ANTIDIABETIC ACTIVITY OF *Berberis aristata* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC MODEL

A Dissertation submitted to

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

CHENNAI - 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch- IV - PHARMACOLOGY

Submitted by

SOUNDARRAJAN.S

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Under the Guidance of

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DEPARTMENT OF PHARMACOLOGY

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KUMARAPALAYAM – 638183

TAMILNADU.

OCTOBER – 2017

CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"ANTIDIABETIC ACTIVITY OF *Berberis aristata* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC MODEL",** submitted by the student bearing **Reg. No: 261525210** to "**The Tamil Nadu Dr. M.G.R. Medical University – Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on……..….

Internal Examiner **External Examiner**

CERTIFICATE

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DECLARATION

I do hereby declared that the dissertation **"ANTIDIABETIC ACTIVITY OF *Berberis aristata* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC MODEL"** submitted to "**The Tamil Nadu Dr.M.G.R Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried out by me during the academic year 2016-2017, under the guidance and supervision of **Dr. C. Kalaiyarasi, M. Pharm., Ph.D.,** Assistant Professor, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam **Mr. SOUNDARRAJAN.S**

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Dedicated to Parents,

Teachers&

My Family

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CONTENTS

1. INTRODUCTION

Diabetes Mellitus (DM)

The Diabetes Mellitus is chronic metabolic disorder, which there are high blood sugar level over a prolonged period. The term **"**mellitus" or "from honey**"** was added by the Briton John Rolle in the late 1700s to separate the condition from diabetes insipidus, which is also associated with frequent urination. It is a fast-growing global problem with huge social, health and economic consequences. It is estimated that in 2010 there were globally 285 million people (approximately 6.4% of the adult population) suffering from this disease. This number is estimated to increase to 430 million in the absence of better control or cure. An ageing population and obesity are two main reasons for the increase. Furthermore it has been shown that almost 50% of the putative diabetics are not diagnosed until 10 years after onset of the disease, hence the real prevalence of global diabetes must be astronomically high.

This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and non ketotic hyperosmolarcoma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes.

Indian physicians around the same time identified the disease and classified it as Madhumeha or "honey urine", noting but the urine would attract ants. This is possibly due to the diet and life-style of the ancient people, or because the clinical symptoms were observed during the advanced stage of the disease. Galen named the disease "Diarrhea of the urine" (diarrhea urinosa). The earliest surviving work with a detailed reference to diabetes is that of Aretaeus of Cappadocia (2nd or early 3rd century CE). Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 CE with type 1 associated with youth and type 2 with being overweight. Effective treatment was not developed until the early part of the 20th century, when Canadians Frederick Banting and Charles Herbert Best isolated and purified insulin in 1921 and 1922. This was followed by the development of the long-acting insulin NPH in the 1940s.

Signs and Symptoms

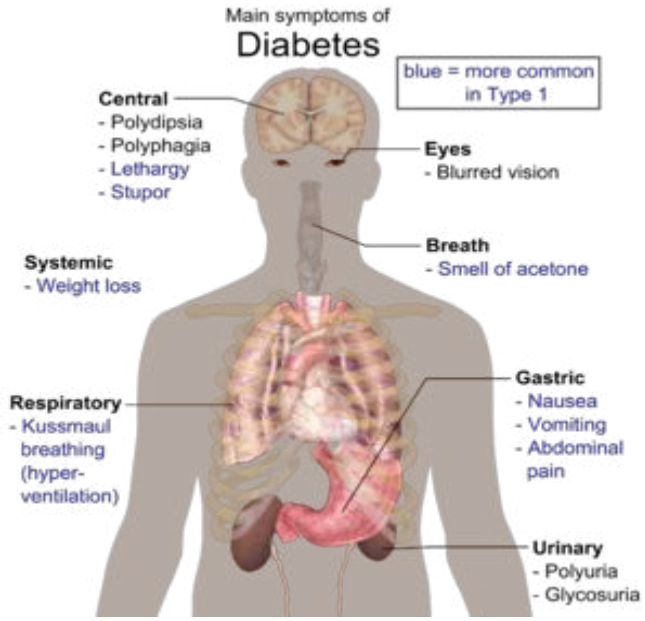


Fig No:1- ***Main Symptoms of Diabetes***

Overview of the most significant symptoms of diabetes

The classic symptoms of untreated diabetes are weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes.

Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes.

People (usually with type 1 diabetes) may also experience episodes of diabetic ketoacidosis, a type of metabolic problems characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness. A rare but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration.

Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary micro vascular complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness. Damage to the kidneys, known asdiabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function.

Diagnosis

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

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

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus.

Per the World Health Organization people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose. People with plasma glucose at or above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl), two hours after a 75 g oral glucose load are considered to haveimpaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease. The American Diabetes Association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause.

The rare disease diabetes insipidus has similar symptoms to diabetes mellitus, but without disturbances in the sugar metabolism (*insipidus* means "without taste" in Latin) and does not involve the same disease mechanisms.

WHO Diabetes Diagnostic Criteria			
Condition	**2 hour glucose**	**Fasting glucose**	**HbA_{1c}**
Unit	mmol/l(mg/dl)	mmol/l(mg/dl)	%
Normal	<7.8 (<140)	<6.1 (<110)	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥ 6.1(≥110) & <7.0(<126)	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥6.5

Prevention and Treatment

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

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).

Learning about the disease and actively participating in the treatment is vital for people with diabetes, since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels. The goal of treatment is an HbA1C level of 6.5%, but should not be lower than that, and may be set higher. Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialised footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet.

Lifestyle:

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure.

Medications:

Metformin is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality. Routine use of aspirin, however, has not been found to improve outcomes in uncomplicated diabetes. Angiotensin converting enzyme inhibitors (ACEIs) improve outcomes in those with DM while the similar medications angiotensin receptor blockers (ARBs) do not.

Type 1 diabetes is typically treated with a combinations of regular and NPH insulin, or synthetic insulin analogs. When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications. Doses of insulin are then increased to effect.

In those with diabetes some recommend blood pressure levels below 120/80 mmHg; however, evidence only supports less than or equal to somewhere between 140/90 mmHg to 160/100 mmHg.

Pancreatic transplantation:

A pancreas transplant is occasionally considered for people with type 1 diabetes who have severe complications of their disease, including end stage renal diseaserequiring kidney transplantation.

Support:

In countries using a general practitioner system, such as the United Kingdom, care may take place mainly outside hospitals, with hospital-based specialist care used only in case of complications, difficult blood sugar control, or research projects. In other circumstances, general practitioners and specialists share care in a team approach. Home telehealth support can be an effective management technique.

