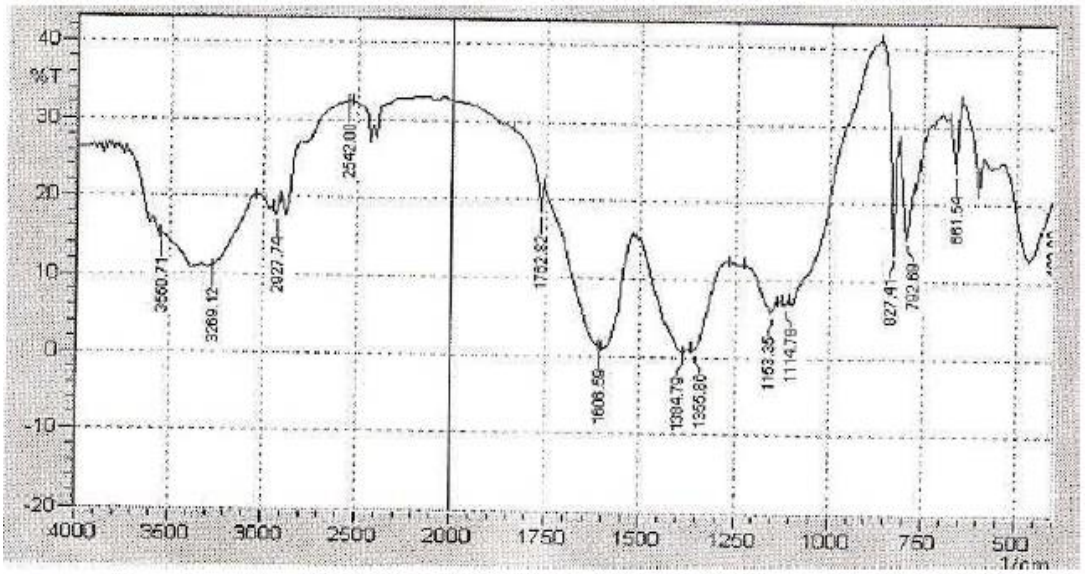
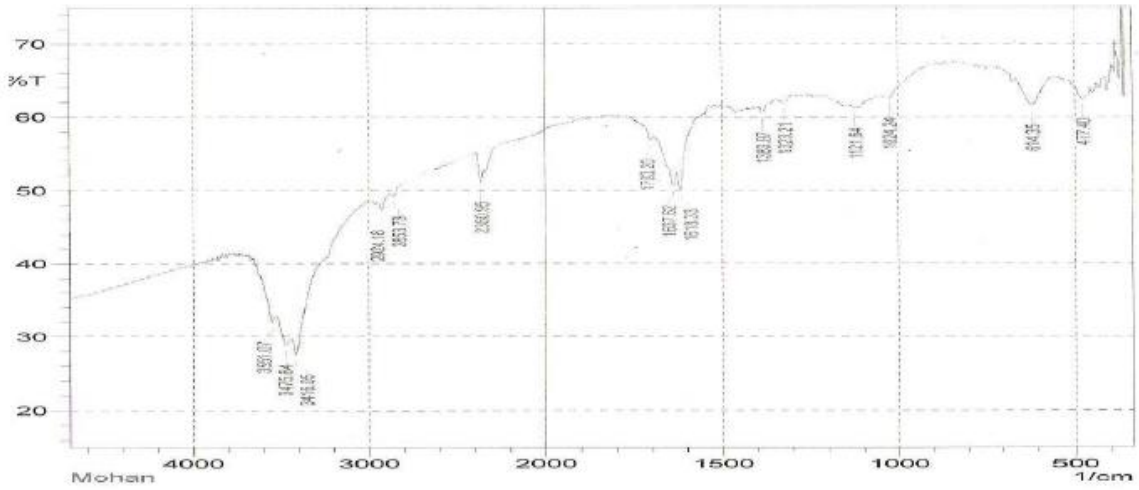


Fig-10

IR Spectroscopy of Isolated Compound



Antidiabetic Activity

Diabetes mellitus is group of metabolic diseases characterized by hyperglycemia, dyslipidemia and protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease is associated with reduced quality of life and increased risk factor for mortality and morbidity. The long term hyperglycemia is an important factor in the development and progression of micro and macro vascular complication, which include neuropathy, nephropathy, cardiovascular and cerebro vascular disease. The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Four major classes of oral hypoglycemic agents have been extensively: insulin secretogogeus, biguanides, thiazolidinediones and α- glucosidase inhibitors[10]**.** Each drug class works on different mechanism of actions, including stimulation of insulin secretion, reduction of hepatic gluconeogenesis, increase in insulin receptor sensitivity and delay of digestion and absorption of carbohydrate, respectively. Unfortunately, these agents could produce severe hypoglycemia, weight gain and

gastro intestinal disturbances. Natural products have been explored for anti diabetic activity[47].

India leads the world with the largest number of the diabetes subject earning the dubious distinction of being termed the "**Diabetic capital of the world**". The most disturbing trend is the shift in the age of onset of diabetes to a younger age in the recent years. This could have long lasting adverse effects on nation's health and economy. As per WHO, India will be the nation with highest number of diabetes in the world by2030 followed by china and then USA. This is an alarming sound as far as the health system of India is concerned.

Table-3

Prediction of Diabetes in 2030

Ranking	Country	Peoples with Diabetes(in Millions)	
		2000	2030
1	INDIA	31.7	79.4
2	CHINA	20.8	42.3
3	USA	17.7	30.3

In developing countries, the majority of people with diabetes are in the 45 to 64 year age range, similar to finding reported previously. In contrast, the majority of people with diabetes in developed countries are >64 years of age. By 2030, it is estimated that the number of people with diabetes >64 years of age will be >82 millions in developing countries and >48 millions in developed countries. India being one of the fastest developing country, and youth being the driving force for it, it will be hampered at the most is not a good sign. Proper patient education and general awareness about the disease can help reducing the extend of this damage.

All the corners of health system viz: Doctors, Pharmacists, Nurses, Government, NGOs etc. have to realize this fact and plan accordingly to tackle this situation.

CLASSIFICATION OF DIABETES MELLITUS

There are different types of diabetes mellitus,

1. TYPE- I (Beta cells destruction, usually leading to absolute insulin deficiency)

- Autoimmune
- Idiopathic

2. TYPE-II (Ranges from predominantly insulin resistant, with relative insulin deficiency, to predominantly insulin secretary defect, with/without insulin resistance)

3. Other specific types of Diabetes mellitus

- Maturity Onset Diabetes Mellitus (MODY) 1 (HNF4); rare
- MODY 2 (Glucokinase); less rare
- MODY 3 (HNF 1); accounts for 2/3 of all MODY
- MODY 4 (IPF-1); very rare
- MODY 5 (HNF-1); very rare
- MODY 6 (neuroD1); very rare
- Mitochondrial DNA

4. Genetic defects in insulin action

- Type A insulin resistance
- Leprechaunism
- Rabson-Mendenhall Syndrome
- Lipoatrophic diabetes

5. Disease of the exocrine pancreas

6. Endocrinopathies

7. Drug or Chemical induced diabetes

8. Other genetic syndromes (Down's, Klinefelter´s, Turner's and others) associated with diabetes[12].

TYPE- 1

- Type 1 diabetes was also called insulin dependent diabetes mellitus (IDDM), or juvenile onset diabetes mellitus. In type 1 diabetes, the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making insulin. Abnormal antibodies have been found in the majority of patients with type 1 diabetes. Antibodies are proteins in the blood that are part of the body's immune system. The patient with type 1 diabetes must rely on insulin medication for survival. Type 1diabetes tends to occur in young, lean individuals, usually before 30 years of age, however, older patients do present with this form of diabetes on occasion. This subgroup is referred to as latent autoimmune diabetes in adults (LADA). LADA is a slow, progressive form of type 1 diabetes.

