EVALUATION OF TOXICITY AND ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *Anisomeles malabarica* (L.) AGAINST DEXAMETHASONE INDUCED HYPERGLYCEMIA IN WISTAR ALBINO RATS

A Dissertation submitted to
THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
BRANCH-IV PHARMACOLOGY

Submitted by
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Under the guidance of
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OCTOBER 2017

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CERTIFICATE

This is to certify that **Mohamed Aboobacker Siddique. M** with University Registration no**. 261526002** carried out the dissertation work entitled "**Evaluation of toxicity and antidiabetic activity of ethanolic extract of aerial parts of *Anisomeles malabarica* (L.) against dexamethasone induced hyperglycemia in wistar albino rats"** for the award of degree in Master of Pharmacy by The Tamil Nadu Dr. M.G.R Medical University**.** The dissertation is a bonafide work done by the above said student under my direct supervision. The work embodied in this thesis is original and has not been submitted in part or in full for any degree of this or any other university.

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Internal Examiner **External Examiner**

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DECLARATION OF THE CANDIDATE

I hereby that the thesis **titled "Evluation of toxicity and antidiabetic activity of ethanolic extract of aerial parts of Anisomeles malabarica(L.) against dexamethasone induced hyperglycemia in wistar albino rats"** submitted in partial fulfillment for the award of degree of **Master of Pharmacy** of the tamil nadu DEr. M.G.R. medical university and carried out at Mohamed Sathak A.J. college of pharmacy, chennai, is my original and independent work done under the direct supervision and guidance of the in Pharmacology is a bonafide individual research work done by **Mr.M.Mohamed Aboobacker Siddique (Reg.No. 261526002),** chennai, under the guidance of **Dr. C. Ronald Darwin, M.pharm.,Ph.D, Department of Pharmacology** during the academic year 2016-2017. This thesis contains no material which has been accepted for the award of any degree or diploma of other universities.

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M.Mohamed Aboobacker Siddique

APPENTIX LIST OF FIGURES

I. INTRODUCTION

DIABETES

Diabetes is a defect in the ability of the body to convert glucose (sugar) to energy. Glucose is the main source of energy in our body. When food is digested it is metabolized into fats, proteins, or carbohydrates. Glucose is then transferred to the blood and is used by the cells for energy production. For transferring of glucose, the hormone - insulin is needed which is mainly secreted by pancreatic beta cells. DM is a type of metabolic disorder that is characterized by increased glucose production in the blood with disturbances in metabolism of carbohydrate, protein and fat mainly due to defects in insulin secretion, its action or both.

If this hyperglycemic stage of diabetes persists for a long time, it is associated with long-term complications like improper functioning and failure of different organs causing deep damage to the eyes, kidneys, nerves, heart, and blood vessels.

CLASSIFICATION OF D.M:

Two major classes of D.M. are proposed by the WHO committee 1980 and named them as insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). These are again named as Type-1 and Type-2 diabetes respectively. In the 1985 study group these type-1 and type-2 are omitted and only IDDM and NIDDM are retained. The other types included in both 1980 and 1985 reports are impaired glucose tolerance as well as Gestational diabetes.

Mainly there are two types of diabetes:

- **Type 1 diabetes:**

  In this type there is β cell dysfunction, usually causing complete insulin deficiency. Again is of two types

  A. Immune mediated

  B. Idiopathic

Type 1 diabetes occurs most frequently in children and young adults. It majorly occurs genetically with no disease history but the correct cause is not yet known

- **Type 2 diabetes:**

This type ranges from predominant insulin resistance with relative insulin deficiency to a defect in secretion of insulin. This is a common type and accounts for 90-95% of all cases. This type primarily affects adults, rarely developing in children. Strong correlation exists between Type 2, physical inactivity and obesity.[5]

INSULIN RESISTANCE

Insulin resistance (IR) is a situation in which cells fail to react to the regular measures of the hormone insulin. The body produces insulin; however the cells in the body develop into resistant to insulin and are incapable to use it as successfully, foremost to hyperglycemia. Beta cells in the pancreas consequently increase their creation of insulin, additional contributing to hyper Insulinemia. This repeatedly remains hidden and can donate to a diagnosis of type 2 diabetes.

One of insulin's functions is to control release of glucose into cells to give them through energy. Insulin resistant cells cannot capture in glucose, fatty acids and amino acids. Therefore glucose, fatty acids and amino acids escape out of the cells. A decrease in insulin or glucagon proportion inhibits glycolysis which in turn decreases energy making. The resultant raise in blood glucose might raise levels exterior the normal range as well as reason adverse health effects depending on dietary situation. Confident cell types such as fat as well as muscle cells need insulin to take in glucose. While these cells not succeed to respond sufficiently to circulating insulin blood glucose levels increase. The liver helps control glucose levels through reducing its emission of glucose in the attendance of insulin. This usual decrease in the liver glucose fabrication may not happen in people through insulin resistance**.**

Insulin resistance within muscle and fat cells reduces glucose uptake while insulin resistance in liver cells consequences in condensed glycogen synthesis along through storage and also a breakdown to restrain glucose production as well as discharge into the blood. Insulin resistance usually refers to condensed glucose lowering special effects of insulin. Though extra functions of insulin can also be exaggerated. For example insulin resistance in fat cells decreases the usual things of insulin on lipids in addition to results in condensed uptake of circulating lipids and increased hydrolysis of stored triglycerides. Improved mobilization of stored lipids within these cells elevates free fatty acids in the blood plasma.

Elevated blood fatty acid concentrations condensed muscle glucose uptake and greater than before liver glucose production all contribute to important blood glucose levels. High plasma levels of insulin and glucose due to insulin resistance are a major constituent of the metabolic syndrome. If insulin resistance exists additional insulin desires to be secreted by the pancreas. If this compensatory enlarge does not happen, blood glucose concentration raise along with type 2 diabetes occurs.

INSULIN RESISTANCE SYMPTOMS[9]

The signs and symptoms of insulin resistance syndrome include:

- Impaired fasting blood sugar, High blood pressure.
- Atypical cholesterol levels.
- Heart disease.
- Obesity.

RISK FACTORS

Several risk factors include

- ❖ Genetic factors (inherited component)
  - family history with type 2 diabetes
  - insulin receptor mutations
- ❖ Particular physiological conditions and environmental factors
  - Obesity
  - Lack of physical exercise, sedentary lifestyle.
  - Hypertension.
  - High triglyceride level.
  - Low level of High density lipoproteins
  - Gestational diabetes during past pregnancies.

CAUSES OF INSULIN RESISTANCE

Even though the correct causes of insulin resistance are not completely understand, scientists believe the main contributors to insulin resistance are overload weight along with physical inactivity.

Excess Weight

Obesity, particularly excess fat approximately the waist, is a major cause of insulin resistance. conversely, studies include revealed that belly fat produces hormones along with other substances that can cause serious health problems such as insulin resistance, high blood pressure, imbalanced cholesterol, and cardiovascular disease (CVD).

Belly fat plays a division in developing chronic, or long lasting, inflammation in the body. Chronic inflammation is able to injure the body more than time, not including any signs or symptoms. This tenderness can make a payment to the growth of insulin resistance, type 2 diabetes, and CVD. Study demonstrate that lose the weight be able to decrease insulin resistance as well as prevent or stoppage type 2 Diabetes.

Physical Inactivity

A lot of studies have shown to facilitate physical inactivity is connected through insulin resistance, frequently leading to type 2 diabetes. During the body, additional glucose is used by muscle than additional tissues. Usually, active muscles burn their stored glucose intended for energy as well as refill their reserves through glucose taken beginning the bloodstream, maintenance blood glucose levels in balance.

Studies demonstrate that after exercising, muscle develops into more responsive to insulin, reversing insulin resistance as well as lowering blood glucose levels. Work out also helps muscles absorb additional glucose with not to be the need for insulin. The additional muscle a remains has, the additional glucose it can glow to manage blood glucose levels.

Other Causes

Other causes of insulin resistance may include ethnicity; certain diseases; hormones; steroid use; some medications; older age; sleep problems, especially sleep apnea; and cigarette smoking.

HYPERGLYCEMIA

Hyperglycemia or **high blood sugar** is a state in which a too much quantity of glucose circulates in the blood plasma. This is normally a glucose level elevated than 11.1 mmol/l (200mg/dl).

Polyuria - increased volume of urination

Polyphagia- frequent hunger, especially pronounced hunger

Polydispia - frequent thirst, especially excessive thirst

Fatigue (sleepiness)

blurred vision

Dry mouth

Weight loss

Erectile disfunction

Cardiac arrhythmia.

Stupor

Seizures

Poor wound healing (cuts, scrapes, etc.)

Dry or itchy skin

Recurrent infection, external ear infections (swimmer's ear)

Tingling in feet or heels

Coma

CAUSES OF HYPERGLYCEMIA

Diabetes mellitus

Chronic hyperglycemia to facilitate persists yet in fasting states is the majority generally caused by diabetes mellitus. In truth, chronic hyperglycemia is the major distinguishing of the disease. Irregular hyperglycemia might be near in prediabetic states. Acute episodes of hyperglycemia not including diabetes mellitus.

Drugs

Some medications increase the risk of hyperglycemia, including and Beta blockers, epinephrine Thiazide diuretics, niacin, pentamidine, protease inhibitors, Corticosteroids, octriotide Some Antipsychotic agents. The sensitive administrations of stimulants such as amphetamine typically produce hyperglycemia. Various of the newer psychotropic medications, such while Olanzapine along with Duloxetine, can also cause considerable hyperglycemia.

Critical illness

A high amount of patients distress an severe stress such while stroke or myocardial infarction might enlarge hyperglycemia, still within the nonexistence of a diagnosis of diabetes. Human as well as animal studies advise to facilitate this is not gentle, furthermore to facilitate stress induced hyperglycemia be connected through a elevated risk of mortality later than both stroke with myocardial infarction.

The following situation is able to also cause hyperglycemia within the deficiency of diabetes.

1. Dysfunction of the thyroid, adrenal, as well as pituitary glands
2. A mixture of diseases of the pancreas
3. Severe increases in blood glucose might be seen within sepsis as well as convinced infections
4. Intracranial diseases are able to also cause hyperglycemia. Encephalitis, brain tumors, brain bleeds, along with meningitis is prime examples. [11]

HYPERLIPIDEMIA

Hyperlipidemia is a situation overload of fatty substances called lipids, mostly cholesterol as well as triglycerides, during the blood. It is moreover called hyperlipoproteinemia since these fatty substances pass through in the blood attach to proteins

In General Hyperlipidemia be able to be separated Into Two Subcategories

1. Hypercholesterolemia, in which here is a elevated level of cholesterol

2. Hypertriglyceridemia, in which near is a elevated stage of triglycerides, the a large amount general form of fat.

SIGNS AND SYMPTOMS OF HYPERLIPIDEMIA

Hyperlipidemia typically has rejection noticeable symptoms as well as tends to be discovered through routine assessment or estimate meant for atherosclerotic cardiovascular disease.

1. Chest Pain
2. Liver Enlarged
3. Abdominal Pain
4. Heart attacks
5. Enlarged Spleen
6. High cholesterol or triglyceride levels
7. Pimple like lesions across body
8. Atheromatous plaques in the arteries
9. Xanthelasma of eyelid
10. Higher rate of obesity and glucose intolerance
11. Xanthoma

CAUSES OF HYPERLIPIDEMIA

1. Diabetes (type 2)
2. Pregnancy
3. Environmental and genetic factors
4. Nephrotic Syndrome
5. Kidney disease
6. An under active thyroid gland
7. Hypothyroidism
8. Alcohol
9. Cushing’s Syndrome
10. Anorexia Nervosa
11. Obstructive Jaundice
12. Estrogen therapy
13. Lipoprotein lipase mutations

14.. High dietary simple carbohydrates

15. Lifestyle contributors include obesity, not exercising, and smoking.

COMPLICATIONS

TYPES OF COMPLICATIONS:

They are two types of complications they are.

MICRO VASCULAR

- Diabetic neuropathy
- Diabetic nephropathy
- Diabetic retinopathy

MACRO VASCULAR

- Atherosclerosis
- Stroke

Diabetic Nephropathy

Diabetic nephropathy is a general and severe complication everywhere kidneys are damaged and fails to function. The cause is due to unrelenting high blood sugar level in the blood. In the premature phase of nephropathy drugs along with diet can manage the condition. The situation while protein start leaking in urine is called as microalbuminuria. The general symptoms are kidney failure and weakness, decreased appetite, nausea and vomiting. Anemia might also be identified in diabetic nephropathy. It has been identified that about 30 to 40 % of Type I diabetics and 20 to 30% of Type 2 diabetics develop reasonable to severe kidney failure. Diabetes myonecrosis might increase previous to or at the occasion of diagnosis of diabetes; normally it is a category of gangrene caused by *Clostridium*

bacteria. The toxins formed with the bacterium leads to tissue diabetic mastopathy frequently seen in premenopausal women distress as of Type 1 diabetes intended for several years among insulin therapy. even though especially rare, it be able to be alive seen in men through diabetes because diabetic mastopathy, which is connected through micro vascular complications such while injure to the eyes, heart and kidneys or further disorders such like thyroid difficulty.

Diabetic neuropathy

Diabetic neuropathies are neuropathic disorders that are linked through diabetes mellitus. This situation is consideration to result since diabetic micro vascular injury involving minute blood vessels that supply nerves (vasa nervorum). Moderately ordinary conditions which might be linked with may be associated with diabetic neuropathy include diabetic amyotrophy, mononeuropathy, a painful polyneuropathy, mononeuropathy multiplex, autonomic neuropathy in addition thoraco abdominal neuropathy.

The major risk factor for diabetic neuropathy is hyperglycemia. period of diabetes, age, hypertension, height and hyperlipidemia are moreover risk factor for diabetic neuropathy.

They are four factors thought to be involved in the development of diabetic neuropathy.

- Micro vascular disease

- Advanced glycation end product,
- Protein kinase C and
- Polyol pathway

Vascular along with neural diseases are directly associated to intertwined. Blood vessels depend going on regular nerve purpose, along with nerves depend resting on sufficient blood flow. The primary pathological adjust within the micro vascular is vasoconstriction. Because the illness progresses neuronal dysfunction correlates strongly through the growth of vascular abnormalities, such like capillary basement covering thickening as well as endothelial hyperplasia which donate toward diminished oxygen stress along with hypoxia. Neuronal ischemia is a fine recognized quality of diabetic neuropathy.

Micro vascular dysfunction occurs near the beginning in diabetes parallels the series of neural dysfunction, along with might survive enough toward carry the strictness of structural, practical, plus medical changes identified in diabetic neuropathy. Important intracellular levels of glucose causes a non enzymatic covalent bonding through proteins, which change their arrangement with demolish their function. a number of of these glycosylated proteins include been implicated within the pathology of diabetic neuropathy as well as additional extended phrase compilations of diabetes.

An investigational fact has so far to conform that the polyol pathway really is responsible for micro vascular injure in the retina, kidney or neurons of

the body. Nevertheless, physiologist are practically certain that it plays various role in neuropathy.

Diabetic neuropathy affects every peripheral nerves pain fibres motor neurons autonomic nerves. But they automatically be able to affect every organs as well as systems because all are innervated. Here are more than a few distinct syndromes based resting on the organ system moreover members exaggerated except these are by no means exclusive. A patient be able to contain sensory motor plus autonomic neuropathy otherwise some new mixture.