Diets And Exercise Treatment

Making healthy food choices is very important to help keep your blood glucose level under control. People with diabetes don't need to buy or prepare special foods. The foods that are best for someone with diabetes are excellent choices for everyone: foods that are low in fat, salt, and sugar, and high in fiber, such as beans, fruits, vegetables, and whole grains. These foods help you reach and stay at a weight that's good for your body. Regular physical activity is important for people with diabetes. Being physically active has been shown to improve blood glucose levels in older people whose levels are high. Exercise is especially good for people with diabetes because it helps control weight, helps insulin work better to lower blood glucose, is good for your heart and lungs, gives you more energy, Regular physical activity improves insulin resistance and lipid profile (reduction in triglyceride and increase in high-density lipoprotein (HDL)) and lowers blood pressure (although blood pressure will rise during exercise),the metabolic benefits in type 2 diabetes are lost within 3-10 days of stopping regular exercise, physical activity also protects against the development of type 2 diabetes.

A1C Test

The A1C test is used to detect type 2 diabetes and prediabetes but is not recommended for diagnosis of type 1 diabetes or gestational diabetes. The A1C test is a blood test that reflects the average of a person's blood glucose levels over the past 3 months and does not show daily fluctuations. The A1C test is more convenient for patients than the traditional glucose tests because it does not require fasting and can be performed at any time of the day.The A1C test result is reported as a percentage. The higher the percentage, the higher a person's blood glucose levels have been. A normal A1C level is below 5.7 percent.An A1C of 5.7 to 6.4 percent indicates prediabetes. People diagnosed with prediabetes may be retested in 1 year. People with an A1C below 5.7 percent maystill be at risk for diabetes, depending on the presence of other characteristics that put them at risk, also known as risk factors. People with an A1C above 6.0 percent should be considered at very high risk of developing diabetes. A level of 6.5 percent or above means a person has diabetes.

Herbal drugs

Allopathic medicines are very costly. In contrast, herbal medicines are very cheap. This cost effectiveness makes them all the more alluring.Herbal medicines can be brought without prescription and they are available in all most all health stores. Some herbs can even be grown at home.For certain ailments, herbal medicines are considered to be more effective than allopathic medicines.

Herbal medicines do not have any side effects, as they are free from chemicals. They are also milder than allopathic medicines.The natural detoxification process of the body is effectively enhanced by herbal medicines. They can be used to cleanse the colon, improve digestion and food absorption. Herbal medicines are also very good in boosting the immune system.

Herbal medicines are very effective in curing various digestive disorders like colitis, indigestion, peptic ulcers, and irregular bowel movements.These types of medicines are best for people who are allergic to various types of drugs.Herbal medicines are also effective in boosting the mental health.Most of the ailments related to blood circulation like high blood pressure, varicose ulcers, and many others can be controlled through herbal medicine.Some herbal medicines are very good in reducing the cholesterol level in the blood stream. They are also used to treat coronary artery diseases.Herbal medicine can be used to reduce weight by regulating appétit.

2. REVIEW OF LITERATURE

Ososki *et al.,* 2002 Reported Ethnobotanical literature survey of medicinal plants in the Dominican republic used for women's health condition on the usage of *Berberis aristata* decotion For uro-genital problems can use as decotion preparation of seeds taken orally or wash bath. The field work in the Dominican republic revealed that herbs were used to treat many women's health condition including uterine fibrosis, excessive uterine bleeding,endometriosis and hot flushes in menopause.

Dasgupta *et al.,* 2013 investigated and reported **b** about the important phytochemicals present in the leaf extract. The preliminary screening showed the presence of many phytochemicals such as alkaloids,flavonoids, terpenoids, saponoin, fixed oils and fats. High Performance Thin Layer Chromatography analysis was carried out with optimized solvent system consisting of ethyl acetate, formic acid, glacial acetic acid and water in the ratio of 8:1:1:2. The densitometric scanning of the chormatograms of hydroaclcoholic extract showed 7peaks at 254 nm 8 peaks at 366 nm. The phytochemicals detected in the present study justify the therapeutic uses of the leaves in the traditional medicines.

Isaac *et al.,* 2007 conducted histopathological and biochemical studies on Short-term administration of an aqueous extract of *Berberis aristata Integra* var. crenata (Andr.) in a 14-day sub-acute toxicity studies using female Sprague-Dawley rats on *Berberis aristata Intergra (Ki)* leaf extract for the treatment of ulcers, pain and adenoma of the prostate gland repoted about its traditionally use for orally administered multipurpose in Ghana and other parts of the world.

Cruz et al., 2012 evaluated and studied on the topic Phytomedicine: International Journal of Phytotherapy & Phytopharmacology about Aqueous extract of *Berberis aristata Pinnata* (Kp) have been found effective in models to reduce acute anaphylactic reactions.They investigated on the effect of Kp and the flavonoid quercetin (QE) and quercitrin (QI) on mast cell activation *in vitro* and in a model of allergic airway disease *in vivo*. Treatment with Kp and QE *in vitro* inhibited degranulation and cytokine production of bone marrow-derived mast cells following IgE/FcεRI crosslinking, whereas treatment with QI had no effect. Similarly, *in vivo* treatment with Kp and QE decreased development of airway hyperresponsiveness, airway inflammation, goblet cell metaplasia and production of

IL-5, IL-13 and TNF. In contrast, treatment with QI had no effect on these parameters. These findings demonstrate that treatment with Kp or QE is effective in treatment of allergic airway disease, providing new insights to the immunomodulatory functions of this plant. *Berberis aristata Pinnata* inhibits mast cell activation and prevents allergic airway disease.

Shazid *et al.,* 2012 reported on the elucidated structure under the title Chemical and biological studies of *Berberis aristata Pinnata* (Lam.) growing in Bangladesh. In this study they isolated compounds from *K. Pinnata* and elucidated their structures and to explore preliminary antioxidant, antimicrobial, cytotoxic and thombolytic activities of extractives of the plant. The methanol extract of whole plant of *K. Pinnata* has been subjected to different chromatographic separation and purification processes to isolate the secondary metabolites. The structures of the isolated compounds have been elucidated by extensive NMR studies.