TYPE -2

- Type 2 diabetes was also referred to as non-insulin dependent diabetes mellitus (NIDDM), or Adult onset diabetes mellitus (AODM). In type 2 diabetes, patients can still produce insulin, but do so relatively inadequately for their body's needs, A major feature of type 2 diabetes is a lack of sensitivity to insulin by the cells of the body (particularly fat and muscle cells). In addition to the problems with an increase in insulin resistance, the release of insulin by the pancreas may also be

defective and suboptimal. In fact, there is a known steady decline in beta cell production of insulin in type 2 diabetes that contributes to worsening glucose control. Finally, the liver in these patients continues to produce glucose through a process called gluconeogenesis despite elevated glucose levels. [13]

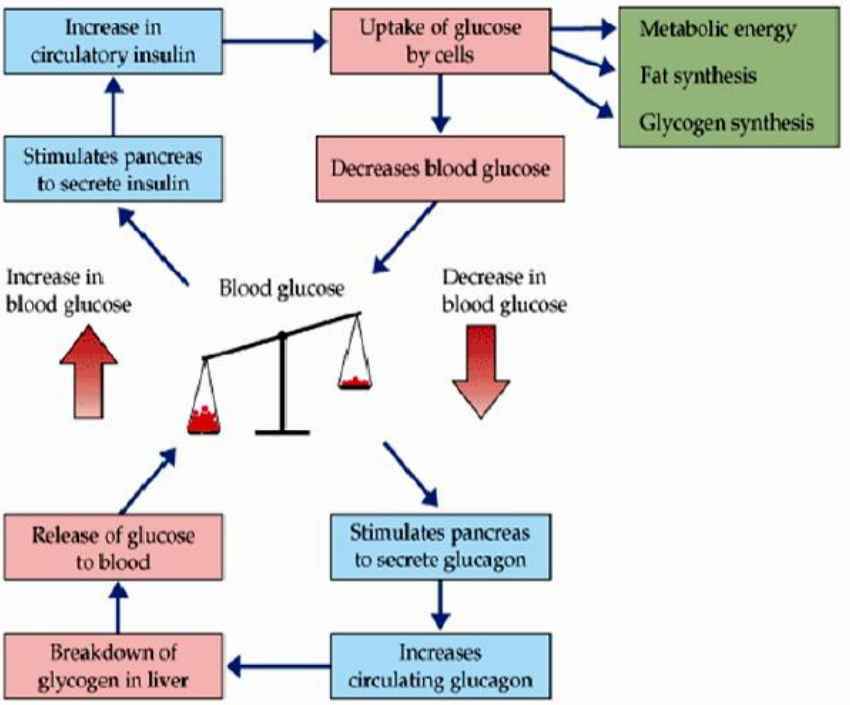
GESTATIONAL DIABETES:

- Diabetes can occur temporarily during pregnancy. Significant hormonal changes during pregnancy can lead to blood sugar elevation in genetically predisposed individuals. Blood sugar elevation during pregnancy is called gestational diabetes. Gestational diabetes usually resolves once the baby is born. However, 25%-50% of women with gestational diabetes will eventually develop type 2 diabetes later in life, especially in those who require insulin during pregnancy and those who remain overweight after their delivery. Patients with gestational diabetes are usually asked to undergo an oral glucose tolerance test about six weeks after giving birth to determine if their diabetes has persisted beyond the pregnancy, or if any evidence (such as impaired glucose tolerance) is present that may be a clue to the patient's future risk for developing diabetes **.**
- Approximately 10% of the patients have Type 1 diabetes and the remaining 90% have Type 2 diabetes. Individuals who are at high risk of developing Type II diabetes mellitus include people who:
- Are obese (more than 20% above their ideal body weight)
- Have a relative with diabetes mellitus
- Have been diagnosed with gestational diabetes or have delivered a baby weighing more than 9 lbs (4 kg)
- Have high blood pressure (140/90 mmHg or above)

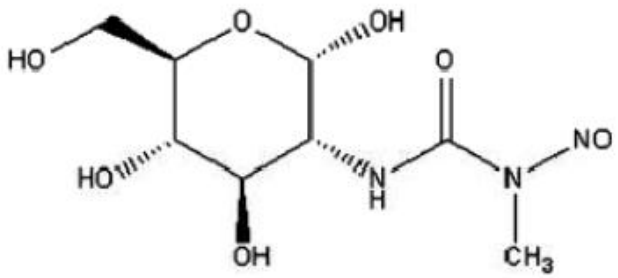
- Have a high density lipoprotein cholesterol level less than or equal to 35 mg/dL and/or a triglyceride level greater than or equal to 250 mg/dL

Fig-11

Glucose Regulation in Human Body



Streptozotocin Induced Diabetic Model:



Streptozotocin

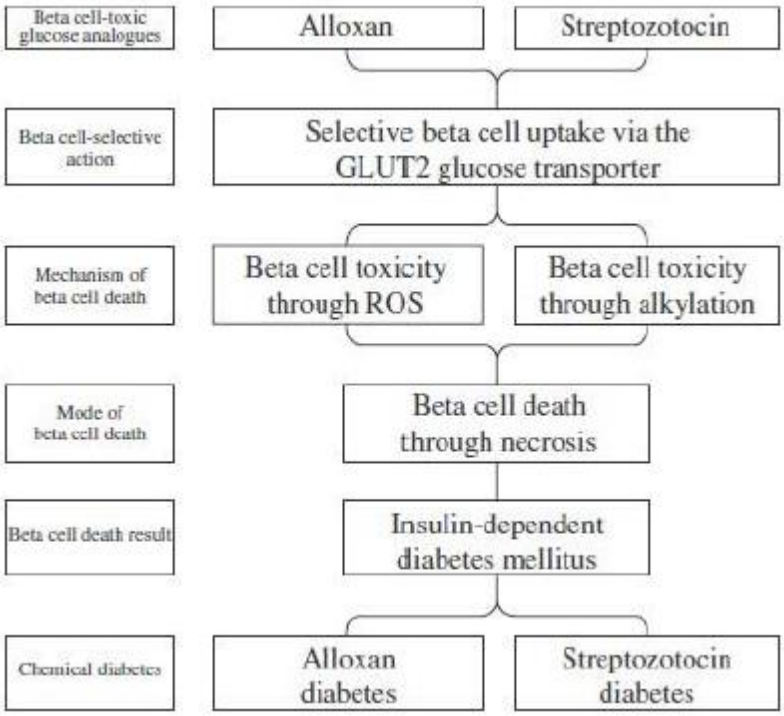
Streptozotocin : Mechanism of action:

*Streptozotocin*inhibits insulin secretion and causes a state of insulin dependent diabetes mellitus. Both effects can be attributed to its specific chemical

properties, namely its alkylating potency. As with *alloxan*, its beta cell specificity is mainly the result of selective cellular uptake and accumulation[50].

Fig-12

Mechanism of Streptozotocin Producing Diabetes Mellitus



Beta cell toxicity of streptozotocin:

It is generally assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnimethylnitrosourea moiety **especially at the O6 position of guanine.** The transfer of the methyl group from *streptozotocin*to the DNA molecule causes damage, which along a defined chain of events **,** results in the fragmentation of the DNA **.** Protein glycosylation may be an additional damaging factor . In the attempt to repair DNA, poly (ADP-ribose) polymerase (PARP) is over stimulated. This diminishes cellular NAD+, and subsequently ATP, stores **.**The depletion of the cellular energy stores ultimately results in beta cell necrosis. Although *Streptozotocin*also ethylated proteins **DNA** methylation is ultimately responsible for

beta cell death, but it is likely that protein methylation contributes to the functional defects of the beta cells after exposure to *streptozotocin*. Inhibitors of poly ADP-ribosylation suppress the process of DNA methylation. Thus, injection of nicotinamide and other PARP inhibitors in parallel with, or prior to the administration of *streptozotocin*is well known to protect beta cells against the toxic action of *streptozotocin*and to prevent the development of a diabetic state [20]. Also, mice deficient in PARP are resistant to beta cell death mediated by *streptozotocin*, in spite of DNA fragmentation. The absence of PARP prevents the depletion of the cofactor NAD+ and the subsequent loss of ATP[21] and thus cell death. The role of alkylation in beta cell damage has also been examined by the use of ethylating agents, which are less toxic than their methylating counterparts, on account of O6- ethyl guanine being less toxic than O6-methylguanine[22]. The fact that N-ethyl-N-nitrosourea and ethyl methanesulphonate are significantly less toxic to insulin-producing cells than MNU and methyl methanesulphonate[22]has been taken as support for the notion that in insulin producing cells, as in other cell types, the mechanism of toxic action is due to alkylation, with methylation of DNA bases being more toxic than ethylation**.**