The mechanisms of diabetic neuropathy are badly implicit. On present management alleviates tenderness as well as be able to control various linked symptoms however the development is normally progressive. Since a difficulty here us a improved threat of damage toward the feet for the reason that loss of sensation. Minute infection is able to progress to ulceration along with this might need exclusion. During adding motor nerve injure be able to psychotic break as well as inequality.

Diabetic Retinopathy

Retinopathy is characterized through improved vascular permeability, with vascular closure mediated through the development of original blood vessels neo vascularization, resting on the retina along with posterior surface of the vitreous. Diabetic retinopathy is a micro vascular disease, characterized through damage of the blood vessels along with retina of the eyes. This situation occurs during mutually in type 1 and type 2

diabetics. It be able to be classify as no proliferative diabetic retinopathy along with proliferative diabetic retinopathy otherwise diabetic macular edema. During diabetic retinopathy, the micro vessel supplying blood toward the retina of eye is exaggerated along which can cause blindness. Retinopathy is connected toward high blood sugar level along with obstructs the stream of oxygen toward the cells of the retina. Intended for the vision of eye, retina receives signals of radiance along with sent them toward the brain forming a three dimensional stature which is recognized. Lastly it is sent backside to the eye through which one can identify the belongings approximately. This functioning machine of transient light throughout the retina is caught up through the elevated glucose levels. The early phase of this disease is recognized as none proliferate Diabetic retinopathy somewhere the same as Proliferative diabetic retinopathy is the higher appearance of diabetic retinopathy during which original as fine as frail blood vessels rupture as well as escape blood into vitreous of the eye causing suspended spot during the eye. Progressively, the distended as well as blemish nerve tissue of the retina is completely damaged along through leads to retinal detachment. The ground reason for blindness between diabetes is owing to the retinal disinterest. Macular edema is regularly a complication of diabetic retinopathy which causes vision loss within public among diabetes. It develops while blood vessels during the retina are leaking fluids. The macula does not utility correctly while it is inflamed along with vision defeat might be calm toward severe, however peripheral apparition remains.[15] Cataracts were reported while a main cause of

blindness as well as diabetic retinopathy. Longer period of diabetes unrestrained diabetes as well as maculopathy be moreover considerably connected through the occurrence of cataracts between these type 2 diabetics.

Insulin action

Insulin is the essential hormone for maintenance the organism on a metabolically reasonable situation and it does so by suggest a multiplicity of response in cells. In the majority cells insulin induces an anabolic action on protein, fat with glycogen as well as it motivate the expression of genes plus synthesis of DNA. In muscle as well as adipose cells, insulin stimulates the uptake of glucose, the most important method for continuation of glucose homeostasis in also. Insulin also induces a numeral of other cellular responses such as simulation of ion and amino acid uptake in cells, rearrangement of the cytoskeleton and instruction of a number of cellular enzymes. The mechanism and Structure of the insulin receptor as well as the protein phosphorylation and dephosphorylation of seryl, threonyl and tyrosyl residues are the central mechanisms by which insulin affects cell function.

Insulin Receptor with Tyrosine Kinase Activity

The insulin receptor is a member of the family of receptors with a tyrosine (Tyr) kinase activity. Tyr-kinase receptors are transmembrane proteins with an extracellular part which contains

the hormone binding site and an intracellular part which consists of a particular amino acid sequence of approximately 300 amino acids encoding the catalytic domain of Tyr-kinase. The various Tyr-kinase domains found in receptors are homologous but not identical. The common property of all these receptors is the activation of the ’Ayr-kinase upon hormone binding. The mechanism of Tyr-kinase activation involves ligand-induced dimerisation of receptors which bring the cytoplasmic regions with the Tyr-kinase domains in close locality to each other. As a result one receptor phosphorylates the other on multiple tyrosine residues and vice versa. This process leads to the so called receptor autophosphorylation. Activation of Tyr-kinase receptors leads to in most cells to a mitogenic response. In addition cells may undergo differentiation. Among the members of the receptor Tyr-kinase family, the insulin receptor and its close re1ative insulin-like growth factor 1 (IGF-1) receptor are unique because only these receptors induce pronounced metabolic responses. In non disease states the insulin receptor is primarily involved in metabolic functions where as the IGF-1 receptor mediates growth and differentiation.

Insulin receptor and signaling

The action of insulin is initiate during its necessary with the target cell surface receptor that activates tyrosine kinase, a element of the receptor molecule. The insulin receptor (IR) is a heterotetramer consisting of two α

subunits and two β subunits that are linked by disulphide bonds into a $\alpha 1\alpha 2$ and $\beta 1\beta 2$ heterotetrameric complex. Insulin binds to the extracellular α - subunit and transduces signals across the plasma membrane which activates the intracellular tyrosine kinase C terminal domain of the β subunit. Binding of insulin to IR effects a series of intermolecular transphosphorylation reactions, where one β subunit phosphorylates its adjacent Partner on a specific C tyrosine residue. Although IRs are present on the surface of virtually all cells, their expression in classical insulin target tissues, i.e. muscle, liver and fat, is extremely high. However, there is very little information on the regulatory mechanism that controls the IR at the level of gene expression. Autophosphorylation of the IR tyrosine residue stimulates the catalytic activity of receptor tyrosine kinase which recruits IRS proteins (IRS-1 and IRS-2).

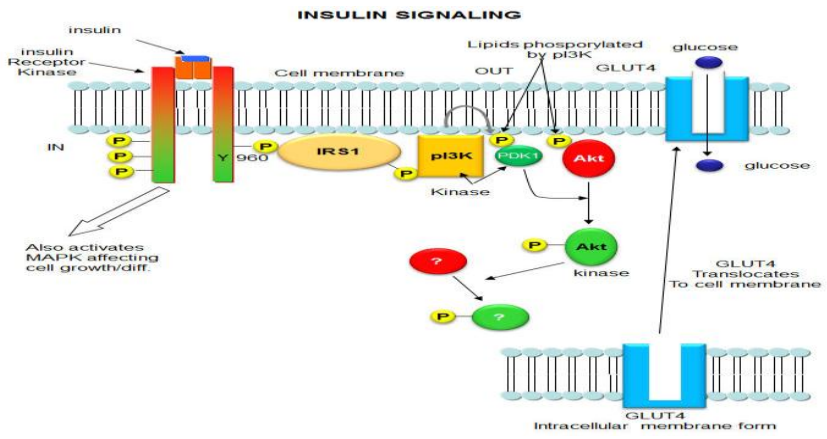


Fig no 1 INSULIN SIGNALING PATHWAY

GLUCOCORTICOID-INDUCED DIABETES MELLITUS

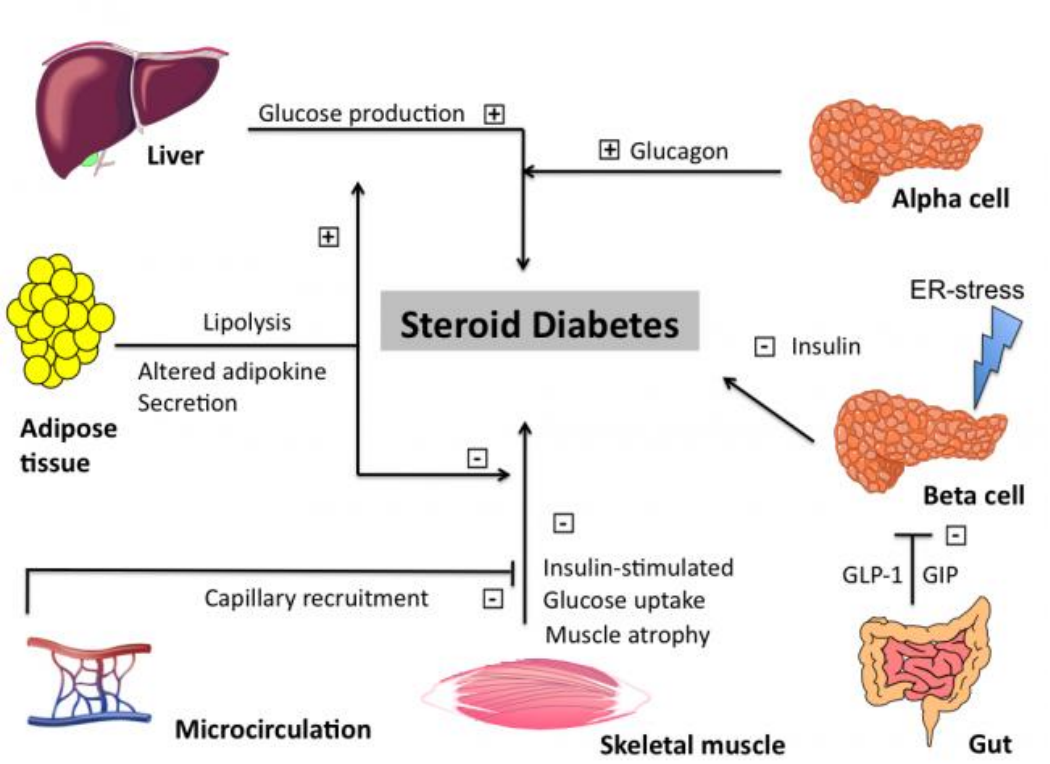
Glucocorticoids have been shown to be potent anti-inflammatory and immunosuppressive drugs; they started to be used for therapeutic purposes in the mid-20th century, and are currently widely used in the treatment of many diseases.

Although they are widely prescribed for their anti-inflammatory and immunosuppressive properties, glucocorticoids have a range of common metabolic side effects including hypertension, osteoporosis, and diabetes. Glucocorticoids exacerbate hyperglycemia in patients with diabetes mellitus, unmask undiagnosed diabetes mellitus or may precipitate the appearance of GIDM, which is an independent risk factor for other complications associated with the use of these drugs. It is expected that the blood glucose levels of non-diabetic patients should normalize after discontinuing glucocorticoid use, this does not always happen, and such patients require close monitoring due to the risk of developing diabetes mellitus in the future.

PATHOPHYSIOLOGY

The effects of glucocorticoids on glucose homeostasis are complex and not completely understood. Their negative effects are believed to be caused by a variety of factors, including increased insulin resistance, increased glucose intolerance, reduced β-cell mass from β-cell dysfunction, and increased hepatic insulin resistance leading to impaired suppression of

hepatic glucose production. The metabolic effects of glucocorticoids on glucose metabolism are seen at numerous stages in the insulin-signaling cascade. Prednisolone administration for 7 days in healthy volunteers led to a 50% reduction in insulin sensitivity, as assessed using the insulin clamp methodology. The effect of glucocorticoids on glucose metabolism likely results from the impairment of multiple pathways, including β-cell dysfunction (sensitivity to glucose and ability to release insulin) and insulin resistance in other tissues.



Glucocorticoids reduce peripheral glucose uptake at the level of the muscle and adipose tissue. Skeletal muscle is primarily responsible for the insulin-mediated capture of postprandial glucose and corticosteroids can induce insulin resistance by interfering directly with various components of the insulin signaling cascade. Chronic glucocorticoid overexposure alters

body composition, including the expansion of adipose tissue depots in the trunk, and impairs metabolism and insulin action, resulting in hyperglycemia and dyslipidemia. The ability of glucocorticoids to induce adipose tissue lipolysis depends on their concentration, duration of exposure, and the specific adipose tissue depot.

The liver plays a major role in the control of glucose metabolism, maintaining fasting euglycemia. Corticosteroids increase endogenous glucose production directly by activating numerous genes involved in the hepatic metabolism of carbohydrates, leading to increased gluconeogenesis. Glucocorticoids also inhibit the production and secretion of insulin from pancreatic β-cells. The abilities of glucocorticoids to induce hyperglycemia depend on their dose and the duration of exposure. In addition to insulin resistance, inflammatory disease states themselves may induce β-cell dysfunction through indirect mechanisms.

GC's provide a substrate for oxidative stress metabolism increasing lipolysis, proteolysis, and hepatic glucose production. The mechanism responsible for glucose intolerance after GC administration is similar to that of type 2 DM since steroids increase insulin resistance, which can be up to 60%-80% depending on the dose and type used. Among the notable factors that modify the biological effects of steroids, there is the enzymatic activity of 11β-hydroxysteroid dehydrogenase, which is classified into two types: type 1, expressed in liver and adipose tissue and amplifies the local action of steroids to convert cortisone to cortisol, and type 2, which predominates in renal tissue and reduces the effect of converting cortisol to cortisone.

Skeletal muscle is responsible for 80% of postprandial glucose storage and represents the largest reserve of glycogen in the body. Its storage is totally dependent on the presence of insulin and the availability of the glucose transporter type 4 (GLUT4) glucose transporters in the cell membrane. Steroids induce insulin resistance by directly interfering with signaling cascades, mainly the GLUT4 transporter, within muscle cells, with the subsequent 30%-50% reduction in insulin-stimulate glucose uptake and a 70% reduction in insulin-stimulated

MECHANISMS OF GLUCOCORTICOID-INDUCED DIABETES MELLITUS

- Reduced peripheral insulin sensitivity and/or promotion of weight gain
- Increase in glucose production through promotion of hepatic gluconeogenesis
- Destruction of pancreatic cells, leading to β-cell injury (inflammation)
- β-Cell dysfunction
- Impaired insulin release
- Impaired insulin release
- Inhibited glyceroneogenesis
- Increase in fatty acids

II. AIM AND OBJECTIVE

AIM

The aim of the present study was to evaluate the anti diabetic activity of ethanolic extract of aerial parts of *Anisomeles malabarica* Linn.

OBJECTIVE:

The objectives of the present research are as follows:

- To prepare and standardize the ethanolic extracts of roots of *Anisomeles malabarica* Linn.
- To establish the safety of the extract by carrying out acute and sub acute toxicity studies and investigating the behavioral changes in Wistar albino rats.
- To investigate the antidiabetic effect ethanolic extracts of roots of *Anisomeles malabarica* Linn. against dexamethasone-induced insulin resistance in wistar albino rats.

III. REVIEW OF LITERATURE

Review on Methodology

Ramu Ravirala et al.,(2016) study was conducted to report the effect of *Echinochloa frumentacea* link on dexamethasone-induced insulin resistance in rats. Rats be treated with a dose of dexamethasone (10 mg/kg) for 10 days with effect of HAEF at the doses of 200, 400 and 600mg/kg, p.o. on serum triglyceride level, plasma blood glucose level, body weight and antioxidant activity of DPPH, hydroxyl, nitric oxide, along with reducing power methods were observed. HAEF contain considerable antioxidant activity in DPPH, hydroxyl, nitric oxide, and reducing power methods. During the model HAEF at 400mg/kg and 600mg/kg p.o. showed considerable effect. Dexamethasone caused an raise in triglyceride levels, serum glucose, and decrease in body weight, thus it can be accomplished that *Echinochloa frumentacea* might prove to be effective in *in vitro* antioxidant property along with the treatment of Type-II Diabetes mellitus due to its capability to decrease insulin resistance.

Adejuwon adewale adeneye et al.,(2015) The study conducts the result of the 10 mg/kg bromocriptine in combination among 20 mg/kg metformin, 1 mg/kg glibenclamide, and both glibenclamide and metformin of oral treatments in combination, in lipids, blood glucose, body weight and cardiovascular risk report in male Wistar rats on

dexamethasone-induced hyperglycemic for 30 days. The resulted the effects of the drug combinations on OGTT treated rats were also evaluated. Repetitive every day dexamethasone injection used for 30 days caused considerable increases in the average body weight, triglycerides, blood glucose, LDL-C, total cholesterol, VLDL-C and cardiovascular risk in the treated Wistar rats. However, the increase is considerably attenuated in rats orally pretreated through bromocriptine and its various combinations. Bromocriptine and its combinations considerably enhanced OGTT in the treated rats.