Patil *et al.,* 2013 reported under Antidiabetic activity of *Berberis aristata Pinnata* in streptozotocin-induced diabetic rats by glucose independent insulin secretagogue action about the anti diabetic action of *K.Pinnata* in wich it was reported that it reduces fasting blood glucose level

Gad *et al.,* 2006[33] studied on Biochemical study of the anti-diabetic action of the Egyptian plants fenugreek and balanites. It proceeded by evaluating the effects of 21 days oral administration of Fenugreek seed and Balanites fruit extracts (1.5 g/kg bw) on the liver and kidney glycogen content and on some key liver enzymes of carbohydrate metabolism in STZ-diabetic rats were studied. In addition, the effects of these two plant extracts on the intestinal alpha-amylase activity in vitro and starch digestion and absorption in vivo were also examined. .

Patil *et al.,* 2013 reported on hypoglycemic activity on *Berberis aristata Pinnata* Lam. (Crassulaceae) under the title Antidiabetic activity of *Berberis aristata Pinnata* in streptozotocin-induced diabetic rats by glucose independent insulin secretagogue action. The probable mechanism for insulin secretagogue action was evaluated through studies using diazoxide and nifedipine. The bioactive component from DCM fraction was studied using HPTLC, GCMS and IR.

Dzhafarova *et al.,* 2009 reported on Antidiabetic action of extract of *Juglans Regia L* study is on the impact of Iuglans Regia L on the pathological processes in alloxan-indeced dibatic animals. The investigation showed that extract from wall nuts leaves decreases the blood sugar level, has a positive impact on lipid metabolism. Antioxidant properties of an extract from leaves of the walnut tree (Juglans regia) are reported.

Radhika *et al.,* 2011[36] Did a major evaluation on Antidiabetic and Hypolipidemic Activity of *Punica Granatum Linn* on Alloxan Induced Rats the study aimed to evaluate the antidiabetic and hypoglycemic activity of *Punica granatum*. Diabetes & hyperlipidemia was induced by the intra peritoneal injection of alloxan mono hydrate (120mg/kg) for 2 consecutive days. Diabetes was confirmed 2days after the last alloxan dose administration by determining the blood glucose concentration. Treatments were started after confirmation of diabetes in rats.During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. So the total hemoglobin level is lowered in alloxan induced diabetic rats. Alloxan induced diabeteshas been observed to cause a massive reduction of the beta cells of the islets of pancreas leading to hyperglycemia. Rats treated with alloxan (120mg/kg), for 2 consecutive days, showed an increase in the concentration of glucose, triglycerides, cholesterol, LDL cholesterol, VLDL cholesterol and a decrease in the level of HDL cholesterol and hemoglobin content. Administration of crude powder of Punica granatum husk reduced the concentration of glucose, triglycerides, cholesterol, LDL cholesterol, VLDL cholesterol and raised the level of HDL cholesterol and hemoglobin content in the blood of both group-I normal and group III alloxan diabetic rats treated.

Saif-Ur-Rehman *et al.,* 2011 presented the work on Antidiabetic Effect of Aloe vera Extract on Alloxan Induced Diabetic Rats. It was carried out to investigate the antidiabetic effect of Aloe vera extract in normal and alloxan induced diabetic rats.

Kuroda *et al.,* 2005 studied on the turmeric (*Curcuma Longa L.* rhizomes) EtOH extract the Hypoglycemic effects of turmeric (*Curcuma Longa L.* rhizomes) on genetically diabetic KK-Ay mice it shows significant suppression and an increase in blood glucose level in type 2 diabetic KK-A(y) mice.

Mondal *et al.,* 2008 did a preliminary and antidiabetic screening on the title Antidiabetic activity of *Diospyros Malabarica* Kostel bark: a preliminary investigation for possible mode of action. The extract exerted significant antihyperglycemic effect in alloxan-induced hyperglycemia and resulted in increase in plasma protein content and decrease in alkaline phosphatase, cholesterol and triglyceride levels when compared with those in the diabetic control group. However there were no significant changes in body and kidney weights of the DM extract-treated animals, compared to those of the untreated diabetic rats as a control.

Grover *et al.,* 2002[40] conducted a research of Medicinal plants of India with anti-diabetic potential on the plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic property. The present paper reviews 45 such plants and their products (active, natural principles and crude extracts) that have been mentioned/used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity.

Rajnish Gupta *et al.,* 2011 reported on Antidiabetic efficacy of *Mangifera Indica* seed kernels in rats. The study was conducted to examine the hypoglycemic potency of seed kernels of *Mangiferaindica* ethanol extract (MIEtE) in streptozotocindiabetic rats. Remarkable abnormalities were observed in serum and tissue parameters in hyperglycemic rats after streptozotocin administration

Sabjan Khaleel Basha and Vinoji Sugantha Kumari.2012 reported on In vitro antidiabetic activity of *Psidium Guajava* seeds extracts. In this they did the evaluation of the glucose uptake of (antidiabetic activity) crude n-hexane, ethanol, ethanol and aqueous leaf extracts of P.guajava. Methods: P.guajava leaf extracts were subjected to inhibitory effect of glucose utilization using specific standard in vitro procedure.

Yoshino *et al.,* 2009 reported on the title Anti-diabetic activity of a leaf extract prepared from *Salacia Reticulata* in mice The effects of a water extract prepared from the leaves of Salacia reticulata on the absorption of sugars in normal and type 1 diabetic mice were investigated.

Akhani *et al.*, 2004 reported on antidiabetic study of *Zingiber officinale Roscoe* as Anti-diabetic activity of *Zingiber Officinale* in streptozotocin-induced type I diabetic rats The fresh and dried rhizome of Zingiber officinale Roscoe (commonly known as ginger) is widely used in traditional medicine.The study is on the effect of the juice of Z. officinale (4 mL kg(-1), p.o. daily) for 6 weeks on streptozotocin (STZ)-induced type I diabetic rats with particular reference to the involvement of serotonin (5-hydroxytryptamine; 5-HT) receptors in glycaemic control.

Ramadan *et al.*, 2013 observed two parameters on the diabetic induced rat on title Hypoglycemic and hepatoprotective activity of *Rosmarinus Officinalis* extract in diabetic rats The present study examined the effect of water extract (200 mg/kg body weight) of Rosmarinus officinalis L. in streptozotocin (STZ)-induced diabetic rats for 21 days. The hepatoprotective effects were investigated in the liver tissues.