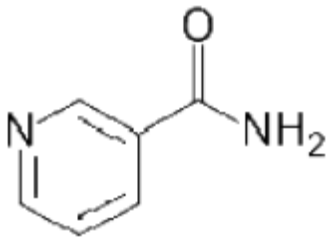
An alternative hypothesis proposes that part of the diabetogenic effect of *streptozotocin*may relate not to its alkylating ability but to its potential to act as an intracellular nitric oxide (NO) donor**.** Both *streptozotocin*and MNU contain a nitroso group and can liberate NO. In fact, *streptozotocin*has been shown to increase the activity of guanylyl cyclase and the formation of cGMP, which are characteristic effects of NO. However, the alkylating agent methyl methane sulphonate is the most toxic compound, though unlike MNU, it is not a NO donor, indicating that NO is not an indispensable prerequisite for the toxic action of the family of alkylating agents that *streptozotocin*belongs to. Finally, some minor generation of ROS, including

superoxide and hydroxyl radicals originating from hydrogen peroxide dismutation during hypoxanthine metabolism[49] may accompany the effect of *streptozotocin*and accelerate the process of beta cell destruction but ROS do not play a crucial role[23..]

Inhibition of insulin secretion by Streptozotocin:

The effects of *streptozotocin*on glucose and insulin homeostasis reflect the toxin-induced abnormalities in beta cell function. Initially, insulin biosynthesis, glucose-induced insulin Secretion and glucose metabolism (both glucose oxidation and oxygen consumption) are all affected. On the other hand, *streptozotocin*has no immediate, direct inhibitory Effect upon glucose transport[31] or upon glucose phosphorylation by glucokinase**.** However, at later stages of functional beta cell impairment, deficiencies in terms of gene expression and protein production lead to the deterioration of both glucose transport and metabolism[28]. Even before the negative effects of mitochondrial DNA and protein alkylation and glycosylation become evident, *streptozotocin*-induced depletion of NAD+ may result in the inhibition of insulin biosynthesis and secretion[16, 18]. Later, inhibition of glucose-induced and amino acid induced insulin secretion[29]**,** as a result of mitochondrial enzyme dysfunction and damage to the mitochondrial genome become apparent. This impairment is more marked for nutrient- than for non-nutrient-induced insulin secretion. This interpretation has been confirmed through studies which have shown that pre-treatment of isolated pancreatic islets with the poly (ADP-ribose) polymerase (PARP) inhibitor nicotinamide prevents early inhibition of beta cell function during the first day after *Streptozotocin*exposure, while long-term inhibition of insulin secretion 6 days after *streptozotocin*exposure was not counteracted by nicotinamide **.**

Nicotinamide



Nicotinamide, also known as niacinamide and nicotinic acid amide, is the amide of nicotinic acid (vitamin B3 / niacin). Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. Nicotinic acid, also known as niacin, is converted to nicotinamide in vivo, and though the two are identical in their vitamin functions, nicotinamide does not have the same pharmacologic and toxic effects of niacin. Nicotinamide is presently tested for its potential benefit in preventing Type 1(insulin- dependent) diabetes. (Wikipedia.com)

Addition of *nicotinamide*to *Streptozotocin*treated beta cells could thus prevent their rapid necrosis through preservation of the cellular NAD and ATP levels, but the surviving cells might be suppressed both in their DNA repair mechanisms and in their mitochondrial functions and could therefore become more susceptible to apoptosic[29].

ANTI DIABETIC ACTIVITY OF *DICHROSTACHYS CINEREA* (L.) WIGHT & ARN

Materials and Methods: Chemicals:

Streptozotocin (STZ) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Carboxy methyl cellulose (CMC) sodium salt, fructose, phosphotungstic acid, 1,1',3,3' tetramethoxy propane, butylated hydroxy toluene (BHT), xylenol orange, dithionitrobis benzoic acid (DTNB), ascorbic acid, 2,2' dipyridyl, p-phenylene

diamine hydrochloride, sodium azide, isopropanol, sodium meta periodate and triethanolamine were obtained from S.D. Fine Chemicals, Mumbai, India. Hesperidin was purchased from Himedia Laboratories Pvt, Ltd, Mumbai. Blood glucose and ELISA kits were purchased from Agappe diagnostics, Kerala, India. All other chemicals and biochemicals used in the study were of analytical grade.

Animals and Animal Care:

Male albino Wistar rats, weighing 150-200g were used in this study. Protocols were in accordance with and approved by the institutional animal ethical committee. These animals were kept in an environment with controlled temperature (25°C), humidity (45–57%), and photoperiod (12/12-hrs light-dark cycle). All the animals were fed free access to the rat chow and also had free access to water.

Induction of Experimental Diabetes:

The animals were acclimatized for one week before initiation of the experiment. After overnight fasting, diabetes was induced by intraperitoneal injection of *streptozotocin* (STZ), 15 min after the administration of 110mg/kg body weight of *nicotinamide* **(**STZ was freshly dissolved in 0.1 m sodium citrate buffer, pH 4.5. at a dose of 65 mg/kg body weight. After 48 hours of STZ administration rats with moderate diabetes having hyperglycemia (>250mg/dL) were used in this study. The treatment was started on the third day after the STZ injection and this was considered the first day of treatment. The treatment was continued for 21 days[51,52]**.**

Experimental Design:

The rats were divided into five groups comprising 5 animals in each group as follows:

Group 1: Control rats given only Carboxy Methyl Cellulose (CMC).

Group 2: Diabetic controls (STZ 65mg/kg + 110mg/kg Nicotinamide body Weight of rats).

Group 3: Diabetic rats treated with *Glibenclamide* (10 mg/kg p.o) for 21days (Sharma et al., 1997).

Group 4: Diabetic rats treated with Ethanolic Extract of *Dichrostachys cinerea* (L.) Wight & Arn (250 mg/kg p.o.) Suspended in CMC for 21 days.

Group 5: Diabetic rats treated with Ethanolic Extract of *Dichrostachys cinerea* (L.) Wight & Arn (500 mg/kg p.o.) Suspended in CMC for 21 days.

After 21 days of treatment period the fasted rats were sacrificed by cervical decapitation and the blood was collected in tubes containing potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting plasma glucose. Plasma and serum were separated by centrifugation. After centrifugation at 2,000 rpm for 10 min, the clear supernatant was used for the analysis of various biochemical parameters. The liver was excised and rinsed in ice-cold saline and kept in formalin solution. Tissues were cut into small pieces and homogenized with a Potter-Elvehjem tight-fitting glass-Teflon homogenizer in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for various measurements.

Statistical Analysis:

All the grouped data were statistically evaluated using the Statistical Package for Social Sciences (SPSS) version 7.5 (Chicago, IL, USA). Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least

significant differences test. P-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as Mean ± Standard Error Mean (SEM) for 5 animals in each group.

The Histological Study:

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

1. Estimation of body weight:

Body weights are estimated for starting day of the experiment and after 21 day of the experiment[53.]

2. Estimation of plasma glucose:

Glucose level in plasma was estimated by glucose oxidase/ Peroxidase method using a commercial kit of SD Check Ozone Biomedical Pvt Ltd[53.]

3. Estimation of Serum Insulin:

Insulin was assayed in plasma using a commercial kit by enzyme linked immunosorbant assay (ELISA) technique **[54].**

Reagents:

1. Monoclonal anti-insulin antibody

2. Enzyme conjugate: Anti-insulin antibodies conjugated to horseradish Peroxidase

3. Standard: Human insulin

4. Solution A: Buffer solution containing hydrogen peroxide

5. Solution B: Tetramethylbenzidine

6. Concentrated wash buffer

7. Stop solution: 2 N HCl

Procedure:

25 l of the plasma was dispensed in micro wells coated with anti-insulin antibody. To this, 100 l of the enzyme conjugate was dispensed into each well, mixed for 5 sec and incubated at 25 C for 30 min. The wells were rinsed five times with washing buffer. Then, 100 l of solution A and then 100 l of solution B were dispensed into each well. This was incubated for 15 min at room temperature. The reaction was stopped by adding 50 l of 2 N HCl to each well and read at 450 nm.