Shyamala Devi et al., (2014) conducted a study states that orally 1 g/kg *Garcinia cambogia* fruit extract along with Dexamethasone10 mg/kg subcutaneously were administered and treatment for 8 days. The treated dexamethasone resulted in distinct increase in the levels of cholesterol, free acids and triglycerides in both liver and plasma. The phospholipids level increased in the plasma but drastically decreased in liver tissue in rats when compared to normal group of rats. After dexamethasone administration the actions of acyl transferase, lecithin cholesterol, and liver lipoprotein lipase are decreased considerably. The levels of HDL cholesterol and HDL triglycerides are remain unchanged, while the VLDL and LDL increased considerably in dexamethasone administered rats. When co treated rats with *Garcinia cambogia* extract and dexamethasone the lipid levels were maintained at normally. The concluded that *Garcinia cambogia* extract shows hypolipidemic property and dexamethasone administration study shows that undesirable changes in lipid profile.

Humberto Nicastro et al.,(2012) We designed to estimate the effect of resistance exercise and leucine supplementation with dexamethasone induced muscle atrophy with insulin resistance. Rats male Wister is randomly separated into four groups. Every group received dexamethasone 5 mg/ kg for 7 days leucine supplemented groups received 0.135 g/ kg through gavages for 7 days. Plasma glucose was considerably increased in the dexamethasone and leucine group compare with the dexamethasone group and resistance exercise considerably decreased hyperglycemia. The dexamethasone and leucine group shows reduced glucose transporter 4 translocation compared among the dexamethasone group and resistance exercise restored this response. Leucine supplementation worsened insulin compassion and did not assuage muscle wasting in rats treated with dexamethasone. On the other hand, resistance exercise modulated glucose homeostasis and fiber type conversion in the plantaris muscle. And Concluded that Resistance exercise but not leucine supplementation promoted fiber type transition and better glucose homeostasis in dexamethasone treated rats.

Michele Barbera et al.,(2012) Reported a research, treated in the aging rats use glucocorticoid to increase insulin resistance in in-vivo and in-vitro outcome of the vanadyl sulfate an insulin mimetic agent. Daily Dexamethasone 0.13 mg/kg b.w. was administered for 13 days to old Sprague Dawley rats and from the 5th day vanadyl sulfate be given oral. Concentrations of Insulin, Plasma glucose, and free fatty acids were measured during these treatments at the end of the experiment the insulin

Secretory effect of the isolated perfused pancreas was assessed In Dexamethasone treatment Both young and aging rats, particularly the latter, hyperinsulinemia and improved in vitro insulin responsiveness to glucose be observed. Simultaneously the concentrations of an increase in plasma FFA. The Beta-cell adaptive answerd occurred in both age groups and could probably be increased circulating FFA In glucocorticoid-treated animals. 70% of aging animals It is lacking to stop hyperglycemia. Oral vanadyl sulfate administration unsuccessful to correct Dex-induced alterations in metabolism of glucose and lipid, even though it prejudiced in vitro b-cell responsiveness to stimuli in aging rats.

Dibyendu Shil et al.,(2012) The plan of this study was to assess the effect of potential hypoglycemic and hypolipidemic effect of *Nepenthes khasiana* Hook leaf extract on Hyperlipidemia and Insulin Resistance in Dexamethasone-induced Rats. The research was done at two dissimilar doses of 250 & 500 mg/kg p.o. on 11 of treatment. Serum glucose and lipid parameters are estimated by collecting the blood samples. 500 mg/kg p.o Standard glibenclamide next to a dosage was compared with the of leaf extract. Leaf extract and glibenclamide appreciably decreased (P<0.05) dexamethasone induced increase of serum glucose while compared to the control group. Dose of 500 mg/kg Leaf extract showed better activity than standard. The research says that the leaf extract of *Nepenthes khasiana* Hook show significant glucose and lipid lowering activity.

O. H. Azee et al.,(2011) the plan of this study was to assess the effect of *Gundelia tournefortii* on various hyperglycemic and hyperlipidemic

biochemical parameters in mice. Daily 1 mg/kg of body weight intramuscularly injected dexamethasone were induced hyperglycemic and hyperlipidemic in Male albino mice. The doses of *G. tournefortii* extract of at a 75, 150, 300 mg/kg.b.w. Orally correspondingly companied through injection of dexamethasone 1 mg/kg body weight intramuscularly. Treatment is for 22 days. Dexamethasone treatment shows that raise in levels of cholesterol, glucose, and triglyceride, and drastically decreases the body weight, with no result on level of overall protein. *G. tournefortii* drug extract treated at dose of 75 mg/kg.b.w. Shows considerable decrease levels of glucose, and body weight. Positive result were seen when mice treated with *G. tournefortii* at a dose of 300 mg/kg.b.w. so as to leads to considerable decrease in levels of triglyceride, glucose, and cholesterol. These outcomes indicate that helpfulness of *G. tournefortii* extract was hypoglycemia along with hypolipidemia during dexamethasone treated mice.

Samir Bhattacharya et al.,(2010) Free fatty acids are well-known to play an important part in promoting loss of insulin sensitivity, in this manner causing insulin resistance and type 2 diabetes. Though, that underlying mechanism involved is undecided. During search intended for the reason of the mechanism, it has been originate that primary palmitate inhibits insulin receptor (IR) gene expression, to a decreased quantity of IR protein in insulin target cells. Causes decreased in insulin receptor gene expression when PDK1-independent phosphorylation of PKCε. throughout the pathways one of which fatty acid can bring insulin resistance in insulin

target cells is recommended by these studies. We give an overview of this essential area, emphasizing the existing status.

Xiaoping Gao et al.,(2011) the special effects of Dioscorea opposita on dexamethasone-induced insulin resistance are investigate in vitro and in vivo. In dexamethasone-induced diabetic rats D. opposita extract decreased considerably the blood insulin along with glucose levels. D. opposita considerably improved insulin stimulated glucose uptake in 3T3-L1 adipocytes in in-vitro. In addition D. opposita increases the mRNA term of GLUT4 glucose carrier in 3T3-L1 adipocytes. These records suggest that D. opposita have insulin sensitivity that is connected with the regulation of GLUT4 expression.

Akhila J. Setty et al.,(2010) evaluated the effect of *costus igneus* leaves on dexamethasone induced hyperglycemia in male Wister rats. Commonly in India *costus igneus* is an Indian insulin plant belongs to the family costaceae. Lowers blood glucose levels, and diabetes and falling the blood sugar levels are the beneficial effects while consumption of the leaf. Animals were divided into 4 groups of six each (n=6) were treated for 20 days with a dose of 10 mg/kg/day of dexamethasone subcutaneously. Animals received *costus igneus* at a dose of 100,200&500 mg/kg/day in distilled water from day 11 to day 20 orally or 500µg/kg of glipenclamide orally. After overnight fasting on 20th day blood samples is collected by retro-orbital puncture for estimating the fasting blood glucose levels after a glucose load of 2.5mg/kg given orally 1 hour before for estimating the post glucose load blood glucose levels. The levels of fasting glucose and post

glucose load blood sugar levels are raised in dexamethasone treated group compared to normal group. Costus igneus at a dose of 250, & 500 mg/kg and 500µg/kg of glipenclamide decrease the dexamethasone induced hyperglycemia. The *costus igneus* leaves reduces the fasting and post prandial blood sugar levels decreases to normal in dexamethasone induced hyperglycemia31

Neeharika V et al.,(2010) To investigate the effect of Madhuriktha a polyherbal formulation on dexamethasone and fructose induced insulin resistance. Administration of 10 mg/kg dose of Dexamethasone for 10 days through S.C rote and 10% w/v aqueous solution of fructose was feed for 20 days in feeding bottles. After completion of treatment sacrifice the animal and collect the blood samples for biochemical parameters like insulin, cholesterol, triglycerides, LDL, HDL, and levels of glucose was estimated. The drug extract Madhuriktha at a dose of 200 and 400 mg/kg of drug shows effect able decreasing all the parameters like like insulin, cholesterol, triglycerides, LDL, HDL, and levels of glucose and better improved the body weights.

Rashid Akthar et al.,(2010) The aim of the study is to investigate the effect of leaves of Tephrosia *purpurea* Linn extract to accepted the lipid lowering properties on experimentally proved dexamethasone induced rat. *Tephrosia purpurea* Linn belongs to a family Fabaceae used for the treatment in diabetes. Low density lipoprotein cholesterol, Total cholesterol, very low density lipoprotein cholesterol, High density

lipoprotein cholesterol, Triglycerides and atherogenic are the lipid parameters studied. Orally the drug extract administered at a dose of 600 and 1200 mg/kg in Dexamethasone induced rats. It shows the levels of low density lipoprotein cholesterol, Total cholesterol, very low density lipoprotein cholesterol and Triglycerides are significantly decreased. But when compared to control group of rats the High density lipoprotein cholesterol (HDLC) are considerably increased. Finally concluded that the leaves of *Tephrosia purpurea* Linn can significantly decreases the lipid levels.

Sankara Sastry Pragda et al.,(2009) Hyper lipidemia is a major problem for developing diabetes mellitus and cardiovascular diseases. The herbaceous weed of *P oleracea* Linn comes under the family of Portulacaceae and having various pharmacological effects like antibacterial, anti inflammatory and antiplasmodial activity. The study is to investigate the effect of antihyperlipidemic activity on dexamethasone induced rats. For rats 10 mg/kg of dexamethasone was administered through subcutaneous route for 8 days shows hyperlipidimea and noticeable increase the level of serum cholesterol and triglyceride levels with increase in atherogenic effects. Here the drug *Portulaca oleracea* Linn are injected at the dose of 200 and 400 mg/kg and finally the result shows that considerable decreases the levels of triglycerides and levels of levels of cholesterol against in rats of dexamethasone induced hyperlipidemia.

C. N. Okwuosa et al.,(2008) To investigate the effect on leaves extract of *Pterocarpus santalinoides* shows decreased level of triglyceride and glucose against induced hyperlipidemia as well as insulin resistance in rats. According to standard methods acute toxicity test was performed. And oral glucose tolerance test was done on the presence of extracts in normal rats. Then after a 12h fasting rats in four groups of respected doses of extracts of aqueous with methanolic extracts of *P.santalinoides* were administered orally and also glucocorticoid 10 mg/kg body wt administered through subcutaneous. In 5^{th} group rats received saline 5 ml/kg p.o and glucocorticoid 10 mg/kg body wt s.c and marked like positive control and sixth group named as normal control. Treatment period are 10 days. The acute toxicity test showed to that the extract had an oral LD50 is more than 5000 mg/kg in rats. When extracts of glucose full normal rats shows significant decreasing of blood glucose levels started with at 90 min after glucose load when compared among the 60 min sample and also when differentiated with the negative control value at 90 min. 10 days administration of dexamethasone resulted hyperlipidemia and insulin resistance because of increasing blood glucose levels and triglycerides levels in positive control when compared with normal control. In dexamethasone induced hyperlipidemia and insulin resistance the *P. santalinoides* extracts shows the properties of triglyceride and glucose lowering effect.

Md. Shalam, M.S. Harish et al.,(2006) To identify the decreasing effect of SH-01D on dexamethasone along with fructose induced

insulin resistance in rats. Here two methods are followed that is drug injected dexamethasone10 mg/kg through subcutaneous route, and orally fructose 10% w/v are given to the rats 10 to 20 days period correspondingly. Two respective doses of 30 and 60 mg/kg of SH-01D was followed. After completion of treatment the biochemical parameters like cholesterol, LDL, triglycerides, HDL, insulin and glucose are studied. After sacrificing the animals muscle and Liver were studied in fructose model. The result shows that 60mg/kg of SH-01D shows significant effect in both models. Serum biochemical parameters are increased in fructose model but in skeletal and liver glycogen levels are decreased. Serum glucose along with triglycerides levels are increased in Dexamethasone induced group. The dose of 60mg/kg SH-01D fructose fed rats is significantly stops the increasing serum biochemical parameters and also greatly reduces glycogen levels. Higher the glucose and triglycerides are prevented in SH-01D in dexamethasone model. Finally concluded that SH-01D are helpful in insulin resistance.

C. Velmurugan et al.,(2006) Reported the effect on the leaves of *Gossypium herbaceum* (200mg/kg) on hypoglycemic and hypolipidemic effect of evaluated ethyl ether along with ethanolic fractions of dexamethasone induced diabetic rats. For animals diabetics' diabetes are induced through dexamethasone 10 mg/kg of body weight s.c. orally treated with various fraction of *Gossypium herbaceum.* Standard drug Glibenclamide is used. As compared to diabetic control the fractions shows

significant ($p<0.01$) anti hyperglycemic and hypolipidemic activity. The fraction shows helpful effects on blood glucose. It also decreases the important biochemical parameters such like low density lipoprotein, triglycerides, total cholesterol and very low density lipoprotein, and maintains the body weight as increases the decreased level of high density lipoprotein. Finally both fractions are good for oral hypoglycemic agents and appear to be hopeful for the improvement of diabetes mellitus.

Atsushi Ogawa al.,(2005) The roles of insulin resistance along with fl-cell dysfunction in Dexamethasone induced Insulin Resistance were studied in Wister rats. All rats are treated with 5 mg/kg of dexamethasone for 25 days shows improved f-cell mass with basal as well as arginine raised insulin secretion, shows insulin resistance, but only 17% become diabetic. The insulin reaction to 20 mM glucose was normal within the fused pancreas of every normoglycemic dexamethasone treated rats but not present in every diabetic rat. In Immunostainable more K. f-cell carrier, GLUT 2, be there in 100% of f-cells of normoglycemic rats, except in only 27% of f- cells of diabetic rats. But there is no reduction of GLUT 2 mRNA. Diabetic and glucose raised insulin secretion was absent in every Zucker rats were treated with 0.2 to 0.4 mg/kg of dexamethasone for 25 days. More glucose transport into islets was 60% of low no diabetic controls. Compared among 100% in controls diabetic rats were GLUT 2 positive up to 35% of cells. Increased two fold Total pancreatic GLUT 2 mRNA be signifying a posttranscriptional irregularity. We terminate that dexamethasone induces insulin resistance, whether it may or not it induces hyperglycemia. At

whatever time presence of hyperglycemia, good GLUT 2 fi cells are decreased, more K^+ glucose transport into the cells is attenuated as well as the insulin reaction to glucose is absent.

Review on Plant

1. **R. Suthar Singh, M. Uvarani and S. Raghu Raman(2003)** "Pharmacognostical and phytochemical studies on leaves of *Anisomeles malabarica"* The present study includes the morphological and anatomical characteristic of the leaf along with the determination of physio chemical constants, phytochemical screening and volatile oil content determination on the leaves of *Anisomeles malabarica* (LAMIACEAE) to provide some pharmocognostical standards and serves as a standard reference for the identification of Anisomeles malabarica.