3. PLANT PROFILE

***Berberis aristata* Leaves**

Plant Profile	
Kingdom	Plantae
Order	Ranunculales
Family	Berberidaceae
Genus	Berberis
Species	*B. aristata*

Berberis aristata

Herbalism or "herbal medicines" have a long history to cure several kinds of human diseases from the various parts of the plants such as leaf, stem, bark, *leaves* , etc. [2] Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicines for some aspect of primary health care. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combining the Earth for phytochemicals and leads that could be developed for treatment of various diseases. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. More than two thirds of the world's plant species at least 35,000 of which are estimated to have medicinal value come from the developing countries [1] . The plant is an erect glabrous spinescent shrub, 3-6 m in height with obovate to elliptic, subacute to obtuse,

entire or toothed leave (3.8-10 × 1.5-3.3 cm long), flowers are yellow in corymbose racemes, petiole distinct up to 4 mm, inflorescence a single drooping raceme (2.5-7.5 cm long), and dense-flowered. Pedicles are stout, 4-6 mm long, fruits are 7-10 mm long, ovoid, bluish black or bright red in color and covered with a thick pale or bluish white bloom and are born in pendulous clusters. *Berberis aristata* belongs to the family Berberidaceae, is an important medicinal plant. It is also called as 'daruharidra', found in the Himalayas and other parts of the world. A very valuable ayurvedic preparation 'Rashut' is prepared by this plant and are used for the curing of diseases such as, jaundice ophthalmic and skin diseases. *Berberis aristata* is characterized by an erect spiny shrub, ranging between 2 to 3 m (6.6 to 9.8 ft) in height. It is a woody plant, with bark that appears yellow to brown from the outside and deep yellow from the inside. The bark is covered with three-branched thorns, which are modified leaves, and can be removed by hand in longitudinal strips. The leaves are arranged in tufts of five to eight and are approximately 4.9 cm (1.9 in) long and 1.8 cm (0.71 in) broad. The leaves are deep green on the dorsal surface and light green on the ventral surface. The leaves are simple with pinnate venation. The leaves are leathery in texture and are toothed, with several to many small indentations along the margin of the leaf. Daruharidra has been observed to be diaphoretic, rejuvenating, antipyretic properties and as Raja Nighantu (bitter tonic). The plant is native to Himalayas at an elevation 2000 to 3500 metres and predominantly found in the Nilgiri mountain range in Southern India. The shrub grows upto 1.5 – 2.0 metres in height, with a thick woody *leaves* covered with a thin brittle bark. The leaves are cylindrical, straight, tapering, very sharp, hard, smooth spine with yellow, numerous, stalked, arranged in drooping racemic flowers and small berry, ovoid and smooth fruits. The flowering season of this plant is observed from April to May. Some of the major Daruharidra formulations are Darvyadi kavatha, Darvyadi leha, Darvyadi taila, Rasanjana and Dasanga lepa. Due to several clinical important formulations, Daruharidra is of trade importance (high volume/high value) and is of conservation concern being an endemic species. Market survey in India indicates that *Berberis asiatica*, Berberis lycium, Cosinium fenestratumand Morinda umbellata are traded as substitutes of B. aristata.

4. AIM AND OBJECTIVE

The Diabetes Mellitus is worldwide problem due to the lifestyle changes lead to decreased physical activity, increased consumption of fat, sugar and calories, and higher stress levels, affecting insulin sensitivity and obesity. Based on the above causative factor to increased tenfold, from 1.2% to 12.1%, between 1971 and 2000 entire world. It estimates is estimated that 61.3 million people aged 20-79 years live with diabetes in India (2011). This number is expected to increase to 101.2 million by 2030. There are many synthetic drugs used for the treatment of diabetes such as Glibenclamide-sulphonylureas were used in India as combination therapy because of its more side effects were reported. Several literatures indicated that the herbal drugs are less adverse effect when compared with synthetic drugs. *Berberis aristata* one of most traditionally using hypoglycemic drug among tribes in Africa and its same species *K.Pinnata* was scientifically reported but *Berberis aristata* is not scientifically validated. Based on the above mentioned reasons, one new research is required to develop one new drug with activity and less side effects, which will be work multifactorial anti diabetic mechanism. Allopathic medicines are very costly In contrast, herbal medicines are very cheap. This cost effectiveness makes them all the more alluring. The work provides scientific validation for the use of leaf against diabetes by revealing the chemical compounds may be present in the plant. The prediction of biological activity of the chemical compounds present in the extract wills also supports the *invitro* results after its phytochemical analysis. Herbal drugs were expelled milde side effects reported in literatues. The present study is attempt to develop a novel plant based antidiabetic drug, which will be evaluated by using *Invitro* and *Invivo* methods.

5. PLAN OF WORK

1. Collection and authentification of the genuine plant material.
2. Shade drying and granulation of the dried leaves.
3. Sequential extraction of the plant material using petroleum ether, ethyl acetate and ethanol.
4. *Invitro* antidiabetic study using α-amylase inhibition assay.
5. Selection of active extract with higher activity.
6. Phytochemical analysis of active extract.
7. Acute toxicity study.
8. *Invivo* antidiabetic study of active extract.

   I) Normal animal
   a) OGTT
   b) Hypoglycemic
   II) Streptazocin induced diabetes mellitus
   a) Multiple dose study

6. MATERIALS AND METHODS

a)Plant collection and authentification

The *leaves* s of *Berberis aristata* were collected from Nilgiri hills Gudalur, Kerala and which was authenticated by Dr.Raghu.A.V Scientist-B,Extension & Training, Kerala Forest Research institute Peechi – 680 653, Thrissur.

b)Preparation of coarse powder and Extraction technique

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered *leaves* was uniformly packed into a thimble in a soxhlet apparatus and extracted with 1000ml Petroleum ether, Ethyl acetate and Ethanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis and pharmacological screenings.

c)*Invitro* antidiabetic activity of *Berberis aristata leaves* extracts

Alpha-amylase inhibition assay

Chemicals or reagents

Potato starch, trichloroacetic acid, Folin-Ciocalteau reagents were purchased from SD Fine Pvt. Ltd., Mumbai, 3,5-dinitrosalicylic acid, Tris buffer, linoleic acid, ammonium molybdate, were purchased from Hi-Media Pvt. Ltd., Mumbai, α-amylase, α-glucosidase enzymes, xanthine oxidase, quercetin, hypoxanthine, pyrocatechol were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Agappe diagnostic Pvt. Ltd., Kerala, Acarbose was obtained from Bicon Pvt. Ltd., Chennai, ferrozine, (2'2-azobis (2-amidino propane) dihydrochloride), butylated hydroxy toluene from Loba Cheme. All other chemicals used in the study were obtained commercially and were of analytical grade.

Instrument used

UV-visible Spectrometer (Systronic double beam- UV-2201).

Preparation of extract

Leaves extraction used in *invitro* and *invivo* studies were prepared by using suitable solvents (Carboxy methyl cellulose).