The values are expressed as µ U/mlplasma.

4. Estimation of glycosylated hemoglobin (Hb A1C):

Glycosylated hemoglobin was estimated by the method using a commercial kit of Asritha quality processes.

Reagents:

1. Lysing reagent

2. Ion exchange resin tube

3. Distilled water

Procedure:

Step A: Hemolysate Preparation

250 µ l of Lysing reagent was taken in a test tube, to this 50µ l of well mixed whole blood was added, mixed well. Incubated for 5 minutes at room temperature to allow complete lysis of R.B.C.

Step B: Glyco Hemoglobin (GHb) Separation

0.1 ml of Hemolysate from step A was added in to ion exchange resin tubes. The resin tube was agitated by vortex mixer for 5 minutes. The resin was allowed to settle. Then the supernatant was separated. The absorbance's of the supernatant was measured at 420 nm in a colorimeter.

Step C: Total Hemoglobin (THb) Estimation

5 ml of distilled water was taken in a glass tube. To this 0.02 ml of hemoglysate from Step A was added, mixed well and the absorbance was measured at 420 nm in a colorimeter [55].

The GHb in % was calculated by the following formula

$$GHb = \frac{\text{Abs. of. GHb}}{\text{Abs. of THb}} \times 4.61$$

Where, 4.61= Assay factor

From this GHb % (A1), HbA1c (A1c) and Mean blood glucose (MBG) was calculated by the standard chart provided with kit.

The values were expressed as mg/g hemoglobin.

Lipid extraction for estimation of lipids:

Serum lipids were extracted by the method [56]

The serum were rinsed in cold physiological saline thoroughly and dried by pressing between the folds of filter paper. A known weight of tissues was homogenized with 2.5 ml of ethanol-ether mixture (3:1 v/v) and contents were digested for about 2 h at 60-65 C. The supernatant was collected 3 ml of ethanol-

ether mixture was added to the residue and digested further for a period of 2 h at 60-65 C. The supernatant was collected. Then 1 ml of chloroform-methanol mixture (1:1 v/v) was added to the residue. It was again digested for 2 h at 50-55 C and the supernatant was collected. The supernatant was pooled and made upto a specified volume. Aliquots of this extract were then used for the estimation of cholesterol, free fatty acids, triglycerides and phospholipids. Serum was also processed similarly.

5. Estimation of total cholesterol

Total cholesterol was estimated by the method[54]

Reagents:

1. Ferric chloride-acetic acid reagent: 0.05% ferric chloride in acetic acid.

2. Concentrated sulphuric acid.

3. Cholesterol stock standard: 1 mg/ml in acetic acid.

4. Cholesterol working standard: 40 g in ferric chloride-acetic acid reagent.

Procedure:

0.1 ml of extract was evaporated to dryness and 5.0 ml ferric chloride- acetic acid reagent was added, mixed and centrifuged. To the supernatant 3.0 ml of concentrated sulfuric acid was added and the absorbance was read after 20 min at 560 nm against a reagent blank. A Standard in the concentration range of 40-200 g was treated similarly. Values were expressed as mg/dL serum.

6. Determination of triglycerides:

Triglycerides were determined by the method[54]

Triglycerides are extracted by isopropanol, which upon saponification with potassium hydroxide yield glycerol and soap. The glycerol liberated is treated with

Meta per iodate, which releases formaldehyde, formic acid and iodide. The formaldehyde released reacts with acetyl acetone and ammonia forming yellow colored compound, the intensity of which is measured at 420nm.

Reagents:

1. Isopropanol.

2. Activated aluminium oxide (Neutral).

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

4. Sodium Meta per iodate reagent - 77 g of anhydrous ammonium acetate was dissolved in about 700 ml of distilled water; 60 ml glacial acetic acid was added to it followed by 650 mg of sodium Meta periodate. The mixture was diluted to 1 liter with distilled water.

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

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

Procedure:

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

reagent followed by 0.5 ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65 C for 1 h, the contents were cooled and absorbance was read at 420nm. A series of standards of concentrations 8-40 g triolein were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 420nm.

The triglyceride content was expressed as mg/dl - serum

7. Cholesterol content in lipoprotein fractions: [57]

Cholesterol in the lipoprotein fractions was also determined by the method as described earlier[53]. HDL cholesterol was analyzed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg2+. LDL cholesterol and VLDL cholesterol were calculated as follows:

VLDL-C = Triglycerides/5

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

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

7. RESULTS AND DISCUSSION

The leaf *Dichrostachys cinerea (L.) Wight & Arn*belonging to the family Fabaceae has been investigated in a systemic way of covering phytochemical and pharmacological aspects in an attempt to rationalize its use as a drug of therapeutic importance.

Preliminary Phytochemical Studies:

The powdered leaf of *Dichrostachys cinerea* (L.) Wight &Arn was subjected for continuous hot extraction with petroleum ether and ethanol successively. The yield was found to be 8% w/w and 26%w/w respectively. The ethanol extract obtained was subjected to various phytochemical tests, to identify the active constituents, which showed the presence of Phytosterol, Carbohydrates, Flavonoids and Terpenoids. The ethanol extract was further partitioned with ethyl acetate and water. The ethyl acetate fraction was further partitioned with chloroform and water. The water fraction was further separated using coloumn chromatography. A fraction was isolated in chloroform: methanol at the ratio of 90:10.

The fraction was dried and was subjected to thin Layer chromatography where aqueous fraction showed a spot with Rf value 0.54 whereas the standard shows Rf value 0.56. At the same was performed to HPTLC for confirmation of chemical constituent present in it. The isolated fraction was then subjected to spectral studies and compared with standard.

The UV absorption range of the isolated fraction shows maximum absorption at 349nm whereas the standard shows the maximum absorption at 366 nm. The IR spectra of standard compound shows the O-H strech-3550, O-H bend at 1384, C=O

stretch at 1762, C-H stretch at 2927. The fractionated compounds shows O-H stretch at 3551, O-H bend at 1383.97, C=O stretch at 1703, C-H stretch at 2924.

Pharmacological Studies:

Table-4

Effect of EEDC on Oral Glucose tolerance Test in Rats

Oral Glucose Tolerance Test

Groups	Fasting	30 min	60 min	90 min	120 min
Control	82.4 ± 3.16	115.74 ± 2.13	112.1 ± 3.84	109.1 ± 2.44	106.35 ± 2.17
Diabetic + *Glibenclamide*(10mg/kg)	77.3 ± 1.77	96.51 ± 2.10a	94.1 ± 1.95a	90.72± 1.65a	89.25 ± 1.03a
Diabetic +EEDC (250mg/kg)	78.2 ± 1.65	107.1 ± 0.85a	105.2 ± 1.19a	100.2 ± 1.95a	98.3 ± 1.65a
Diabetic +EEDC (500mg/kg)	76.25 ± 0.825	102.15 ± 1.25a	98.25 ± 1.25a	96.8 ± 2.32a	94.8 ± 1.795a

Values are given as Mean ± Standard Error Mean for groups of 5 animals each. Values are statistically significant at (a -P<0.05)

Glucose (control) groups were compared with Extract treated and standard drug group**.**