2. **R.Jeyachandran, A.Mahesh, L.Cindrella.(2007)** "DEN-induced cancer and its alleviation by *Anisomeles malabarica*(L.) R.Br. ethanolic extract in male albino mice". Anti-cancer activity was evaluated by measuring the activities of total protein, Glutamate pyruvate transaminase(GPT), glutamate oxaloacetate transaminase(ALP). The ethanolic extract at an oral dose of 100mk/kg exhibited a significant ($p<0.05$), protective effect by reduce liver and serum levels of total protein, GPT, GOT, ACP, ALP as compared to DEN induced mice.

3. **Ushir Y et al**.,(2011) "HPTLC Fingerprint Profile For Quantitative Determination Of Various Phytoconstituents In Anisomeles Species". Finger print of two ethano-botanically important Anisomeles species has been developed. A sensitive and reliable densitometric High Performance

Thin Layer Chromatography (HPTLC) method has been developed for the quantification of quercetin, β-sitosterol, stigmasterol, catechin and ovatodiolide present in *Anisomeles indica* and *Anisomeles malabarica*. Chromatographic analysis was performed using methanol, chloroform, acetone and ethanol extract of these plants were developed in the different solvents such as toluene, chloroform, ethyl acetate, methanol at various proportions. Detection and quantification of all phytoconstituents was done by densitometric scanning at different wavelengths. They reported finger prints would be helpful in the standardization of these species.

4. **R. Ranganathan and R. Vijayalakshmi(2012)** "Effect of Anisomeles malabarica (L.) R.Br. Methanolic extract on DMBA - induced HBP Carcinogenesis". The effect of *Anisomeles malabarica*(L.) R.Br. Whole plants extract has been studied on cellular redox status during hamster buccal pouch carcinogenesis. The buccal pouch AMME reversed the susceptibility to lipid peroxidation while simultaneously increasing GSH-dependent antioxidant enzyme activities, whereas in the liver and erythrocytes, the extent of lipidperoxidation was reduced with elevation of antioxidants. Thus, modified oxidant status together with antioxidant adequacy in the target organ as well as in the liver and erythrocytes induced by AMME may significantly reduce cell proliferation and block tumor development in the HBP.

5. **Kavitha T, Nelson R, Thenmozhi R, Priya**,(2012)"Antimicrobial activity and phytochemical analysis of *Anisomeles malabarica* (L.) R.Br". *Invitro* antibacterial activity of leaf extracts of *A. malabarica* against E.coli,

S.aureus, P.mirabilis, P.aeruginosa, k.pneumonia. The preliminary phytochemmical analysis of both Ethanolic and Diethyl ether extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides. It was found that the ethanolic extract exhibited a maximum antibacterial activity at 200μg/ml and produced 25mm zone of inhibition against S.aureus whereas Diethyl ether extract produced 30mm zone of inhibition in the same concentration. The results provide justification for the use of A. malabarica to treat various infectious diseases.

6. **M. Ismail Shareef, S. Leelavathi, Anis Ahmed Shariff. (2012).** "Inhibition of *Invitro* TNF-α Production by *Anisomeles malabarica* R.Br. Reinforces Its Anti-Rheumatic and Immunomodulatory Properties". Anti-rheumatic and immunomodulatory role of aerial parts, leaves and roots in lipopolysaccharide (LPS) mediated signaling in macrophage and mouse connective tissue cell cultures were observed. Study employed tumor necrosis factor-α (TNF-α) bioassay, all the three extracts viz., aerial parts, leaves and roots inhibited TNF-α production in LPS (1 μg/mL) stimulated RAW-32 cells. 38.75 % inhibition of TNF-α was observed at 200 μg extracts of the aerial parts of the plant followed by 17.64 and 14.94 % by the roots and leaves respectively. These findings from the present in vitro studies suggest the anti-rheumatic and immunomodulatory properties of the methanolic extracts of A. malabarica.

IV. SCOPE AND PLAN OF WORK

4.1 SCOPE OF WORK

Diabetes is one of the commonest diseases, which is characterized by a selective and progressive degeneration of beta cells. The goal of therapeutic measures is to achieve conventional glucose levels (euglycemia), to lower the incidence of vascular and neuropathic complications, to reduce hypoglycemia, and to affect the patient's lifestyle and activities as little as possible. The scope of the present work stays with the fact to prevent the beta cells from getting destroyed, the plant *Anisomeles malabarica* L. is expected to protect the damage generated.

4.2 PLAN OF THE WORK

The plan of the work is given below.

1. **Collection and authentification of *Anisomeles malabarica* Linn.**
2. **Preparation of ethanolic extracts by cold maceration**
3. **Preliminary phytochemical screening of extracts.**
4. **Toxicological investigation of the extracts.**
    a. **Acute toxicity study.**
    b. **Sub acute toxicity.**

5. **Evaluation of anti diabetic activity of the aerial parts of *Anisomeles malabarica* Linn. against Dexamethasone induced hyperglcemia.**

V. MATERIALS AND METHODS

5.1 Plant Material

Binomial Name: *Anisomeles malabarica* Linn.

Fig 14: *Anisomeles malabarica*(L.)R.Br.

Scientific classification

Kingdom	:	Plantae
Phylum	:	Charophyta
Superorder	:	Asteranae
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	Anisomeles
Species	:	Malabarica

Vernacular Names

Tamil	:	Peyimarutti
Malayalam	:	Perumpumpa, Karintumpa
English	:	Malabar Catmint

Distribution: Found throughout south India, in higher elevations.

Plant Description

A small perennial shrub grows upto 1.5 meters in height. Leaves simple, opposite, very thick, aromatic, oblong-lanceolate, acute, pale above, white below, crenate-serrate, soft woolly, flowers purple, in dense whorls of more or less interrupted spikes, fruits nutlets, bearing ellipsoid and compressed seed

Properties and uses

The plant is acrid, bitter, aromatic, intellect promoting, neurprotective, stomachic, antihelminthic, febrifuge and sudorific. It is useful in hailotis, epilepsy, hysteria, amentia, anorexia, diabetes, dyspepsia, colic flatulence, intestinal worms, fever arising from teething in children, intermittent fevers, vitiated conditions of vata and kapha, gout, swellings and diarrhoea.

Authentification:

Anisomeles malabarica Linn., was authenticated by Prof. Dr. P. Jayaraman Director, Plant Anatomy Research centre Tambaram. The specimen of the plant was maintained with voucher number PARC/201/1397.

5.2 Experimental Animals

Albino Wistar rats of either sex approximately same age group were used after being acclimatized for a week at laboratory conditions. They were provided standard rodent pellet diet (Lipton India) and water *ad libitum*. The animals had free access to food and water and maintained under 12:12 hr light and dark cycle. All experiments were carried out during day time from 09.00 to 17.00 hr. The protocol was approved by Institutional Animal Ethical Committee and care of the animals was taken as per guidelines of committee for the purpose of control and supervision in experiments on animals (CPCSEA), representative of Animal Welfare, Government of India. Department of Biotechnology Sri Venkateswara College of Engineering, Paranur, Chennai - (SVCE/BIO/2017/004). Wistar Albino rat of 43 were sanctioned for the study (weight about 150-200 gm)

Female rats of 3 numbers were used for the acute toxicity study; five male and female animals were used for the sub acute toxicity study. Male rats (n=6 x 6 Groups) were used for the antidiabetic study.

5.3 Instruments

S.NO	INSTRUMENTS	MANUFACTURED COMPANY
1	UV-Visible Spectrophotometer	UV-1800 Shimadzu, Model, Mfg by Shimadzu Carporation.
2	Centrifuge	Research centrifuge, Mfg by Remi Instruments Ltd, Mumbai
3	Tissue Homogenizer	Type: RO-127A, Mfg by Rajendra Elect, IND.Ltd, Remi Instruments Division, Vasai
4	Sonicator	Pci made in Mumbai.
5	Milli pore water collector	Mfg by TKA smart pure made in Made in Germany
6	Soxhlet apparatus	Agarwal
7	Rotory evaporator	Medika instrument Mfg co.
8	UV chamber	Singhla sciences, Ambala

5.4 Chemicals Used In This Study

S.NO	DRUGS AND CHEMICALS	MANUFACTURED COMPANY
1	Dexamethasone	Sigma Aldrich life sciences, Bangalore
2	glipinclamide	Titan biotech limited, Bhiwadi
3	Heparin sodium injection I.P	Biological E. Limited, Hydarabad
4	Sodium lauryalsulphate (L.R)	Molychem, Mumbai
5	Acetic acid(L.R)	Thermo fisher scientific pvt.ltd Mumbai
6	Thiobarbicturic acid (L.R)	Hi Media Laboratories pvt.Ltd. Mumbai
7	Tri chloro acetic acid (L.R)	Molychem, Mumbai
8	Phosphate buffer (K_2HPO_4) (L.R)	Titan biotech limited, Bhiwadi
9	5,5[1]-dithiobis(2-nitrobenzoic acid) 99% extra pure(L.R)	Kemphosol Bombay
10	Sulphosalicylic acid (L.R)	Burgoyne Burbidges& co Mumbai
11	Sodium pyro phosphate di basic (L.R)	Sigma Aldrich life sciences, Bangalore
12	PhenazineMethosulphate (R & D)	Sigma Aldrich life sciences, Bangalore
13	Nitro blue tetrazolium (L.R)	Molychem, Mumbai
14	Coomassie brilliant blue (Bradford Reagent) (R & D)	Sigma Aldrich life sciences, Bangalore
15	Ethanol absolute (L.R)	Changshuyanguan Chemical, China

16	Sodium Phosphate dibasic (L.R)	Sigma Aldrich Life sciences, Bangalore
17	Potassium phosphate mono basic (R & D)	Sigma Aldrich, Bangalore
18	Ortho-phosphoric acid (L.R)	Universal Laboratory pvt limited, Mumbai
19	Pyridine (L.R)	Merck Specialitiespvtlimt Mumbai
20	Sodium chloride (L.R)	Molychem, Mumbai
21	Sodium citrate purified (L.R)	S d Fine-chem limited, Mumbai
22	NADH (R&D)	Sigma Aldrich life sciences, Bangalore
23	n-Butanol (L.R)	Karnataka Fine Chem, Bangalore
24	Sodium nitrite (L.R)	S d Fine-chem limited, Mumbai

5.5 Extraction and Identification of Phytoconstituents

5.5.1 Extraction of plant material

The air dried Roots of *Anisomeles malabarica* Linn., 500gms each were coarse powdered and extracted with ethyl alcohol. The crude extract was further filtered and evaporated by the aid of rotary evaporator. The final mass is weighed and preserved for further use.

5.5.2 Preliminary phytochemical screening

Preliminary phytoconstituents present in the hydroethanolic extract of *Anisomeles malabarica* (L.) and *Echinops echinatus* Roxb., plants were identified based on the chemical test.

1. Test for alkaloids

Treated with dilute Hydrochloric acid and filtered. The filtrate was treated with various alkaloidal agents.

a) **Mayer's–Test**

   Treated with Mayer's reagent and cream colour indicates the presence of alkaloid.

b) **Dragendroff's–Test**

   When little amount of the sample was treated with the Dragendroff's reagent, the presence of reddish brown precipitate reveals the presence of alkaloid.

c) Hager's–Test

Treated with the Hager's reagent and presence of yellow colour precipitate indicates the presence of alkaloid.

d) Wagner's–Test

Treated with the Wagner's reagent, the appearance of brown colour precipitate indicates the presence of alkaloid.

2. Test for carbohydrates

The extracts were treated with 3ml of alpha–Napthol in alcohol and to the sides of the test tube concentrated sulphuric acid was added carefully. Formation of violet colour ring at the junction of two liquids shows the presence of carbohydrates.

a) Fehling's–Test

The extracts were treated with Fehling's solution A and B and heated. Presence of reddish brown colour precipitate indicates the presence of reducing sugars.

b) Benedict's–Test

The extracts were treated with Benedict's reagent and heated and presence of reddish orange colour precipitate indicates the presence of reducing sugars.

c) Barfoed's–Test

The extracts were treated with Barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicates the presence of non reducing sugars.

3. Test for proteins

a) Biuret's–test

When the extracts were treated with copper sulphate solution, followed by the addition of sodium hydroxide solution, appearance of violet colour indicates the presence of proteins

b) Millon's–Test

When the extract was treated with Millon's reagent, appearance of pink colour indicates the presence of proteins.

4. Test for steroids

a) Libermann Burchard Test

When the extracts were treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, appearance of green colour indicates the presence of steroids.

5. Test for sterols

When the extracts were treated with 5% potassium hydroxide solution, appearance of pink colour indicates the presence of sterols.

6. Test for phenols

When the extracts were treated with neutral ferric chloride solution, appearance of violet colour indicates the presence of phenols. When the extracts were treated with 10% sodium chloride solution, the appearance of cream colour indicates the presence of phenols.

7. Test for tannins

a) When the extracts were treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins.
b) When the extracts were treated with aqueous bromine solution, appearance of white precipitate indicates the presence of tannins.

8. Test for flavanoids

a) 5ml of the extract solution was hydrolyzed with 10 % v/v sulphuric acid and cooled. Then, it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1 ml of diluted sodium carbonate, 1 ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) **Shinoda's test**

The extract was dissolved in alcohol, to that one piece of magnesium followed by concentrated HCl was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

9. Test for gums and mucilage

The extracts were treated with 25ml of absolute alcohol, and then solution was filtered. The filtrate was examined for its swelling properties.

10. Test for glycosides

When a pinch of the extracts were dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

11. Test for saponins

Foam test

1ml of the extracts are diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

12. Test for terpenes

When the extracts were treated with tin and thionyl Chloride, appearance of pink colour indicates the presence of terpenes.