Experimental procedure for α-amylase inhibition assay

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

Calculation of 50% inhibitory concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I\ \% = \frac{(Ac-As)}{Ac} \times 100$$

Where,

Ac = Absorbance of the control

As = Absorbance of the sample.

d) Thin layer chromatographic profiling

Practical requirements

- Stationary phases
- Glass plates
- Mobile phase
- Preparation and activation of TLC plates
- Application of sample
- Development tank
- Development technique
- Detecting or visualizing agents

Glass plates

Specific dimensions like 20 cm*20cm (full plate), 20cm*10cm (half plate), 20 cm*5cm (quarter plate) can be used. It should be good quality and can withstand temperature used for drying.

Preparation and activation of TLC plates

Mixture of stationary phase and water forms slurry. TLC plates prepared by pouring, dipping , spraying or spreading. In pouring technique, slurry prepared and poured on the glass plate. Slurry spread uniformly on glass plate. Plates are dried in oven. In dipping technique two plates dipped in slurry, separated and dried. Disadvantage is that large quantity slurry required.

Spreading technique

In this technique TLC spreader is used. The glass plates are stacked on base plate. Slurry poured in the reservoir of TLC spreader. Thickness adjusted using knob in the spreader. 0.25 mm is normally used thickness for analytical purposes. Spreader rolled on the plates and air drying the plates. Plates are activated by keeping at 100 degree Celsius to 120 degree Celsius for one hour. Activated plates can be stored in thermostatically controlled oven for further use.

Application of sample

The concentration of sample of standard should be minimum for good spots. 2- 5ug of 1% solution of standard or sample spotted using capillary tubes. Spots should be 2 cm above the base of plate and spotting area should not be immersed in mobile phase. At least 4 spots can be spotted on quarter plate.

Development tank

The developing tanks require more solvent for developing tank –having hump in the middle and require less solvent. It should be lined inside with filter paper moistened with mobile phase

Mobile phase

The solvent or mobile phase depends on various factors.

Nature of substance
Nature of stationary phase
Mode of chromatography
Separation to be achieved. Analytical or preparative

Procedure

Commercial sheet pre coated with silica gel are available. Select a solvent by testing out the samples in various solvents. Dissolve a small quantity of ethanolic extract of *Berberis aristata* leaves of the unknown in different flask containing solvents of different polarity. Place the TLC plates, the spotted side down in to the chamber so that the lower the pencil line about the solvents. Remove the plate from the development chamber and allowed to dry. Plate is placed under UV light, dark spots are observed.

Quantitative analysis

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

R_f = retardation factor

e) Preliminary phytochemical screening of ethanolic leaves extract of *Berberis Aristata*

The ethanolic leaves extract of *Berberis aristata* was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

- Molisch's test: Dissolved small quantity of 300mg alcoholic and dried *leaves* extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test.
- Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution.
- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavanoids

- Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.
- Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.

Test for tannins

- Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for steroid/terpenoid

- Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids

- Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.
- Hager's test: The extract was treated with few ml of Hager's reagen.
- Wagner's test: The extract was treated with few ml of Wagner's reagent.

Tests for Glycosides

- Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

Test for Saponins

- Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Anthraquinones

- Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids

- Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

g) Acute toxicity study

In a research study when a drug is administered to a biological system there will be some interactions may happen .In most case these are desired and usefull,but many effects are not advantageous. Acute, subacute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug.Acute toxicity is involved in estimation of LD_{50} (It is the lethal dose (cuasing death)to 50% of tested group animals)[54].

LD_{50} (median lethal oral dose)

LD $_{50}$ (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of animal (mg/kg)

In this study acute toxicity study was carried out in wistar albino rats. The procedure was followed by using OECD 423(Acute toxic class method).The rats are fasted overnight, prior to dosing. The three dose levels are administered by the help of oral feeding needle over the prior of 24 hours. After the drug has been administered, food may be with held for a further 3-4 hours in rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg).Based on the mortality result on 14^{th} day of observation, the doses for in vivo study are selected.

h) *Invivo* antidiabetic activity of *Berberis aristata* leaves extract in streptozotocin induced diabetic wistar albino rats.

Wistar albino rats (150- 200 grams) of both sexes were procured from Dr. Samsun laboratories, Tirupur-TN, India. Prior to the experiment the rats were housed in a clean polypropylene cages (6 rats/ cages) for a period of 7 days under standard temperature (25 - 30^{o} c) , relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of Organisational Animal Ethics Committee (OAEC) (DAEC/TNA/965/345/16). The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of water.

Chemicals:

Streptozotocin from Loba Chemie. Standard Glibenclamide (Daonil) from Aventis Pharma. Ethanol (Analytical grade) and 5% Dextrose solution Glucose Estimation Kit from Gluco Dr Super sensor

Hypoglycemic Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2mg /kg), Group III served as aqueous ethanolic extract of *Berberis aristata*– (200mg/kg), Group IV served as aqueous ethanolic extract of *Berberis aristata*– (400mg/kg). Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2 mg /kg), Group III served as aqueous ethanolic extract of *Berberis Aristata*– (200mg/kg), Group IV served as aqueous ethanolic extract of *Berberis aristata*– (400mg/kg). All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been adminisratered to different groups and 30mins later all the groups of rats were treated with glucose orally at dose 10gm/kg body weight by using oral

feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals

A single dose (100 mg/kg b.w., i.p.) of streptozotocin monohydrate dissolved in sodium citrate buffer was used for the induction of diabetes in rats after overnight fasting. After 1 hr of streptozotocin monohydrate administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycaemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Experimental design

Five groups of rats six in each groups received the following treatment schedule for 14 days.

GROUP I - Normal control (normal saline 10 ml /kg, P.O)

GROUP II - Streptozotocin treated control (100 mg/kg, I.P)

GROUP III - Streptozotocin (100 mg/kg, I.P) +

Standard drug Glibenclamide (2 mg/kg, P.O).

GROUP IV - Streptozotocin (100 mg/kg, i.p.) + EEBAL.(200 mg/kg, P.O)

GROUP V - Streptozotocin (100 mg/kg, i.p.)+ EEBAL. (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control rats. Group IV and Group V (which previously received streptozotocin100mg/kg) were given fixed doses of ethanol leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Berberis aristata*and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (EEBAL- Ethanolic extract of *Berberis aristata* Leaves).

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of rats at weekly intervals till the end of the study 1, 7, and 14 days.