Fig-13

Effect of EEDC on Oral Glucose tolerance Test in Rats

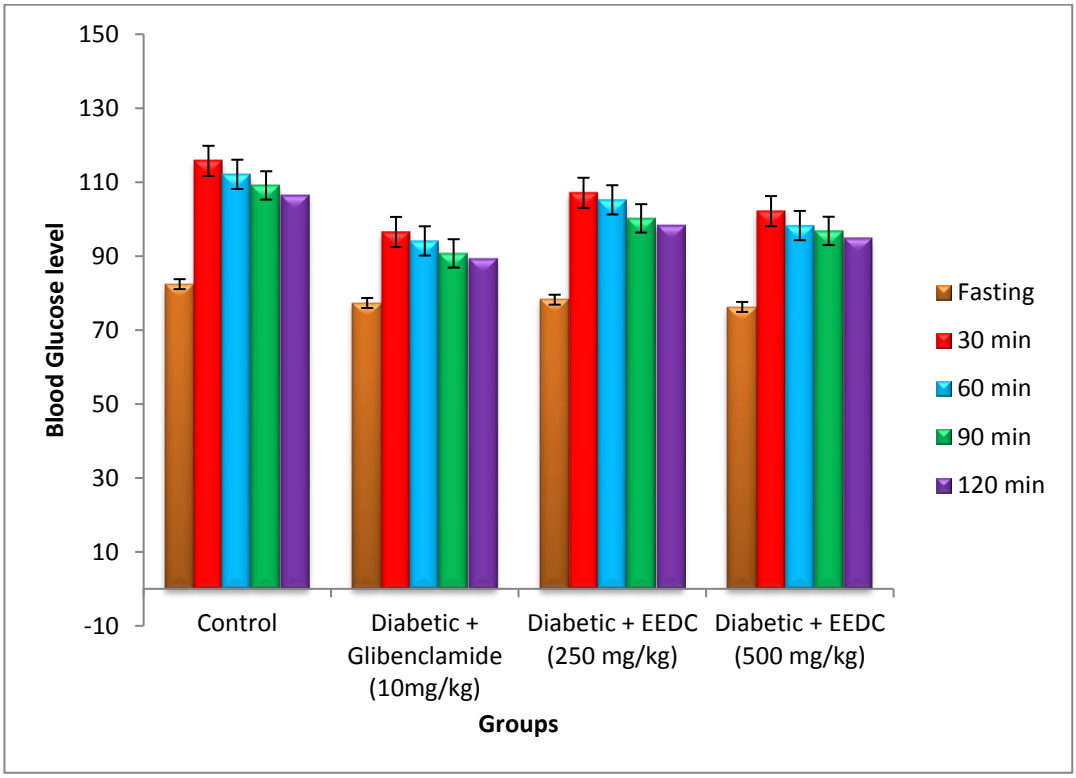


Table- 5

Body weight changes of EEDC on control and experimental groups of rats

Treatment	Body weight (g) Initial	Body weight (g) Final
Control	179±3.3	194.8±3.9
Diabetic Control	181±3.1	165.2±3.3
Diabetic + Standard drug(10mg/kg)	175±5.6b	192±5.04b
Diabetic + Extract 250mg/kg(EEDC 250mg/kg)	179±3.6a	183±3.4a
Diabetic + Extract 500mg/kg(EEDC 500mg/kg)	175±3.0b	189.75±3.9b

Values are given as Mean ± S.E.M for groups of 5 animals each. Values are statistically significant at (a -P<0.05, b - P<0.01)

Glucose (control) groups were compared with Extract treated and standard drug group**.**

Fig- 14

Effect of EEDC on Body weight of control andexperimental groups of rats

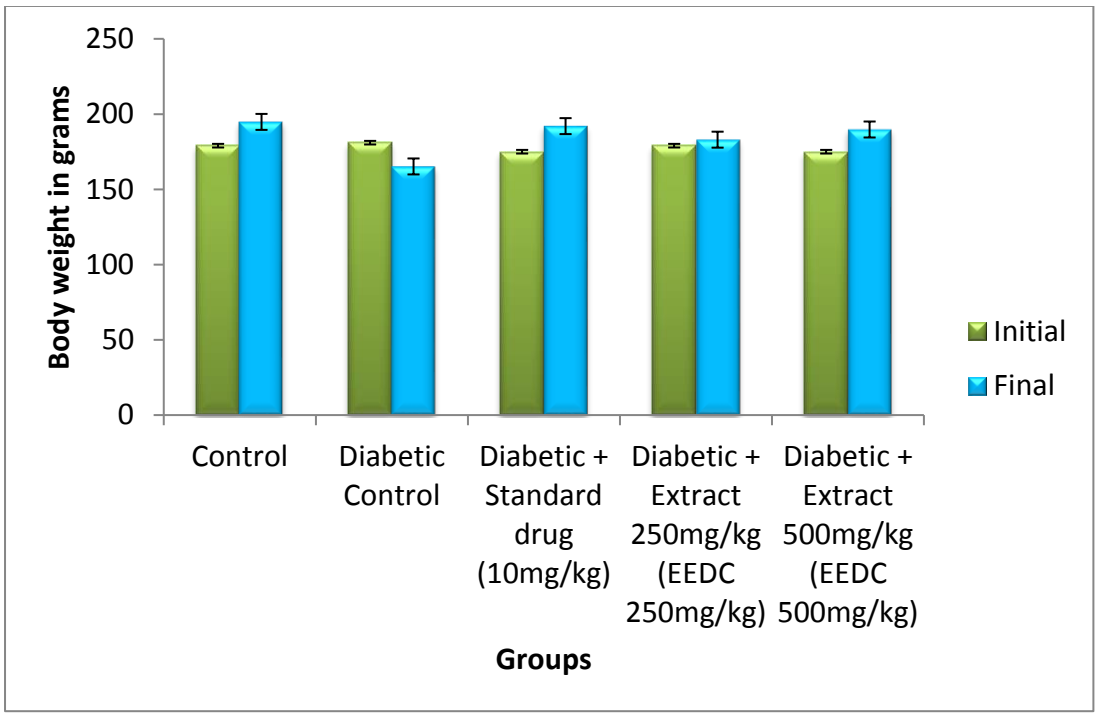


Table- 6

Effect of EEDC on Blood glucose of control and experimental groups of rats.

Groups	Treatment dose/kg, b.wt	Blood glucose level (mg/dl)			
		0 Day	**7th Day**	**14th day**	**21st day**
I	**Normal control**	92.16 ± 3.25	96.13 ± 2.73	94.8 ± 4.65	97 ± 1.81
II	**Diabeticcontrol**	365.83 ±1 .10c	374.66 ± 4.38c	390.2 ± 4.25c	405.1 ± 7.95c
III	**Diabetic +*Glibenclamide* 10 mg/kg**	305 ± 4.59a	250.4 ± 4.26c	204.4 ± 5.24c	139 ± 9.33c
IV	**Diabetic +EEDC250mg/ kg**	306.6 ± 4.02a	265.4 ± 12.26c	217.6 ± 9.67c	178.5 ± 6.41c
V	**Diabetic +EEDC500mg/ kg**	307.83 ± 3.58a	253.4 ± 7.54b	202.4 ± 4.86b	141.8 ± 3.42c

Values are given as Mean ± S.E.M for groups of 5 animals each. Values are statistically significant at (a -$P<0.05$, b - $P<0.01$)

Normal control groups were compared with diabetic control and EEDC – treated diabetic rats were compared with diabetic rats; *Glibenclamide*– treated diabetic rats were compared with diabetic control rats.