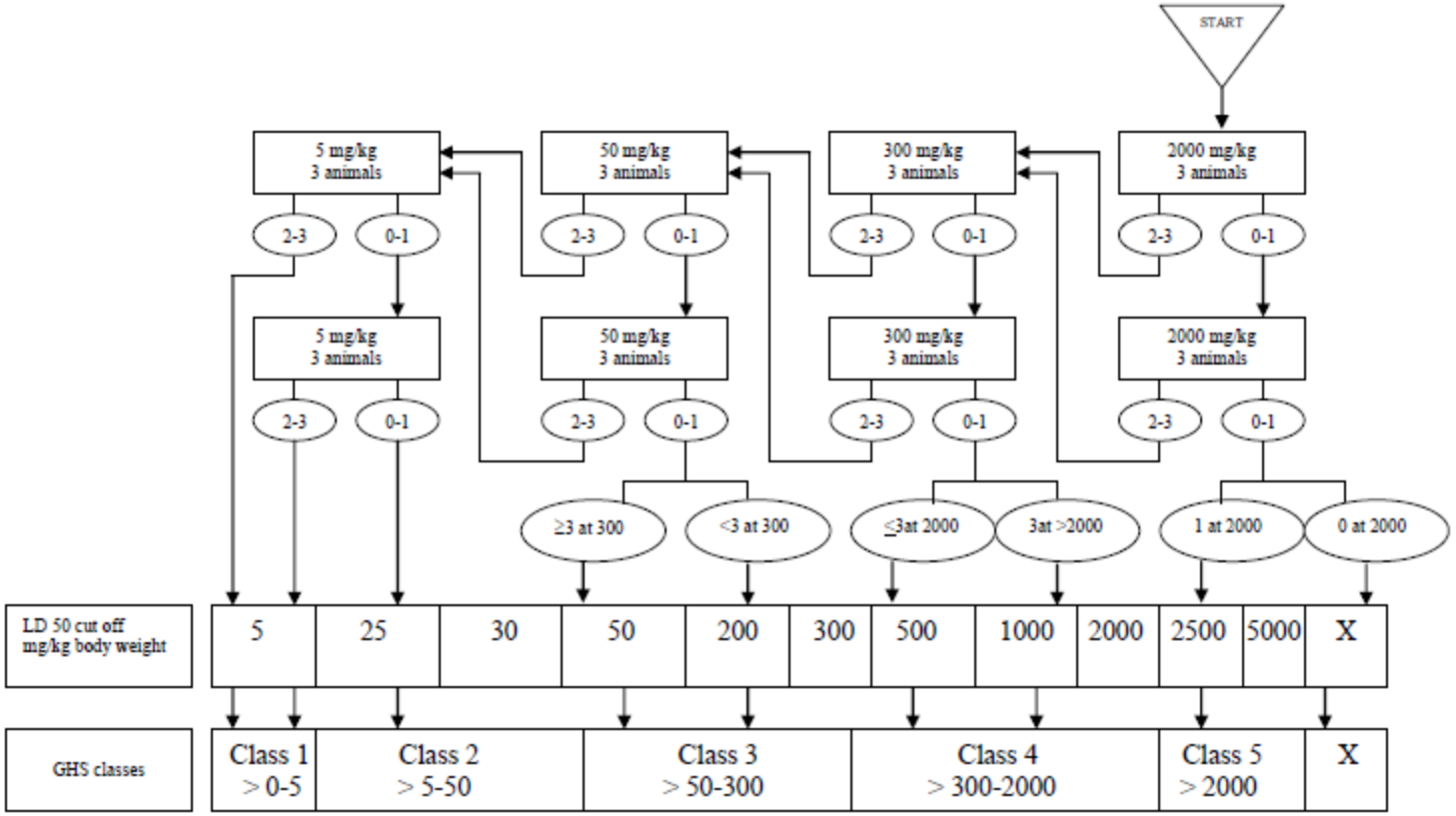
5.6 TOXICITY STUDY

5.6.1 Acute oral toxicity study (OECD 423)

For carrying out oral toxicity study OECD guidelines 423 was followed. It is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked morbidity of the animals a few steps may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data.

The method uses defined doses (5and classified according to the globally harmonized system. The starting dose for ethanolic extract was 2000mg/kg bodyweight (p.o). The dose was administered to the rats which were fasted overnight with water ad libitum and observed for signs of toxicity. The same dose was once again tried with another three rats and were observed for 72 hours for symptoms like change in skin colour, salivation, diarrhea, sleep, tremors, convulsions and also respiratory, autonomic and CNS effects.

Fig-14 Flow Chart for acute toxic class method (OECD guidelines 423) at starting dose of 2000 mg/kg body weight/p.o



*0,1,2,3: Number of moribund or dead animals at each step *GHS: Globally Harmonized classified system (mg/kg b.w) *X-Un classified

5.6.2 Sub acute toxicity study (OECD 407)

For carrying out sub acute oral toxicity study OECD guidelines 407 -Repeated Dose 28-Day oral toxicity study in rodents was followed. The duration of the Study was 28 days. Rat were administered with at the dose level of 200 mg/kg. Each group consists of ten animals (five animals/sex/group). The drug was administered orally once daily for 28 days. On 29^{th} day the animals was anaesthetized and blood was collected by retro orbital puncture. Hematological parameters were evaluated. Serum was separated and biochemical parameters were estimated. Animals were sacrificed and organs were removed and weighed. The organs were kept in 10% formalin and used for histo-pathological analysis.

Change in body weight

The change in body weight was observed for a time period of 28 days at an interval of 5 days. Body weight gains were determined from the final and initial body weights.

Organ weight

After sacrifice, organs were quickly excised and weighed and relative organ weights were computed.

Hematological studies

The following hematological parameters were estimated by standard procedures.

Blood samples were drawn by cardiac puncture and haematological parameters were analyzed by autoanalyzer.

i. Total R.B.C. count
ii. Total W.B.C. Count
iii. Differential leukocyte count
iv. Haemoglobin (Hb) concentration

Biochemical studies

Blood samples were drawn by cardiac puncture. Blood from three animals was pooled for serum separation. Each serum sample was analyzed by auto analyzer.

i. Aspartate Aminotransferase (ASAT)
ii. Alanine Aminotransferase (ALAT)
iii. Alkaline Phosphatase (ALP)
iv. Total Bilirubin (TB)
v. Total protein.

Histopathological study

After blood collection rats were sacrificed for tissue studies. The internal organs like liver, kidney, lungs, brain and heart were isolated and blotted free of blood, weighed immediately to determine relative organs weights and observed for gross lesions. Histological examination was performed on the tissue preserved in 10% buffered formalin solution with particular emphasis on those which showed gross pathological changes.

5.7 EXPERIMENTAL DESIGN - ANTI DIABETIC

Experimental Protoco

30 Rats were divided in to 5 groups (n=6) and the duration of the experiment was 21 days with overnight fasting.

Groups	Treatment
Group-I	Rats received normal distilled water for 21 days.
Group-II	Rats received Dexamethasone 10 mg/kg i.p for a period of 10 days
Group –III	Rats received Dexamethasone 10 mg/kg i.p with low dose of drug 100mg/kg p.o
Group –IV	Rats received Dexamethasone 10 mg/kg i.p with high dose of drug 200mg/kg p.o
Group -V	Rats received Dexamethasone 10 mg/kg i.p along with standard drug Glipenclamide 500mg/kg p.o

At the end of the treatment period, rats were deprived of food overnight and sacrificed on day 22nd day by light ether anesthesia followed by decapitation after recording the final body weight. Blood was collected from each rat for biochemical estimation and pancreas was quickly isolated immersed in ice cold saline and weighed. Pancreas was stored under freezer (-20 °C) for estimation of tissue antioxidant parameters

5.7.1 Estimation of blood glucose: (GOD/POD Method)

Span diagnostics kit was used for the estimation of serum/plasma glucose. The estimation was carried out by GOD/POD method. The enzyme glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide as follows:

$$\textbf{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \textbf{Gluconic acid} + H_2O$$

Addition of the enzyme peroxidase and a chromogen such as 4 Aminoantipyrine results in the formation of a colored compound quinoneimine that can be measured.

$$2H_20 + \textbf{4-Aminoantipyrine phenol} \xrightarrow{\text{peroxidase}} \textbf{Quinonimine} + 4H_2O$$

The red color of quinoneimine is measured at 505 nm and is directly proportional to glucose concentration.

Glucose oxidase is highly specific for β – D glucose because 36% and 64% of glucose in solution are in alpha and beta forms respectively, complete reaction requires mutarotation of the α to β form . The second step, involving peroxidase, is much specific than the glucose oxidase reaction. Various substances, such as uric acid, ascorbic acid, bilirubin, hemoglobin, tetracycline and glutathione, inhibit the reaction (presumably by competing with chromogen for $H_2O_{2)}$ producing lower values. Some glucose oxidase preparations contain Catalase as a

contaminant; Catalase activity decomposes peroxide and decreases the intensity of the final color obtained.

Programme:

The basic assay parameters

Mode	**End point**
Wavelength	505 nm (490 – 550 nm)
Temperature	37 ^{o}C or R.T.
Optical path length	1 cm
Blanking	Reagent blank
Incubation time (minutes)	10 at 37 ^{o}C or 30 at R.T.
Sample volume	10 µl
Working reagent volume	1000 µl
Concentration of standard	100 mg/dL
Linearity	Upto 5oo mg/dL
Stability of color	1 hour
Maximum absorbance limit	2.000
Units	mg/dL

Procedure:

Pipette into tubes marked	Blank	Standard	Test
Serum or Plasma	-	-	10 µl
Glucose standard	-	10µl	-
Working glucose reagent	1000 µl	1000µl	1000µl

The above were mixed well , incubated at 37 ^{o}C for 10 min or R.T. for 30 min and the absorbance of sample (As) and of standard (Astd) was read against blank.

Calculation:

Glucose (mg/dL) = (As / Astd) x concentration of the standard

5.7.2 Estimation of serum cholesterol

Span diagnostics kit was used for the estimation of serum cholesterol.

Method:

Enzymatic, (Cholesterol Oxidase – Peroxidase), Endpoint colorimetry.

Single Reagent Chemistry, with LCF (Lipid clearing factor)

Principle:

The estimation of cholesterol involves the following enzymatic reactions

Cholesterol esters $\xrightarrow{CE}$ Cholesterol + Fstty acids

Cholesterol + 0_2 $\xrightarrow{CO}$ Cholesten – 3 – one + H_2o

H_2o_2 + Phenol + 4-APP $\xrightarrow{POD}$ Quinoneimine dye + H_2o

CE = Cholesterol esterase

CO = Cholesterol oxidase

POD = Peroxidase

4APP=4-Aminoantipyrine

Absorbance of quinoneimine was measured at 505nm which proportional to cholesterol Concentration in the specimen.

Programme:

The basic assay parameters are:

Mode	**End point**
Wavelength	505 nm (490 – 550 nm)
Temperature	37 ^{o}C
Optical path Length	1 cm
Blanking	Reagent Blank
Incubation time (minutes)	10 at 37 ^{o}C
Sample volume	10 µl
Working reagent volume	1000µl
Concentration of standard	200 mg/dL
Linearity	750 mg/dL
Stability of color	1 hour
Maximum absorbance limit	2.000
Units	mg/dL

Procedure:

Pipette into tubes marked	Blank	Standard	Test
Serum	-	-	10µl
Standard	-	-	-
Cholesterol Reagent	1000µl	1000µl	1000µl

The above were mixed well, incubated at 37 ^{o}C for 10 minutes, the absorbance of standard and sample were read against reagent blank at 505 nm within 60 minutes .

Calculation:

$$\text{Cholesterol concentration (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Cholesterol concentration (mmol/L) = concentration (mg/dl) x 0.0259

5.7.3 Estimation of serum triglycerides

Span diagnostics kit was employed for the assessment of serum TG.

Method:

Enzymatic (GPO/Trinder), Endpoint Colorimetry, Single Reagent Chemistry with LCF (Lipid Clearing Factor)

Principle:

The estimation of triglycerides involves the following enzymatic reaction:

$$\text{Glycerol} + \xrightarrow{\text{LPL}} \text{Glycerol} + \text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol} - 3 - \text{Phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{Phosphate} + o_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$2H_2O_2 + 4\ \text{APP} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\ H_2o$$

Absorbance of the quinoneimine dye measured at 550 nm is increased with increase in the triglycerides concentration.

Programme:

The basic assay parameters

Mode	Endpoint
Wavelength	505 nm (490 – 550 nm)
Temperature	37 ^{o}C
Optical path length	1 cm
Blanking	Reagent Blank
Incubation time (minutes)	10 at 37 ^{o}C
Sample Volume	10 µl
Working reagent Volume	1000 mg/dL
Concentration of standard	200 mg/dL
Linearity	1000mg/dL
Stability of color	1 hour
Maximum absorbance limit	2.000
Units	mg/dL

Procedure;

The above were mixed well, incubated at 37 ^{o}C for 10 minutes, the absorbance of standard and sample were read against blank at 505 nm within 60 minutes.

Pipette into tubes marked	Blank	Standard	Test
Serum or Plasma	-	-	10 µl
Glucose Standard	-	10µl	-
Triglycerides Reagent	1000µl	1000µl	1000µl

Calculation:

$$\text{Triglycerides (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Triglycerides (mmol/L) = Concentration (mg/dL) x 0.014

5.7.4 Estimation of serum HDL-Cholesterol and LDL-Cholesterol

S.D Kit was employed for the assessment of serum HDL – cholesterol.

Method:

Polyethylene glycol – CHOD – PAP Endpoint Colorimetry. Two – Reagent Chemistry with LCF (Lipid clearing Factor)

Principle:

Low and Very low density lipoproteins (LDL and VLDL) are resulted out by adding a supplementary poly ethylene glycol 6000. Iin this procedure the HDL are sorted out. HDL Cholesterol is predictable by separating the upper layer and also by observing the sequence of enzymatic reactions starting from the conversion of cholesterol to cholestenone catalysed by cholesterol oxidase in the oxidation process and also by the creation of H_2O_2 figure out a red tinted quinoneimine . Absorbance 505 nm is increased with increasing HDL Cholesterol incidence.

Procedure:

STEP – A: HDL – CHOLESTEROL SEPERATION

Pipette into Centrifuge tube	Quantity
Sample	0.2 ml
Precipitating reagent	0.2ml

The above were mixed well, kept at R. T. and then centrifuged at 2000 rpm for some time and then centrifuged at 2000 rpm for 15 minutes to attain a apparent upper layer and then continued to step – B

Step – B Colour Development:

Pipette into tubes manifest	Blank	Standard	Test
Supernatant from step A	-	-	100µl
HDL Cholesterol Standard	-	100µl	-
Cholesterol Reagent	1000µL	1000µL	1000µL

The above were mixed well, incubated at 37 ^{o}C for some time and absorbance was spectrophotometrically for reagent adjacent to blank at 505 nm within 1hour.

Programme:

The basic assay parameters

Mode	End Point
Wavelength	505 nm (490 – 530 nm)
Temperature	37 ^{o}C or R.T.
Optical path Length	1 cm
Blanking	Reagent Blank
Incubation time (minutes)	10 at 37 ^{o}C or 30 at R.T.
Sample volume	100µl
Working reagent volume	1000µl
Concentration of standard	50 mg/dL
Stability of color	1 hour
Maximum absorbance limit	2.000
Units	mg/dL

Calculation:

$$\text{HDL Cholesterol (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 50 \times 2$$

5.8 Evaluation of antioxidant status

5.8.1Estimation of TBARS

TBARS levels were determined by a modified version of the method described by Ohkawa.

Principle

Acetic acid was used to detach the lipid and protein of the tissue and the protein in the reaction mixture were dissolved by the addition of sodium lauryl sulphate. Thiobarbituric acid reacts with lipid peroxides, hydroperoxide and oxygen double bond to form a coloured adduct with absorption maximum at 532 nm, which was then measured.

Reagents

Sodium lauryl sulphate	-	8.1% (W/V)
Acetic acid	-	20% (pH – 3.5)
Thiobarbituricacid	-	0.8% (w/v)
Butanol:Pyridine	-	(15:1) (v/v)
Trichloracetic acid (TCA)	-	10%

Procedure

Liver was homogenized with 2 ml of Trichloroacetic acid (TCA). 0.2 ml of whole homogenate was taken to which 0.2 ml of 8.1% sodium lauryl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml 0.8% thiobarbituric acid were added. Volume was made up to 4 ml with double distilled water. It

was heated at 95°C for 60 min. After cooling, 1 ml of double distilled H2O and 5 ml of butanol– pyridine mixture was added. The solution was shaken vigorously in a vortex and centrifuged at 4000 rpm for 10 min in a cold centrifuge. The organic layer was separated and absorbance was observed at 532 nm in a spectrophotometer.

Standard curve

Various concentrations of 1, 1, 3, 3-Tetra methoxy propane (TMP) were used as external standard (1–10 nm) and were subjected to the steps mentioned in the procedure section. The readings of absorbance were plotted against the concentration of TMP to derive a linear standard graph. Data expressed as nmole/g wet wt. tissue

Calculation

The concentration of TBARS was determined from the linear standard graph.