Estimation of biochemical parameters Serum blood glucose

On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis

Statistical analysis was done by using GRAPHPAD PRISM 5.0.All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

7. RESULTS

a)Appearance and percentage yield of EEHC (Ethanolic Extract of *Berberis aristata* Leaves)

EEBAL was a semisolid brownish colour extract and the percentage yield was found to be 14.35%

b) *invitro* antidiabetic study

Table No:1, α-Amylase Inhibition of Petroleum Ether extract of *Berberis aristata* leaves

Concentration(µg/ml)	Percentage Inhibition (%)
0	0
25	29
50	33
75	42
100	53
125	56

Fig No :3α-Amylase Inhibition of Petroleum Ether extract of *Berberis aristata* leaves

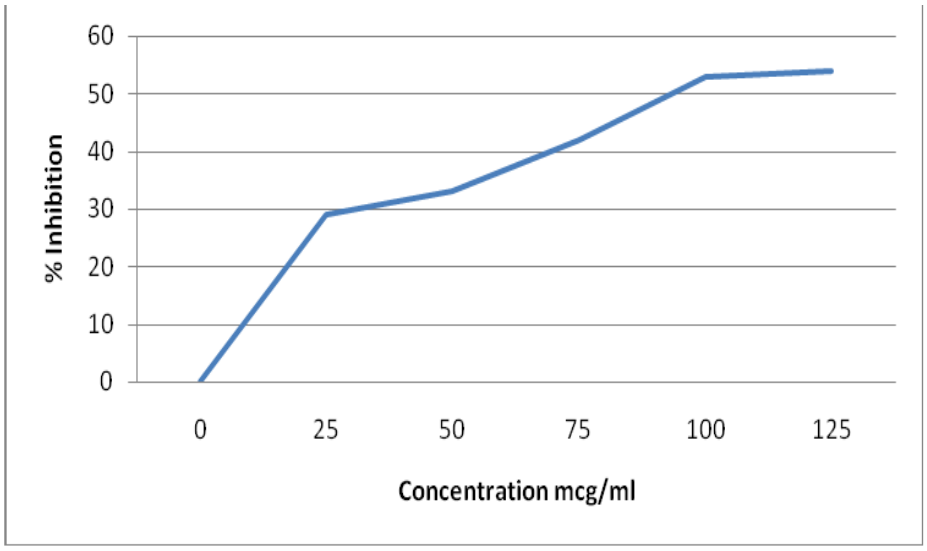


Table no:2, α-Amylase Inhibition of Ethyl acetate Extract of *Berberis aristata* leaves

Concentration(µg/ml)	Percentage Inhibition(%)
0	0
25	34
50	41
75	48
100	56
125	60

Fig No:4 α-Amylase Inhibition of Ethyl acetate Extract of *Berberis aristata* leaves

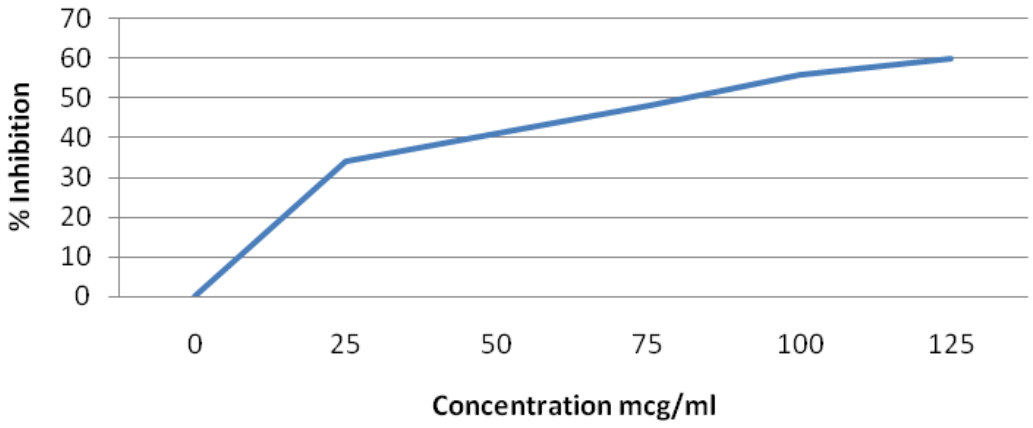


Table no:3, α-Amylase Inhibition of Ethanolic Extract of *Berberis aristata* leaves

Concentration(µg/ml)	Percentage Inhibition(%)
0	0
25	30
50	36
75	50
100	56
125	61

Fig No:5 α-Amylase Inhibition of Ethanolic Extract of *Berberis aristata* leaves

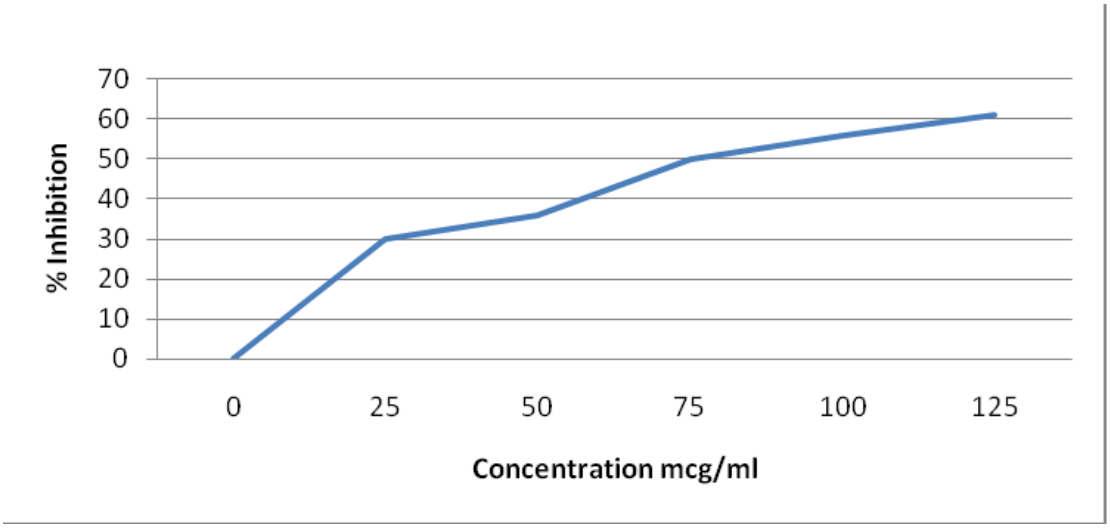
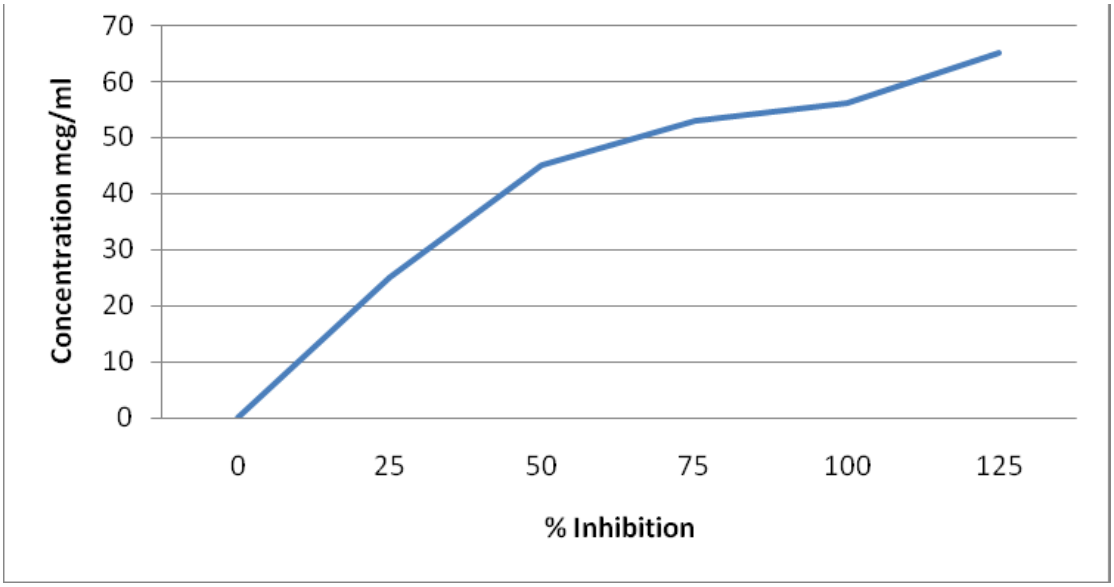