Fig- 15

Blood Glucose level

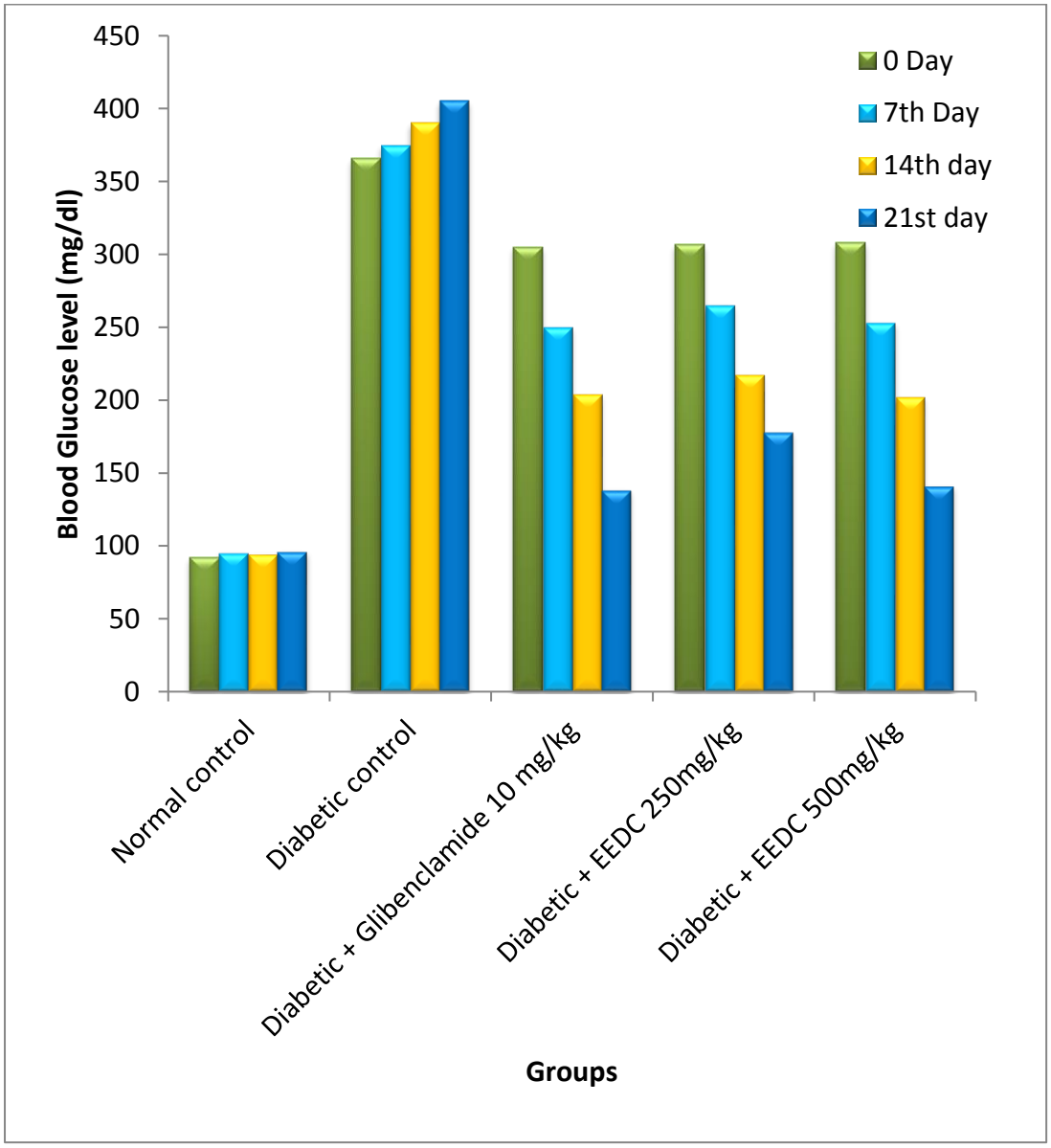


Table-7

Effect of EEDC on Serum Insulin of control and experimental groups of rats

Group	Serum Insulin	HbA1C
	Final	**Final**
Control	29.07±0.46	1.92 ± 0.17
Diabetic Induced	19.86±0.59b	5.70 ± 0.37b
Diabetic + *Glibenclamide*10 mg/kg	27.02±0.47 a	2.00 ± 0.15b
Diabetic + EEDC 250mg/kg	23.22±0.68 a	4.20 ± 0.38 a
Diabetic + EEDC 500mg/kg	26.51±0.68a	3.20 ± 0.44 b

Values are given as Mean ± S.E.M for groups of 5 animals each. Values are statistically significant at (*a -P<0.05, b - P<0.01)*

Normal control groups were compared with diabetic control and EEDC – treated diabetic rats were compared with diabetic rats; *Glibenclamide*– treated diabetic rats were compared with diabetic control rats.

Fig-16

Effect of EEDC on Serum Insulin of control and experimental groups of rats

Serum Insulin Level

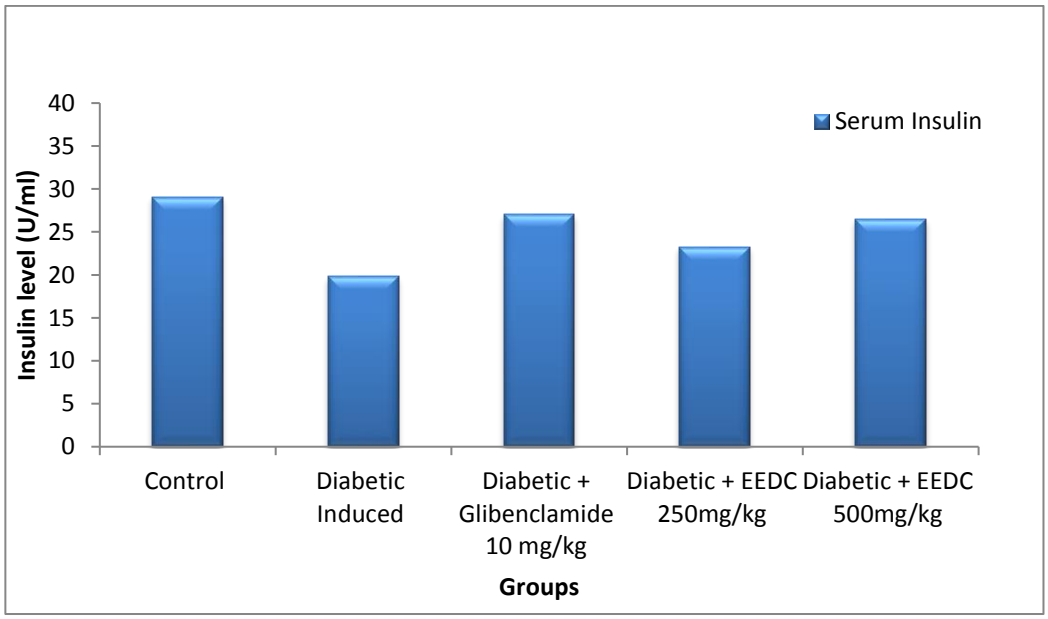


Fig-17

Effect of EEDC on Glycosylated Haemoglobin of control and experimental Groups of rats

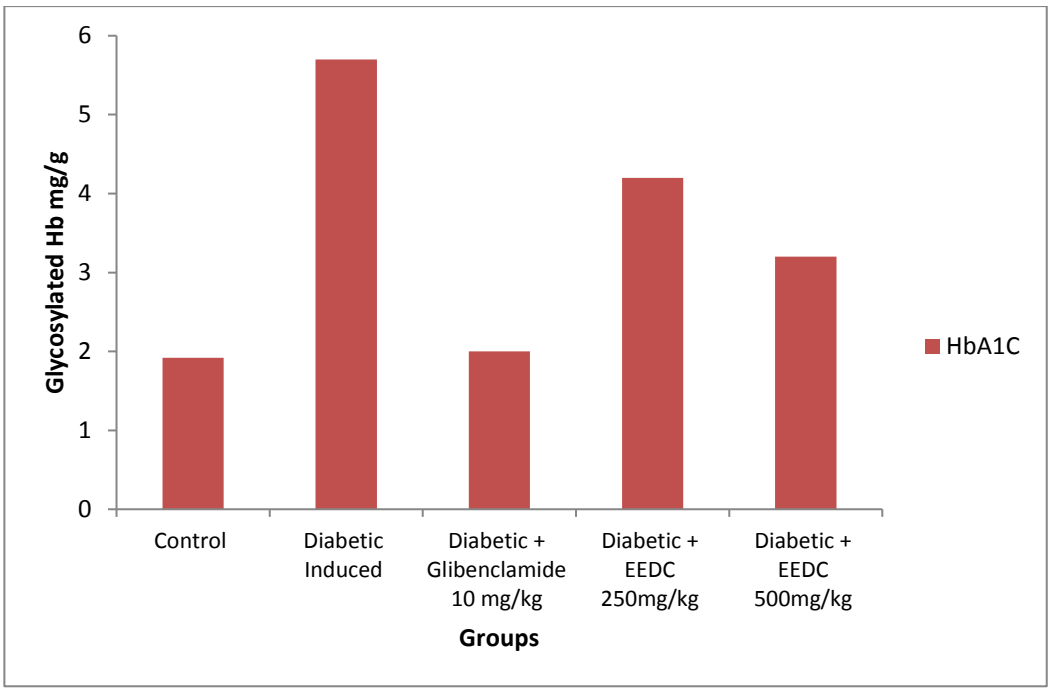


Table- 8

Effect of EEDC on Total cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol of control and experimental groups of rats