5.8.2 Glutathione [GSH]

Glutathione was estimated by the method described by Ellman

Principle

Bis (p-nitro phenyl) disulphide reacts with aliphatic thiol compounds at pH 8.0 to produce one mole of p-nitrothiophenol anion per mole thiol. Since this anion is highly coloured ($\lambda m \approx 13,6000$ at 412 nm), it can be used to measure the thiol concentration.

Reagents

Phosphate buffer (K_2HPO_4) - 0.3 M (pH-8.4)

5, 5` - Dithiobis (2- nitrobenzoic acid) - 0.2% w/v (DTNB)

Sulfosalicyclic acid -10% v/v

Procedure

The Liver were homogenized with 10% TCA and centrifuged at 3000 × g for 10 min. The reaction mixture contained 0.1 ml of supernatant, 2.0 ml of 0.3 M phosphate buffer (pH-8.4), 0.4 ml of double distilled water and 0.5 ml of DTNB [5, 5dithiobis (2-nitrobenzoic acid)]. The reaction mixture was incubated for 10 min and the absorbance was measured at 412 nm in a spectrophotometer.

Standard Curve

Various concentrations of the standard glutathione (1–10 µg) were subjected to the steps mentioned above. The readings of absorbance were plotted against the concentration of GSH to produce a linear standard graph.

Calculation

The concentration of GSH was determined from the linear standard graph. Data are expressed as µmole per gm wet weight.

5.8.3 Estimation of SOD

SOD levels in the hearts were determined by the method of McCord and Firdovich method (1969) and modified by Kakkar

Principle

Superoxide anions were generated in a system comprising of NADH and phenazinemethosulphate. This superoxide anion reduced into blue

tetrazolium forming a blue Formozan, which was measured at 560 nm. SOD inhibited the reduction of nitrobluetetrazolium and thus the enzyme activity was measured by monitoring the rate of decrease in optical density at 560 nm.

Reagents

Sodium pyrophosphate - 0.052 M, pH 8.3

Phenazinemethosulphate - 186 mM

Nitrobluetetrazolium - 300 mM

Glacial acetic acid - 60.05 M

Tris buffer with sucrose - 0.25 M

Tris HCL buffer - 0.0025 M, pH 7.4

Procedure

The Liver was homogenized in 0.25 M Tris–sucrose buffer. Then the homogenized liver was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected, to which 50% ammonium sulphate was added, vortexes, and the reaction mixture was kept for incubation at 4°C for 4 h. After the incubation period it was again centrifuged at 12,000 rpm for 30 min at 4°C. The samples were then kept overnight for dialysis in 0.0025 TrisHCl buffer.

The next day, appropriate volumes of samples to a maximum of 1.2 ml were taken. To it, 1.2ml sodium pyrophosphate, 0.1 ml of phenazinemethosulphate, 0.3 ml of NBT and 0.2 ml NADH were added. The final volume was made up to 3 ml with distilled water. After adding

NADH, it was immediately incubated for 90 sec at 30°C and the reaction was stopped by adding 1ml acetic acid to the reaction mixture. 4 ml of butanol was added and after 10 min, centrifuged at 3000 rpm, for 10 min. The organic layer was separated and absorbance was observed at 560 nm in a spectrophotometer.

Standard curve

Various concentrations of standard SOD (0.1 to 2.0 µg) were subjected to the same steps as the samples mentioned in the above procedure. The reading of absorbance was plotted against the concentrations of SOD to derive a linear standard graph.

Calculation

The concentration of SOD was determined from the linear standard graph. Data expressed as IU/mg protein.

5.8.4 Estimation of Catalase

Principle

In the ultraviolet range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in extinction at 240 nm. The difference in extinction at 240 nm was measured. The difference in extinction per unit time is a measure of the catalase activity.

Reagents

Phosphate buffer - 50 mM at pH 7.0 (0.05 M KH_2PO_4) and 0.053M Na_2HPO_4 mixed in a ratio of 1:1.55

Hydrogen peroxide - 30 mM solution Isotonic buffer - pH 7.4 (0.9% NaCl and 0.01 M phosphate buffer)

Ethanol - 99.99%

Triton X - 10%

Procedure

The liver was homogenized with isotonic buffer and centrifuged at 3000 rpm for 10 min. The supernatant liquid was collected and 0.01 ml of ethanol per ml of supernatant liquid was added. Then the samples were incubated for 30 min in an ice water bath. At the end of the incubation period, 10% Triton X was added to 0.1/ml of supernatant and used for catalase estimation. 2 ml of sample was appropriately diluted in phosphate buffer and 1 ml H_2O_2 was added and extinction was read at 240 nm at 15 sec interval for a total of 30 sec. Standard curve Various concentrations of standard catalase (1–20 µg) were subjected to the steps mentioned above. The readings of absorbance were plotted against the concentrations of catalase to derive a linear standard graph. Calculation.The concentrations of catalase in the samples were

determined from the linear standard graph. Data expressed as IU/mg protein.

5.9 Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dunnett's Multiple Comparison testusing computer based fitting program (Prism, Graph pad.). Statistical significance was determined at $P < 0.0001$

VI. RESULTS AND ANALYSIS

6.1 The percentage yield of ethanolic extracts of roots of *Anisomeles malabarica* (L.) was 16%.

6.2 Preliminary phytochemical tests

Table:- 4 shows the phytochemical analysis of ethanolic extract of *Anisomeles malabarica*(L.) revealed the presence of alkaloid, glycoside, sterols, phenols, tannins, flavanoids, terpenes, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, and phytosterols.

Preliminary Phytochemical Screening of ethanolic extract of *Anisomeles malabarica(L.)*

S.NO	Constituents		ETHANOLIC EXTRACT
1	Alkaloids	Mayer's Test	+ve
		Dragendroff's Test	+ve
		Hager's Test	+ve
		Wagner's Test	+ve
2	Carbohydrates	Fehling's Test	-ve
		Benedict's Test	+ve
		Barfoed's Test	+ve
3	Proteins	Biuret's test	+ve
		Millon's Test	-ve
4	Steroids	Libermann Burchard Test	-ve
5	Sterols		-ve
6	Phenols		+ve
7	Tannins		+ve
8	Flavonoids		+ve
9	Gums and Mucilage		-ve
10	Glycosides		+ve
11	Saponins	Foam test	+ve
12	Terpenes		-ve

-ve- indicate the absence of compound
+ve- indicate the presence of compound

6.3.1 Acute Toxicity studies of ethanolic extract of the plant *Anisomeles malabarica*(L.)

Parameters observed	**Ist hr**	**IInd hr**	**IIIrd hr**	**IVth hr**
Aggressiveness	+	+	+	+
Alertness	-	-	-	-
Alopecia	-	-	-	-
Circling	-	-	-	-
Diarrhoea	-	-	-	-
Edema	-	-	-	-
Eye closure at touch	+	+	+	+
Grip strength	+	+	+	+
Grooming	+	+	+	+
Lacrimation	-	-	-	-
Loss of writing reflex	-	-	-	-
Mortality	-	-	-	-
Nasal sniffing	-	-	-	-
Piloerection	-	-	-	-
Rearing	-	-	-	-
Righting reflex	-	-	-	-
Seizures	-	-	-	-
Straub tail	-	-	-	-
Urine stains	-	-	-	-

4 hours observation in acute toxicity studies of 2000mg/kg of *Anisomeles malabarica* (L.)

Parameters observed	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Day-6**	**Day-7**	**Day-8**	**Day-9**	**Day-10**	**Day-11**	**Day-12**	**Day-13**	**Day-14**
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

6.3.2 Observation in acute toxicity studies at the dose of 2000mg/kg bw p.o dose of *Anisomeles malabarica* (L.)

I. SUB-ACUTE TOXICITY STUDIES

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into groups of 10 animals (5 male and 5 female). The suspension of ethanolic extract was administered to rats at the dose of 100 & 200 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.

6.3.3 Signs of toxicity in sub acute toxicity (28 days)

Parameters observed	Day-2	Day-4	Day-6	Day-8	Day-10	Day-12	Day-14	Day-16	Day-18	Day-20	Day-22	Day-24	Day-26	Day-28
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Treatment	0th day	5th day	10th day	15st day	20th day	25th day	28th day	% increase
Control	175.83±6.84	179.50±6.28	181.83±6.46	184.83±6.31	187.16±6.01	190.66±6.46	193.66±5.70	10.3637
100mg/kg	177.00±4.43	180.50±4.47	182.83±5.02	186.16±5.40	190.00±6.04	192.500±5.70	195.500±5.63	10.1695
200mg/kg	177.16±8.02	178.83±8.17	182.83±8.23	186.00±7.85	189.000±8.11	192.16±8.43	194.83±8.34	09.6044

6.3.4 Change in body weight after the drug treatment *Anisomeles malabarica*(L.)

6.3.5 Haematological Parameter

Haematological parameter	Control	*Anisomeles malabarica* (L.)	
		100 mg	200mg
Total R.B.C. count (×10^6 mm−3).	9.09±0.15	8.90±0.12	9.11±0.16
Total W.B.C. Count (×10^3 mm−3).	12.67±0.22	12.35±0.15	11.23±0.23
Haemoglobin (Hb) (g/dl)	15.61±0.36	14.07±0.30	15.63±0.36
Hematocrit (%).	44.21±1.01	43.61± 1.72	36.4±1.36
Platelets (×103 mm−3).	834.91±24.01	867.21±23.25	739.81±26.86
Lymphocytes(%).	84.7±1.32	81.8±1.33	72.8±1.43
Neutrophils (%).	20.6±0.65	12.6±0.52	19.2±0.91

Data are expressed as mean ± SEM

6.3.6 Biochemical Parameters

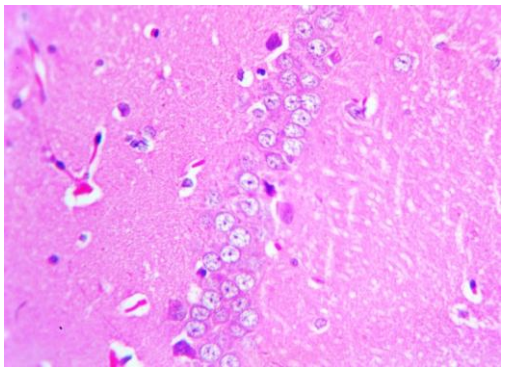
Biochemical parameter	Control	*Anisomeles malabarica* (L.)	
		100 mg	200mg
Creatinine (mg/dl)	0.5890±0.079	0.6600 ± 0.049	0.5540 ± 0.074
Urea (mg/dl)	15.30 ± 0.47	14.50 ± 0.40	15.20 ±0.57
Triglycerides (mg/dl)	52.20±1.13	51.40±1.08	47.10±1.62
Total Cholesterol (mg/dl)	46.60±1.21	51.40±1.08	54.03±1.67
Total protein (mg/dl)	4.40±0.26	4.20±0.35	3.70±0.26
Albumin (g/dl)	3.20±0.41	3.70±0.33	3.20±0.29
AST (IU/L)	121.41±2.68	121.3±1.65	116.61±2.045
ALT (IU/L)	69.40±1.57	67.60±1.301	68.60±1.108
ALP (IU/L)	112.6±4.67	117.01±0.714	117.41±0.718
T. Bilirubin (mg/dl)	0.2569±0.32	0.267±0.029	0.254±0.023

Data are expressed as mean ± SEM

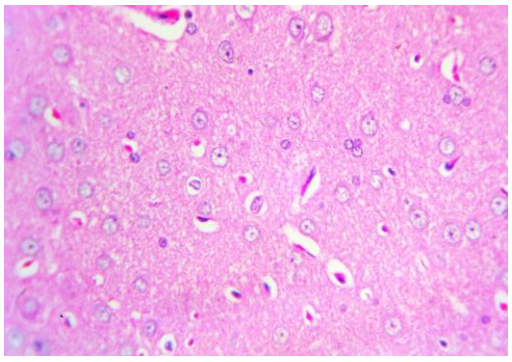
6.4 HISTOPATHOLOGY OF VITAL ORGANS

At the end of the 30th day Liver, Kidney, Heart, Lungs and Brain were subjected for routine Histopathological examination and fixed in 10 % formal saline (10 parts of formaldehyde and 30 parts of normal saline). Tissues were processed and embedded in paraffin wax. Sections were cut at 5 micron thickness and stained with Haematoxylin and Eosin. Light microscopic examination of the sections was then carried out and micrographs produced using Vanox-T Olympus photographing microscope. The histopathological examinations were reviewed by the pathologist.