Table no:) 4, α-Amylase Inhibition of Acarbose (Positive control

Concentration(µg/ml)	Percentage Inhibition(%)
0	0
25	25
50	45
75	53
100	56
125	65

Fig No:6 α-Amylase Inhibition of Acarbose (Positive control



Result

%Inhibition of Petrolium ether extract of *Berberis aristata leaves* = 91 µg/ml
%Inhibition of Ethyl acetate extract of *Berberis aristata* leaves = 80 µg/ml
%Inhibition of Ethanolic extract of *Berberis aristata leaves* = 75 µg/ml
%Inhibition of Acarbose (Positive control) = 61 µg/ml

Minimum % Inhibition was found in Ethanolic extract of *Berberis aristata leaves* which resemblance to %Inhibition of positive control, So Ethanolic extract of *Berberis aristata* contain active constituents of antidiabetic.

c) TLC study

Table No: 5, Results of ethanolic extract of *Berberis aristata* leaves

Si No.	**Solvents**	**Concentration**	**R_f value**
1.	Toluene + Ethyl acetate	7:3	0.62
2.	Toluene + Ethyl acetate + Glacial acetic acid	5:5:1	0.76
3.	Petrolium ether+Chloroform	7:3	0.56
4.	Ethyl acetate + Methanol	1:1	0.88
5.	Hexane+Dichloro methane	1:1	0.67
6.	Ethyl acetate + Methanol	3:1	0.59
7.	Dichloro methane +Hexane	3:1	0.72

R_f Value range high-Polar substances present.

R_f value range low-Low polar substance present.

Solubility of compounds depend upon the polarity of solvents.

Obtained R_f values were confirmed by standard R_f values.

R_f values obtained for my extract ranges from 0.56 to 0.88,So my extract may contain compounds like Flavanoids,Glycosides and Alkaloids.

d) Phytochemical studies

Table No : 6, Results of ethanolic extract of *Berberis aristata* leaves

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test Fehling's test	-
Phenols	Phosphomolybdic acid test	+
Flavonoids	Shinoda test Lead acetate test	+ +
Tannins	Braemer's test	–
Alkaloids	Wagner's Mayer's Draggendrof's test	+ + +
Glycosides	Legal's test Brontranger's test	+ + +
Saponins	Foam test	+
Sterols	Salkowski's test	–
Amino acids	Ninhydrin test	–
Terpenoids	Lieberman Burchardt test	+

+Present in moderate amount

-Absence

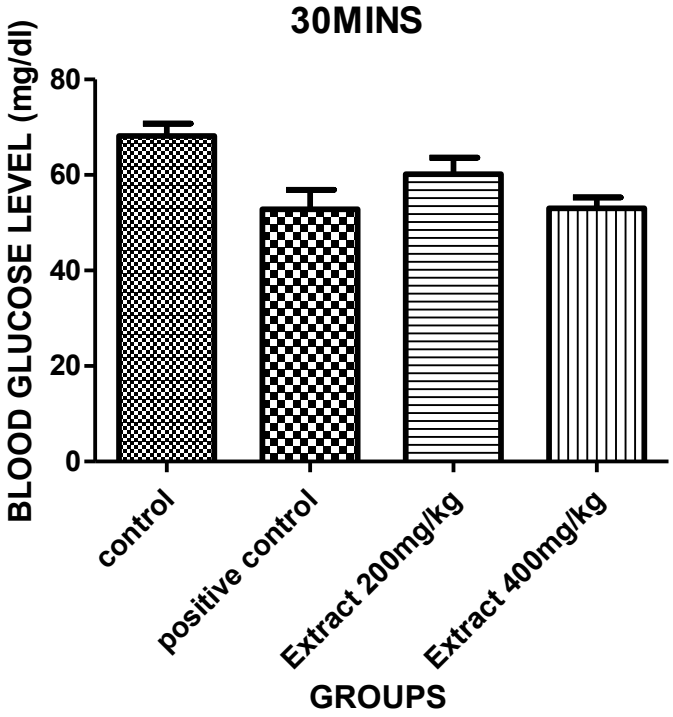
The phytochemical studies results revealed that the Molisch's test no characteristic observation indicated the absence of carbohydrates, by phosphomolybdic acid test Blue coloration of the spot indicated the presence of phenols. Shinoda test and Lead acetate test gave pink or red coloration of the solution indicated the presence of flavonoids Flocculent white precipitate also indicated the same. There is no dark blue or greenish grey coloration of the solution indicated the absence of of tannins in the drug. No characteristic observation for steroids and dark pink or red coloration of the solution indicated the presence of terpenoids. Orange coloration of the spot indicated the presence of alkaloids. Yellow or reddish brown precipitation precipitation indicated the presence of alkaloids. Pink to red colour solution indicates the presence of glycosides. No layer of foam formation indicates the absence of Saponins. If the response to the test is indicated table-1high it can be noted or which indicates that the particular group is present as the major class. If the response is average then note it as indicates the presence in moderate quantity and note it as indicating the presence of only in traces. If no response is then negative.