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
Control	132±3.1	82±1.68	48.61±1.86	90.8±1.56	19±1.14
Diabetic control	255.3±2.73c	206.6±4.78c	28.28±1.854b	200±1.64c	34.2±1.71c
Diabetic + Standard Drug (10 mg/kg)	160.78±1.27b	87.6±2.274b	52.8±2.358b	96±1.54b	20.4±1.28b
Diabetic + Ethanol Extract (250 g/kg)	180±2.31a	119.4±2.239a	38.17±1.77a	133±2.24a	29.6±1.07a
Diabetic + Ethanol Extract (500 mg/kg)	165.66±2.13b	95.8±1.97b	43.45±2.27b	101±1.14b	24.2±2.227b

Values are given as mean ± S.E.M for groups of 5 animals each. Values are statistically significant at (*a -P<0.05, b - P<0.01, c- P<0.001).*

Normal control groups were compared with diabetic control and EEDC – treated diabetic rats were compared with diabetic rats; *Glibenclamide*– treated diabetic rats were compared with diabetic control rats.

Fig-18

Effect of EEDC on Total cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol of control and experimental groups of rats

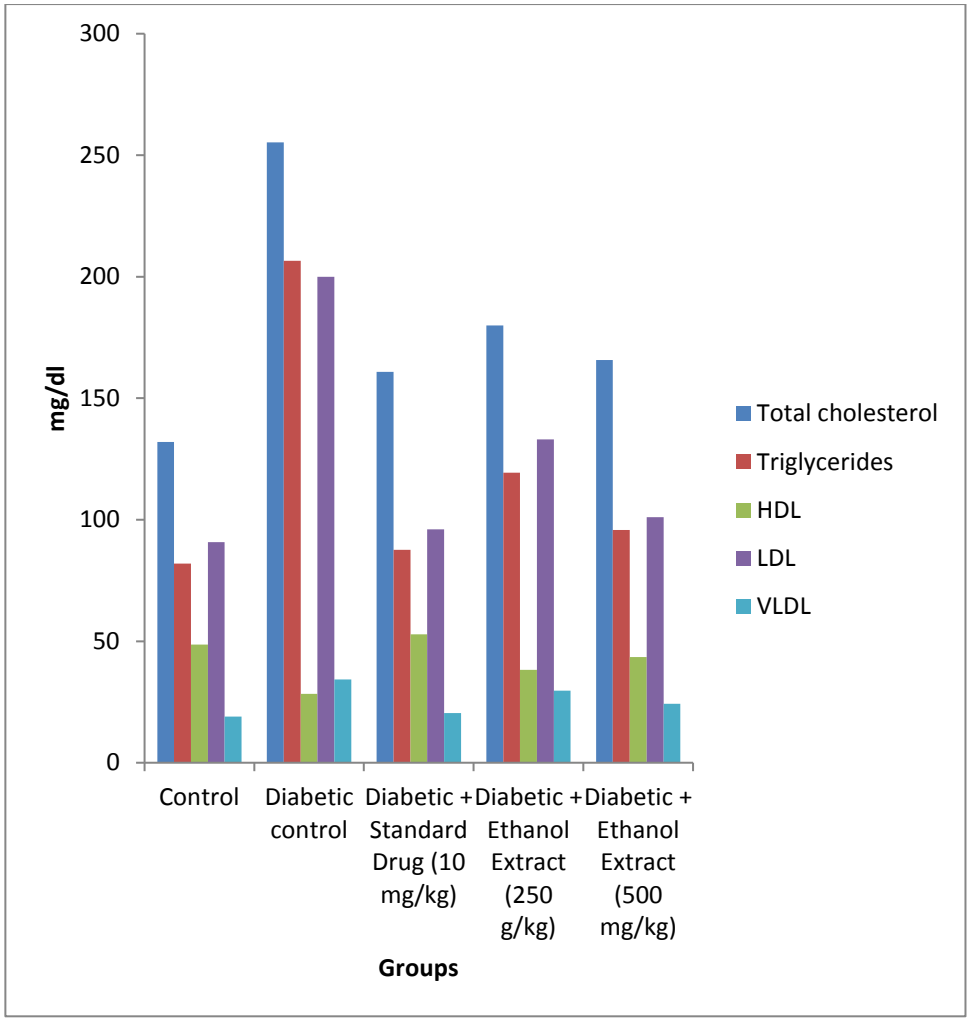
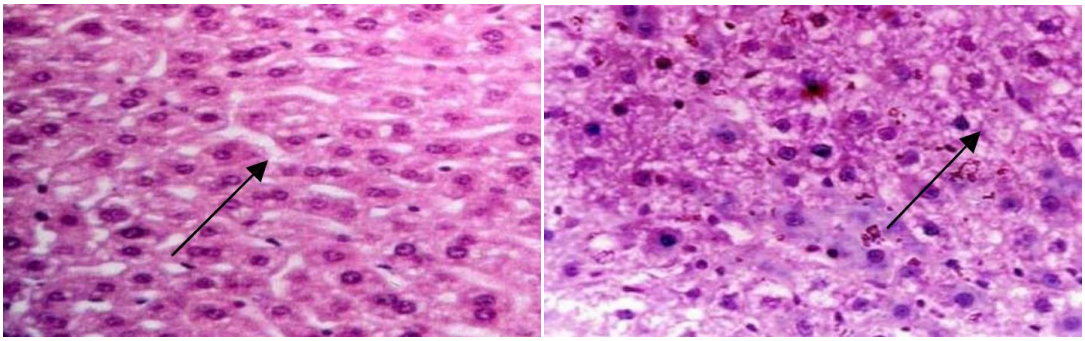
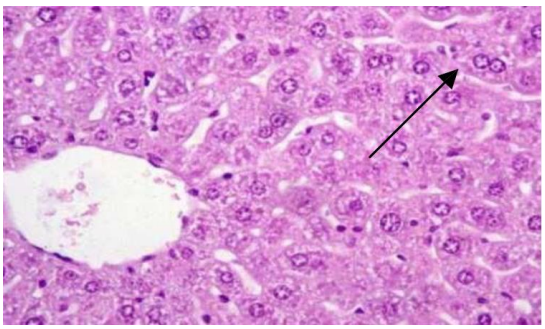


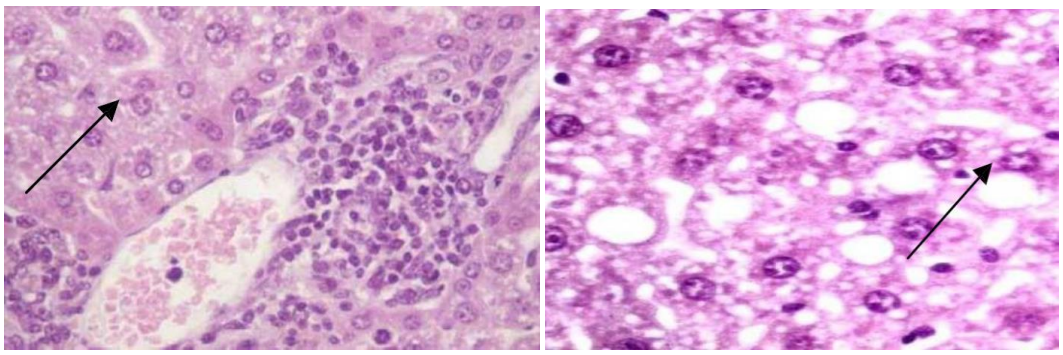
Fig-19 Histopathology of liver



Group 1 **Group 2**



Group 3



Group 4 **Group 5**

Description of liver: Group-1:

Tissue section shows normal architecture of liver cells. Hepatocytes show normal size and shape. The hepatocytes are well arranged. The nuclei are round and uniform.