BRAIN

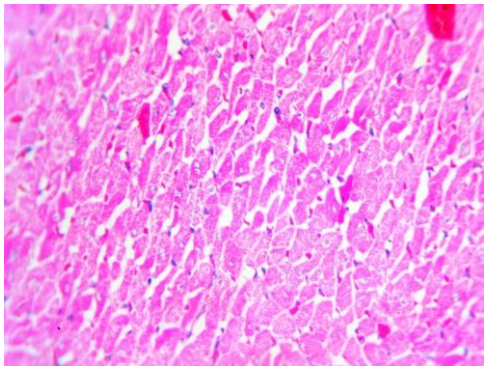


100mg/kg

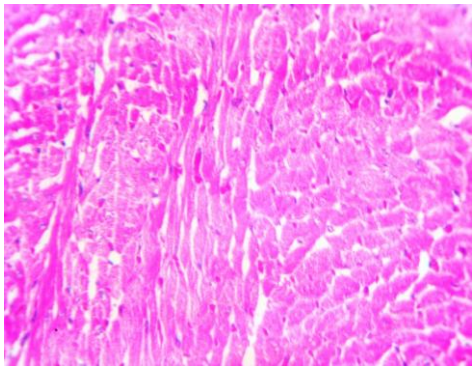


Control

HEART

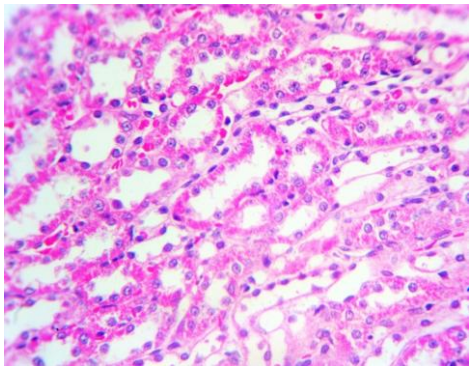


100mg/kg

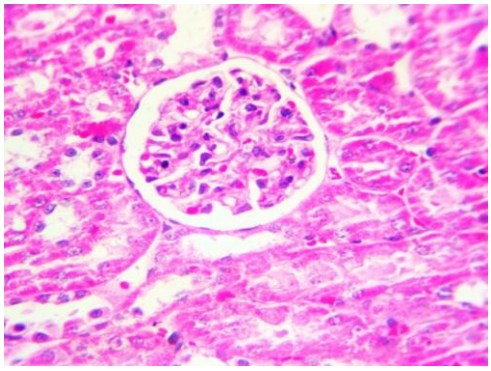


Control

KIDNEY

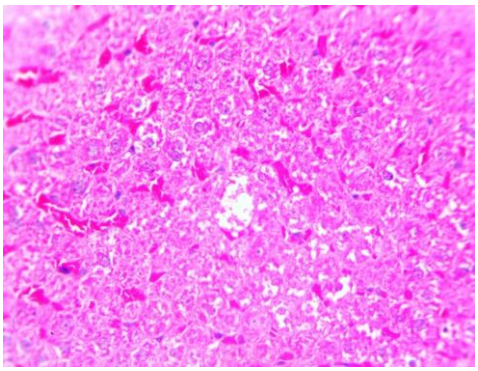


100mg/kg

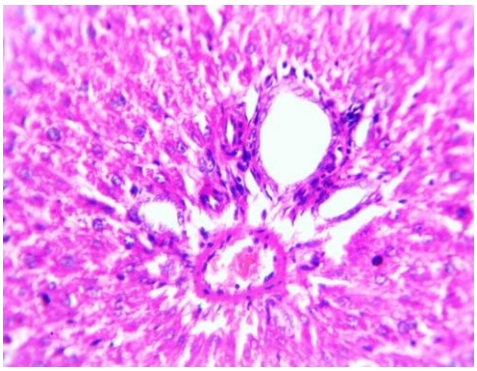


Control

LIVER

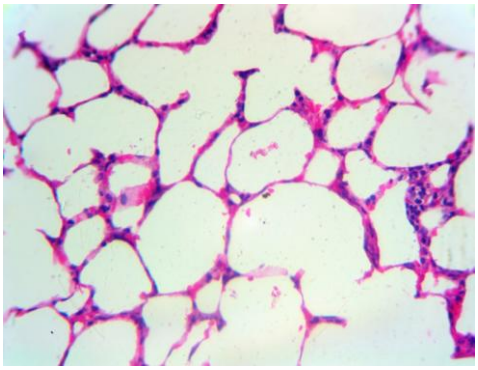


100mg/kg

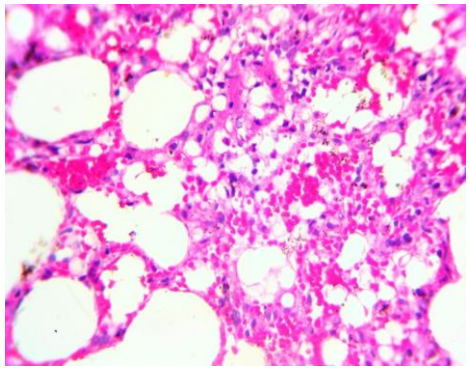


Control

LUNG



100mg/kg



Control

6.5 Dexamethasone induced diabetic model

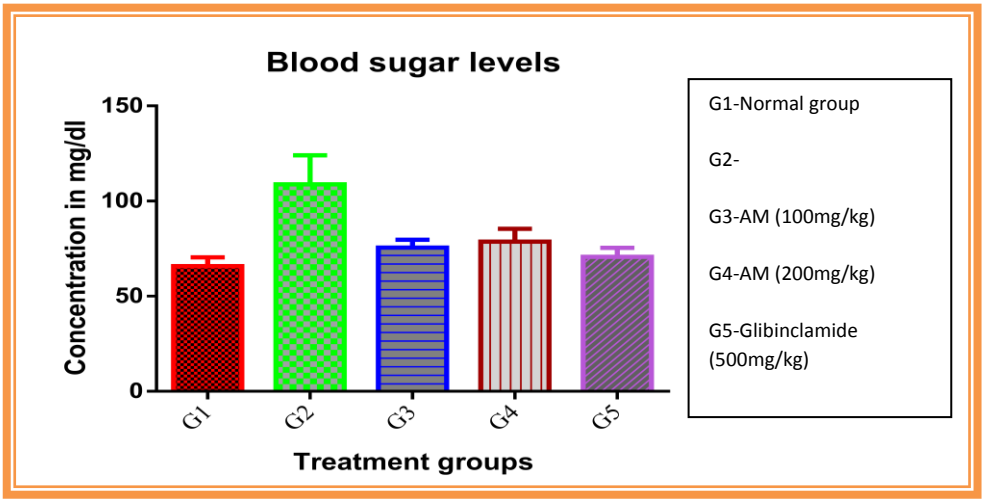
Blood was collected from each rat for biochemical estimation and pancreas was quickly isolated immersed in ice cold saline and weighed. Pancreas was stored under freezer (-20 °C) for estimation of tissue antioxidant parameters.

5.2 Effect of *Anisomeles malabarica Linn* on serum biochemical parameters

Blood glucose, Total cholesterol, HDL, LDL, VLDL, Triglycerides, levels were estimated in serum. The results were presented.

6.5.1 Serum level of Blood glucose

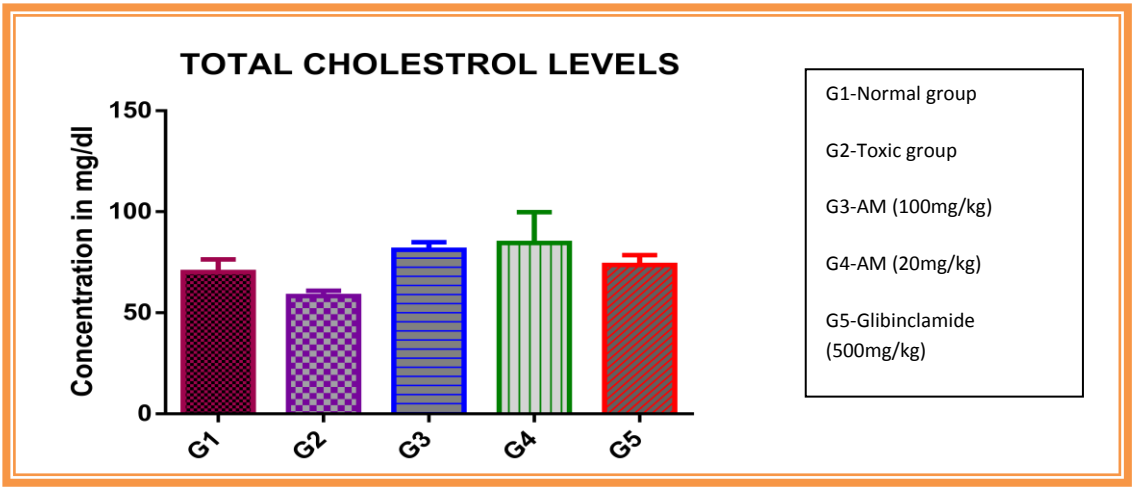
There is significant (P<0.0001) increase in the level of Blood glucose at group 2 when compared with Group 1. There is significant (P<0.001) decrease in the level of Blood glucose at group 3, 4 and 5 when Compared with group 2.



6.1. Effect of *Anisomeles malabarica Linn* on blood glucose levels

6.5.2 Serum level of Total cholesterol

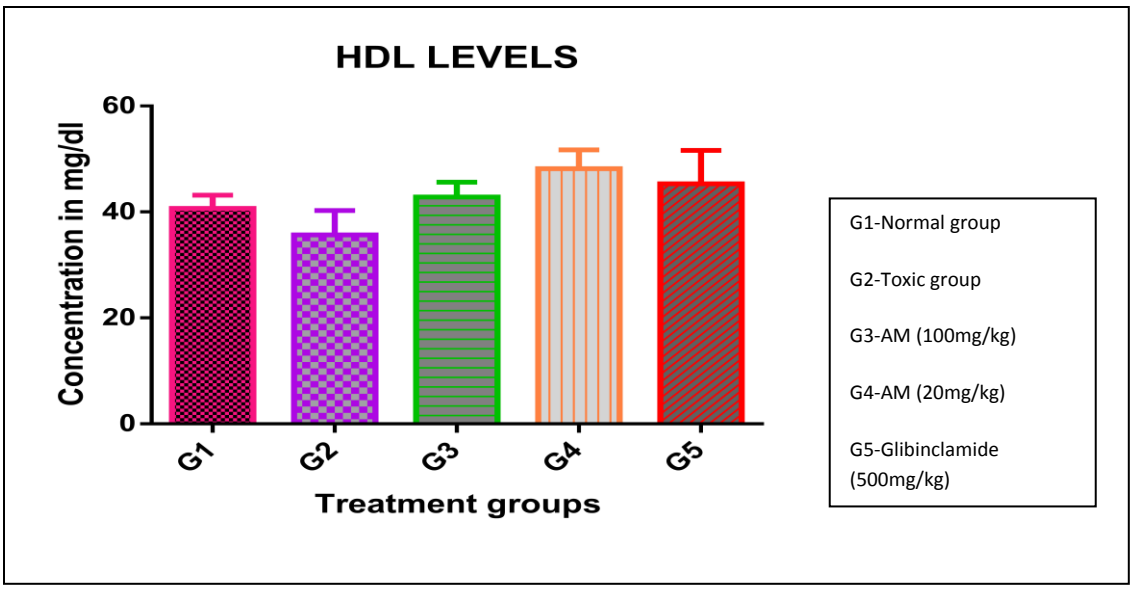
There is no significant increase in the level of Total cholesterol at group 2 when compared with group 1. There is significant increase (P<0.001) in the level of Total cholesterol at group 3 when compared to group 2. Group 4 showed significant increase (P<0.0001) in the level of Total cholesterol compared to group 2. There is significant (p<0.001) increase in the level of Total cholesterol in group 5 compared to group 2.



6.5.3 Effect of *Anisomeles malabarica Linn* on HDL

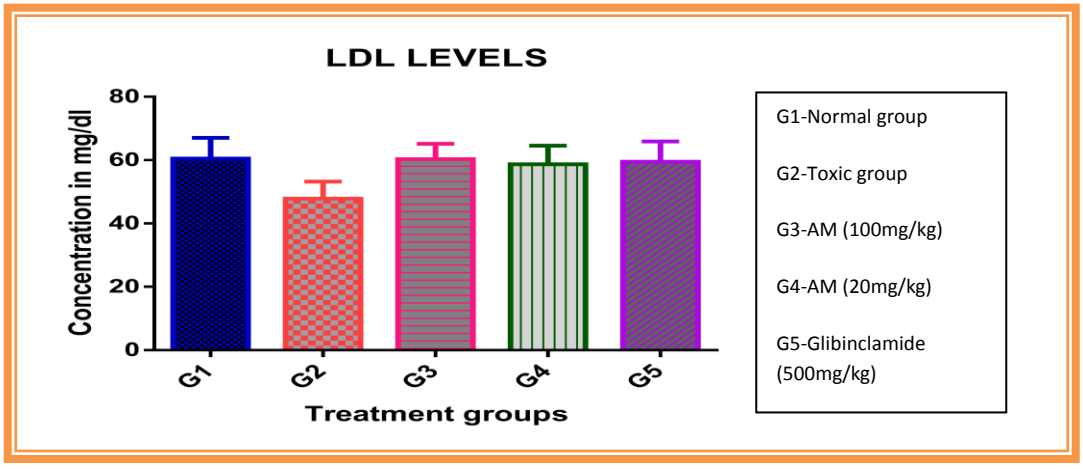
There is significance (p<0.01) increase in the level of ALP at group 2 when compared with group 1. There is significant (p<0.0001) decrease in the level of ALP at group 3 when compared to group 2. There is significance (p<0.01) increase in the level of ALP at group 4 when compared to group 2. There is no significance increase in the level of ALP at group 5 when compared to group 2.

6.5.4 Serum level of HDL



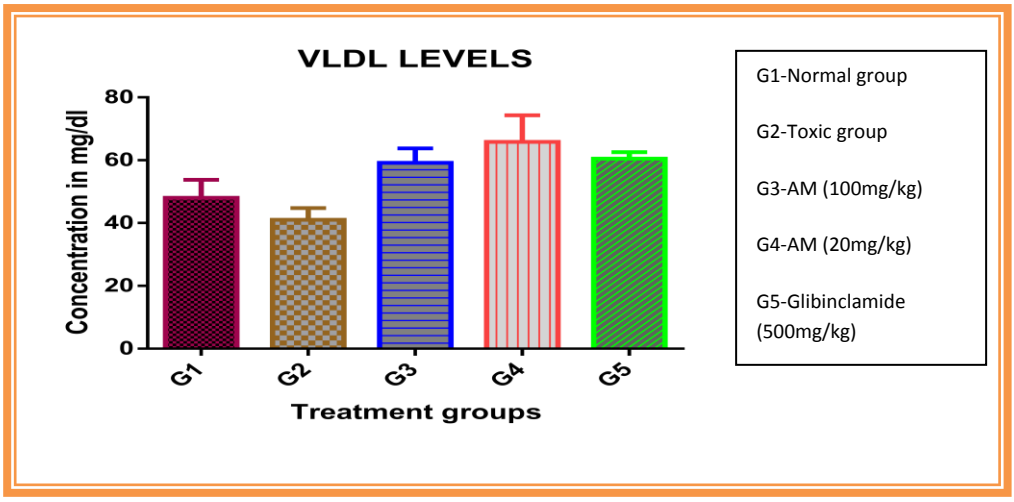
6.5.5 Serum level of LDL

There is no significance increase in the level of LDL at group 2 when compared with group 1. There is significant increase ($p<0.0001$) in the level of LDL at group 3 when compared to group 2. There is significance ($p<0.01$) increase in the level of LDL at group 4, when compared to group 2. There is significance ($p<0.0001$) increase in the level of LDL at group 5 when compared to group 2.

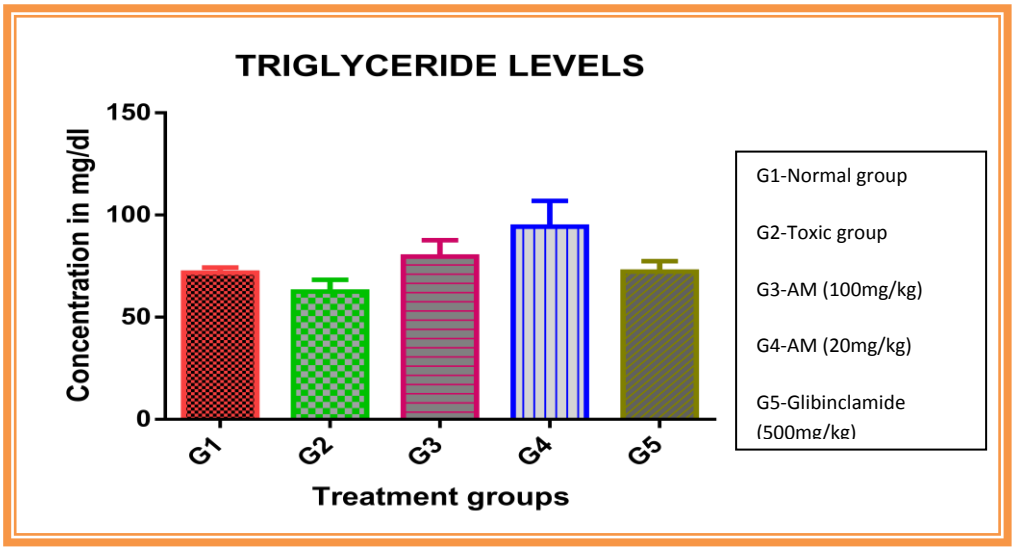


6.5.6 Serum level of VLDL

There is no significance increase in the level of VLDL at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of VLDL at group 3, 4 and 5 when compared with group 2.



6.5.7 Serum level of Triglycerides



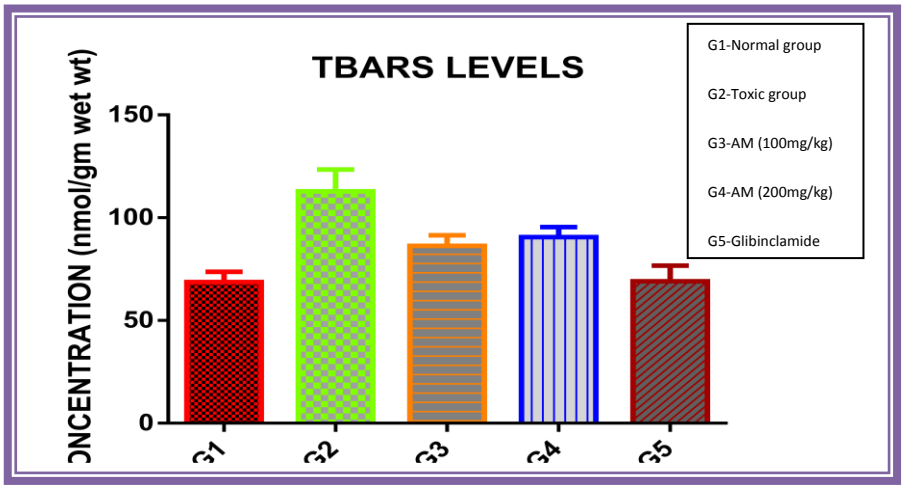
There is no significance increase in the level of Triglycerides at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of cholesterol at group 3, 4 and 5 when compared with group 2.

6.6 Effect of *Anisomeles malabarica Linn* on tissue parameters

Pancreas are homogenised and TBARS, GSH, SOD, Catalase, levels were estimated. The results were presented in Table 5.5 and Graph no. 5.4, 5.5 and 5.6

6.6.1 Effect on TBARS

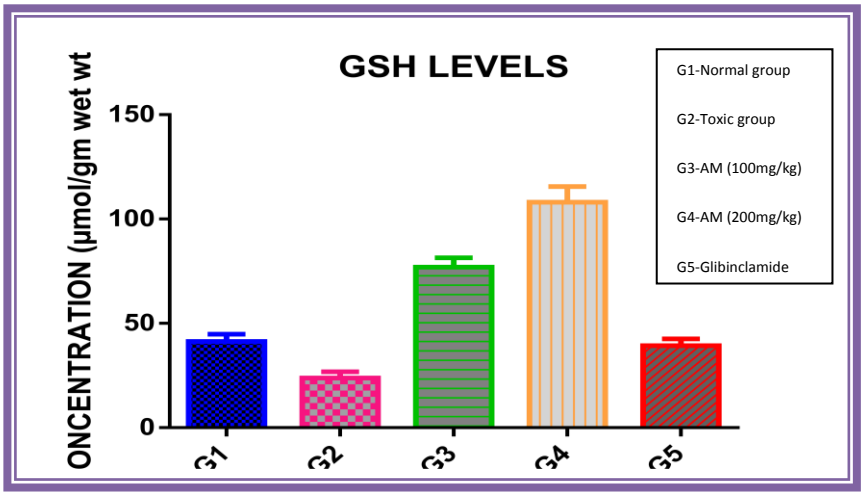
There is significance (P<0.0001) increase in the level of TBARS at group 2 when compared with group1. There is significance (P<0.01) decrease in the level of TBARS at group 3, 4, and5 when compared with group 2.



Effect of *Anisomeles malabarica Linn* on TBARS levels

6.6.2 Effect on reduced glutathione (GSH)

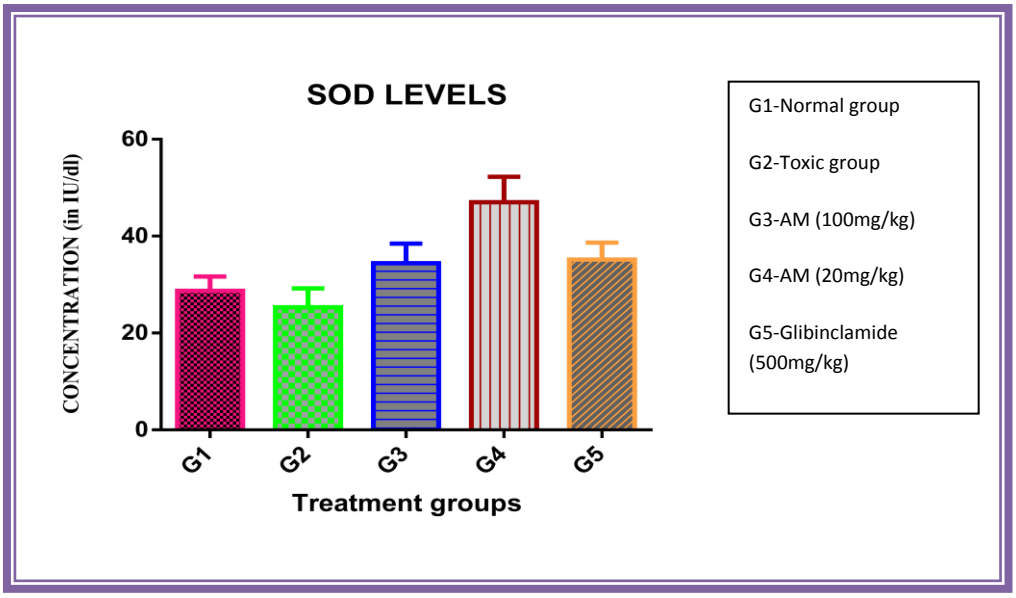
There is significance decrease in the level of GSH at group 2 when compared to group 1. There is significance (P<0.0001) increase in the level of GSH at group 3 when compared to group 2. There is significance (P<0.0001) increase level of GSH at group 4, 5 when compared with group 2.



Effect of *Anisomeles malabarica Linn* on GSH levels

6.6.3 Effect on superoxide dismutase (SOD)

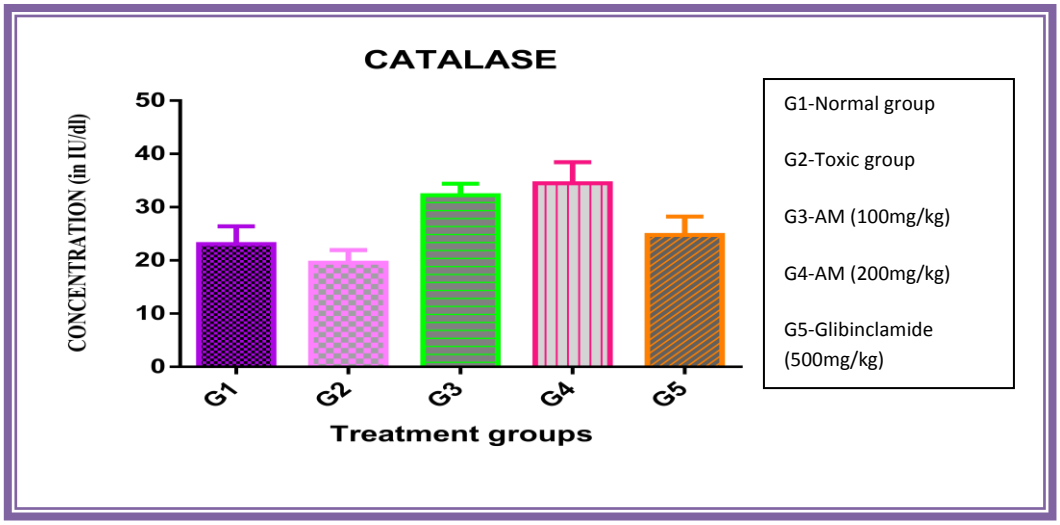
There is significance decrease in the level of SOD at group 2 when compared with group 1. There is significance (P<0.0001) increase in the level of SOD at group 3,4 and 5 when compared with group 2.



Graph 5.10 Effect of *Anisomeles malabarica Linn* on SOD levels

6.6.4 Effect on Catalase (CAT)

There is significance decrease in the level of catalase at group 2 when compared to group 1. There is significance (p<0.0001) increase in the level of catalase at group 3 when compared with group 2. There is significance (P<0.0001) increase level of catalase at group 4, 5 when compared with group 2.



VII. DISCUSSION

D.M. is a very common metabolic disorder affecting three fourth of the world's population in advanced age and is the fourth leading causes of death in the world. The occurrence is very common and not in any one hands. Neither diet nor long term usage of medicines leads to this disease. They are only minor causes and major reason is only inheritance. Once affected there is no cure and is the major border line for many micro and macro vascular complications.

The usage of carbohydrates, proteins and amino acids play a major role in our body as they are the major source to generate ATP for energy purpose. There is imbalance in this major metabolic process of carbohydrates which is linked to low insulin levels or insulin sensitivity in people affected with the disease. The energy fuel glucose shows imbalances in the disease causing hyperglycemia.

Many allopathic drugs are there to be used as anti-diabetic agents to lessen the commonness of the disease as there is no permanent cure for the disease. But these allopathic medicines have equal side effects than the pharmacological effects. Hence the usage of these drugs can be replaced by using herbal drugs that don't possess any side effects and sometimes the diseases can be cured permanently. But yet they have to be commercially

proved as modern medicines. Even though there are many anti diabetic agents, the research in finding herbal drugs for diabetes has not been stopped and is a continuous process because of intolerable adverse effects of allopathic medicines.

Glucocorticoids are broadly used therapeutic tools, mainly in treatment meant for anti-inflammatory as well as immunomodulatory purposes. Glucocorticoid treatment Side effects include steroid diabetes. Glucocorticoid shows hyperglycemia is partly owing to improved hepatic glucose production as well as insulin resistance of peripheral tissues. in addition, glucocorticoids are recognized to reduce insulin secretion. The primary mechanism involves increased α2- adrenoceptor signaling and enlarged Potassium channel activity and impaired glucose metabolism. even though reduced insulin secretion through glucocorticoid treatment can be overcome by stopping adrenoceptor signaling or by reduction of potassium channel convincing evidence suggests that the proper functioning of β-cells that's depends on cell survival. Therefore, a reduction of β-cell mass in longstanding glucocorticoid therapy may give up the subsequent development of steroid diabetes

Insulin resistances in humans are shown in situation like obesity, non-insulin dependent diabetes mellitus (NIDDM) and Dyslipidemia. So, interventions to reduce insulin resistance might push back the development of

NIDDM and the complications. Treatment through natural herbs is probable to be filled with smaller side effects compared to the currently use synthetic oral anti diabetic agents. several medicinal plants encloses has been reported to posses considerable antidiabetic effect in both type 1 and II diabetes mellitus along with improved glucose tolerance. Dexamethasone is a powerful synthetic element of the glucocorticoid group of steroid hormones. Dexamethasone administration leads to causes hyperglycemia, insulin resistance, and Dyslipidemia in human and experimental animals. Insulin resistance and hyperinsulinemia are often linked with a group of risk factors such as impaired glucose tolerance, obesity, hypertension and Dyslipidemia.

Dexamethasone is an effective and greatly selective glucocorticoid used in the treatment of inflammation. Large exposure of glucocorticoids impairs insulin sensitivity, leads to the generation of metabolic syndrome as well as insulin resistance and hypertension. The mechanism which dexamethasone induces peripheral insulin resistance is in inhibiting GLUT-4 translocation, and rising lipase activity in adipose tissue leads to cause impairment of Endothelium-dependent vasodilatatio. Dexamethasone increases the triglycerides levels causing an difference in lipid metabolism leads to hyperlipidemia and increases glucose levels causes to hyperglycemia Pharmacological doses of glucocorticoids induces ob gene expression in rat

adipose tissue within 24 hrs and is followed by a complex metabolic changes ensuing in decrease in food consumption causing reduction in body weight and also occurred with by diabetes and generation of Insulin resistance with improved glucose and triglycerides levels. In this experiment administration of dexamethasone for 10days resulted in improved glucose, triglycerides, cholesterol, insulin, levels. Anisomeles malabarica Linn at a dose of 10mg/kg and 20 mg/kg drastically prevented the rise in blood glucose levels, triglycerides (TG), total cholesterol levels (TC), low density lipoproteins (LDL), and very low density lipoproteins (VLDL). Thus the above results indicate that Anisomeles malabarica Linn has preventive effect on dexamethasone induced insulin resistance.

VIII. SUMMARY & CONCLUSION

In the current research, we have investigated the effect of *Anisomeles malabarica* Linn on dexamethasone induced insulin resistance models. Various biochemical estimations like glucose, cholesterol. Triglycerides, LDL and HDL levels and anti-oxidant estimations like TBARS SOD, CAT, and GSH were estimated in two different doses of Anisomeles malabarica Linn (100 and 200mg/kg p.o.) and then compared with the standard and induced compound.

Anisomeles malabarica Linn at a dose of 100mg/kg and 200mg/kg prevented the development of hyperglycemia, hypercholesteremia and hypertriglyceridemia in dexamethasone induced insulin resistance models. Oral administration of Anisomeles malabarica Linn 100mg/kg and 200mg/kg reduces serum glucose, triglyceride, total cholesterols and LDL concentration and improve the concentration of HDL in dexamethasone administered rats. The lignin Anisomeles malabarica Linn showed significant anti-diabetic effect in rats after oral administration.

The present study demonstrated that Anisomeles malabarica Linn could be useful in Management of diabetes associated with abnormalities in lipid profiles. Further study need to isolate, identify the active compounds and find out the possible mechanism of actions.

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APPROVAL CERTIFICATE

This is to certify that the project title **"Evaluation of *Anisomeles malabarica*(L.) for its toxicological and Anti Diabetic activity in Wistar Albino rat."** has been approved by IAEC and the details are furnished under

Approval no	Name of the species	Breakup sex wise	Weight	No's approved
SVCE/BIO/2017/004	Wistar Albino rat.	23 female& 20 male	150-200gms	43
Forty three wistar albino rats only				

31/5/17

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PARC PLANT ANATOMY RESEARCH CENTRE

Dr.P. Jayaraman, Ph.D.
Director, PARC,
Retd. Professor, Presidency College

Herbal PARC

AUTHENTICATION CERTIFICATE

Based upon the ~~Organolep~~tic /macroscopic /~~microsco~~pic examination of fresh /~~market~~ sample, it is certified that the specimen given by

is identified as below:

Binomial: Anisomeles malabarica (L.) R.Br.

Family: Lamiaceae

Synonym(s): Nepeta malabarica L.

Regional names: Tam: Peyamaratti

Reg.No of the certificate: PARC/201 /1397

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Ibid. III: _____ .1989.

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National Conference on "RATIONAL USE OF MEDICINES - CURRENT SCENARIO AND WAY FORWARD"

Certificate

This is to certify that Dr. / Mr. / Ms. MOHAMED ABOOBACKER SIDDIQUE.M

has participated as a Delegate / Presented Poster in the ***"National Conference on Rational use of Medicines - Current Scenario and Way Forward"*** *organized by the Department of Pharmacy Practice, SRM College of Pharmacy, SRM University held on 8th and 9th August 2016.*

Dr. K.S. Lakshmi	Dr. N. Chandraprabha	Dr. K. Sridhar
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Society for Ethnopharmacology, Chennai Chapter

CERTIFICATE

This is to certify that Dr./Mr./Ms. M. MOHAMED ABOOBACKER SIDDIQUE
has participated as a ~~Resource person~~ / ~~Oral~~ / ~~e poster Presenter~~ / Delegate in the National Conference on "Current Perspectives in Herbal Drug Regulations - Global Scenario" held on 28th - 29th June 2017, organized by Sri Ramachandra University, Porur, Chennai.

This carries 8 Credits.

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Dr. D. CHAMUNDEESWARI
Principal &
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K.V. Somasundaram
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Dean of Faculties