Group-2:

Tissue section shows marked distortion of hepatic architecture by sub massive

Necrosis near the portal area.

Portal veins are dilated and congested.

Disarrangements of hepatocytes with mild inflammation.

Hepatocytes show atypical proliferation.

Group-3:

Tissue section shows regeneration of liver cells.

Portal tracts are normal.

The nuclei are round and uniform with little variation in size.

Atypical proliferation was reduced to normal.

Group-4:

Tissue section shows degenerative changes partly reduced.

Hepatocytes show regaining of normal size and shape.

Hepatocytes are well arranged.

Portal veins are near to mild normal congestion.

Group-5:

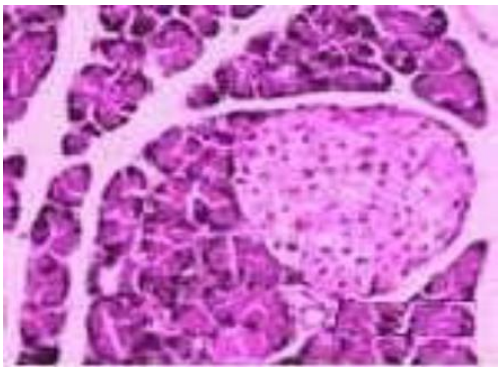
Tissue section shows regeneration to normal liver cells.

Hepatocytes shows normal size in shape and well arranged. Portal veins are appearing to be normal.

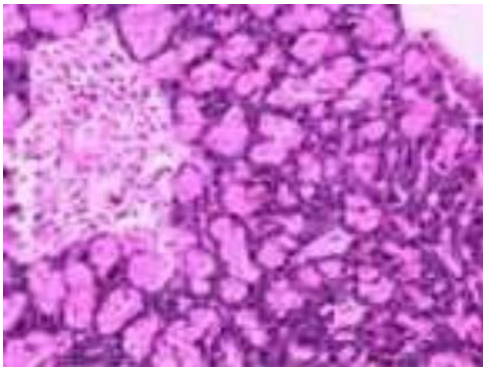
The nuclei are round with very little variation.

Fig-20

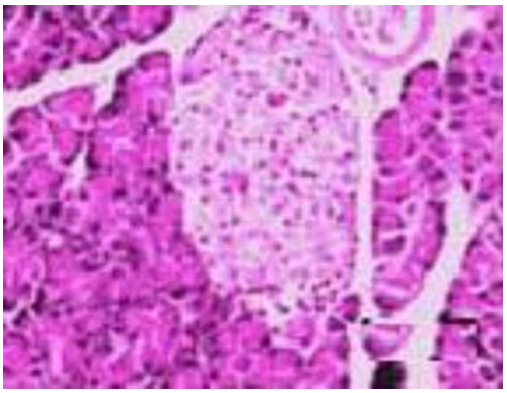
Histopathology of Pancreas



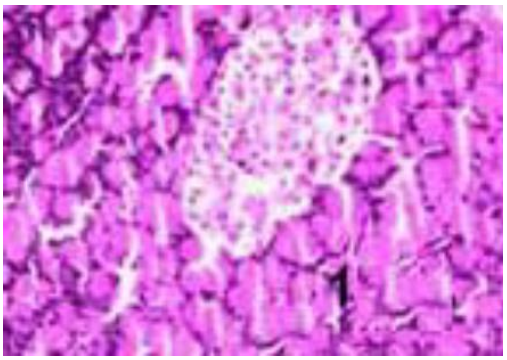
Group-1



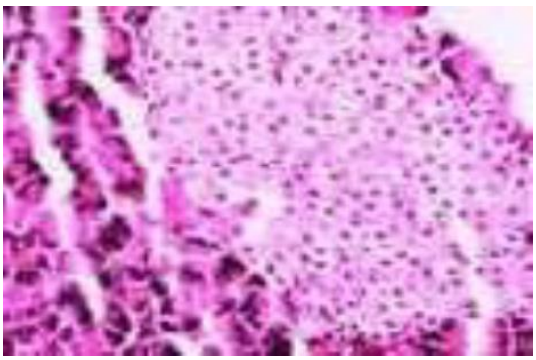
Group-2



Group-3



Group-4



Group-5

Photomicrograph of pancreas stained with haematoxylin and Eosin (H & E). (Magnification × 200)

Description of Pancreas:

Group-1:

The section shows exocrine acini and endocrine islets.

The islets were compactly arranged within negligible intercellular space. The islets are quit abundantly distributed.

Group-2:

The pancreatic islets are damaged and shrunken in size. The pancreas shows depleted islets.

Significant architectural disarray and extended into surrounding exocrine tissue.

Necrosis of the cells is very clear.

Group-3:

Shows exocrine acini and small preserved islets. Decreased in intracellular space.

Less occurrences of peripheral widening between pancreatic acini and islets.

Group-4:

Shows less preserved islets.

Cells are irregular, not well defined in compared with diabetic group. Varying in sizes in the same lobule of pancreas.

Group-5:

The islets and acini cells are improved restored.

The islets were compactly arranged, widening was decreased between acinar and islet cells.

8. SUMMARY AND CONCLUSION

The leaf *Dichrostachys cinerea* (L.) Wight and Arn belonging to family fabaceae has been studied to compare and give detailed reports on, preliminary phytochemical analysis, isolation of active constituents and pharmacological studies which has done in it.

The leaf *Dichrostachys cinerea* (L.) Wight and Arn has been collected from Kolli Hills, Namakkal and taxonomically identified by the Botanical survey of India, Coimbatore.

The literature survey revealed that very less amount of pharmacological work has been carried out on *Dichrostachys cinerea* (L.) Wight andArn. Also it was observed from Ayurvedic literature and ethno botanical studies shows that the leaf is useful in the treatment of diabetes. But no scientific investigation has been done in such direction. Therefore it was a through work to carry out Preliminary Phytochemical screening, isolation and characterization of isolated compound by UV, IR and HPTLC analysis and pharmacological investigation of anti diabetic property.

The phytochemical test of ethanol extract shows the presence of Phytosterol, Flavonoids, Carbohydrates and Terpenoids.

Our observations are in complete with several workers that STZ induced diabetic mellitus causes insulin deficiency that leads to Hyperglycemia.Evidence has accumulated suggesting that STZ induces oxidative stress .Oxidative stress is caused by a relative overload of oxidants ie. Reactive oxygen species. Once, STZ enters inside the cell, it is able to spontaneously decompose to form an isocyanate compound and a methyl diazohydroxide. Isocyanate compound and methyl

diazohydroxide undergoes intra molecular carboxylation and alkylation of cellular components respectively. The DNA damage of β cells of pancreas mainly by alkylation with carbonium ion produced by methyl diazohydroxide (Weiss et al., 1982; Wilson et al., 1990). STZ is not only damaging the β cells all so in hepatocytes, nephrons and cardiomyocytes. The Nicotinamide on coaddition with STZ partially prevents hepatocyte damage

In 21 days treatment of *Dichrostachys cinerea* (L.) Wight &Arn extract on the STZ induced diabetic rats significantly reduced the elevated blood sugar levels. This indicates that the extract may not able to produce the effect by one dose and by continuous treatment it acts effectively. Overall results show that ethanolic extract of *Dichrostachys cinerea* (L.) Wight &Arn possess marked anti hyperglycaemic activity (by improvement of glucose tolerance test and by lowering the blood glucose levels in STZ-induced diabetic rats). The hypoglycaemic effect of *Dichrostachys cinerea* extracts on fasted normoglycaemic rats were dose dependent manner.

Prolong administration might have stimulated the β-cells of the islets of langerhans to produce insulin. From the result it is assumed that the extract could be responsible for the stimulation of insulin release and observed restoriation of metabolic activities. Further pharmacological and biochemical investigation are to done to find out the active constituent responsible for the anti-diabetic activity and elucidate the possible mechanism of action.

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