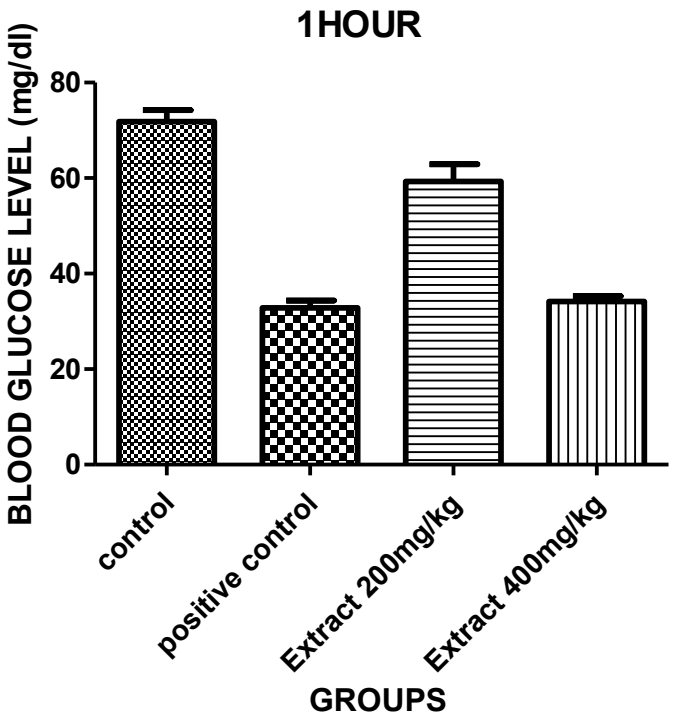
TABLE-I, Hypoglycemic Test

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	0.5hr	1 hr
CONTROL CARBOXYMETHYL CELLULOSE(CMC)	0.5 %	68.00±2.429	68.17±2.587	71.83±2.372
POSITIVE CONTROL Glibenclamide	2	69.00±0.6325	52.83±4.037**	32.83±1.515***

AQUEOUS ETHANOLIC EXTRACT OF *BERBERIS ARISTATA*	200	68.80±2.245	60.17±3.497*	59.33±3.584*
AQUEOUS ETHANOLIC EXTRACT OF *BERBERIS ARISTATA*	400	68.00±2.429	53.00±2.309**	34.17±1.138***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test.





The hypoglycemic test results have shown Table No:I, which indicated aqueous ethanolic extract of *Berberis aristata*treated animals 200 & 400, significantly decreased in blood glucose level (0.84± 1093↓ & 18.83±3.879↓) (P<0.05)*, (P<0.001)**& (P<0.0001)*** when compared to control and positive control

g) *Invivo* antidiabetic study

Table No: 13, Results of the effects of ethanolic extract on blood Glucose levels

SL. No.	TREATMENT	Blood glucose level (mg/dl) day		
		Day 1	Day 7	Day 14
1	Normal control 10 ml/kg P.O	79.83±2.833	75.7±4.014	76.7± 4.944
2	Negative control	265.2±3.85	270.1±2.9	275.2±2.5
3	Possitive control (Glibenclamide 2mg/kg) P.O	255.83±2.386	135.63±3.8***	112±2.8***
4	EEBAL 200 mg/kg P.O	260±3.5	250.3±3.138**	245.2±3.250**
5	EEBAL 400 mg/kg P.O	263±4.55	173.1±2.88***	162.1±1.8***

EEHC-Ethanoloc extract of *Berberis aristata* Leaves

(The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

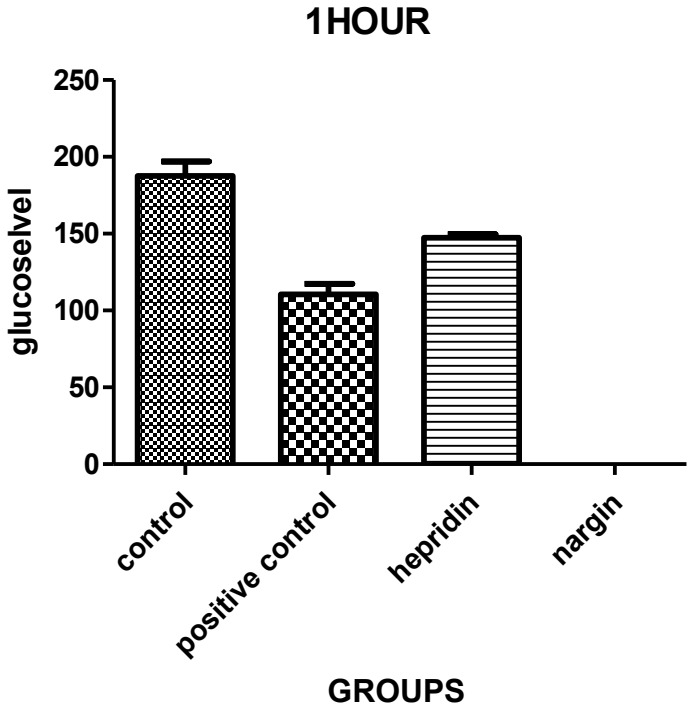
The experimental results have indicated on Table No:13. The negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level (4.13±1.207↓& 1.±0.93↑) for normal control group at 7th and 14th days . On day 7th glucose levels were significantly decreased glibenclamide 2mg/kg treated group (120.2±1.414↓& 23±1↓) (P<0.05)*, (P<0.001)**& (P<0.0001)*** when compared with control group at 7th and 14th days. The ethanolic leaves extract of *Berberis aristata*treated groups 200 & 400 mg/kg were dose dependent manner decreased (P<0.001)**& (P<0.0001)*** (10±0.362↓& 90±1.67↓) when compared with control group but positive control have more anti diabetic activity at 7th day. The aqueous ethanolic leaves extract of *Berberis aristata* at the dose level 400mg/kg have equipotent activity (90±1.67↓&120.2±1.414↓when compared with positive control at 7th day. The ethanolic leaves extract of *Berberis aristata* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action (P<0.001) **& (P<0.0001) *** when compared to control and positive control. On day 14th, ethanolic leaves extract of *Berberis aristata*

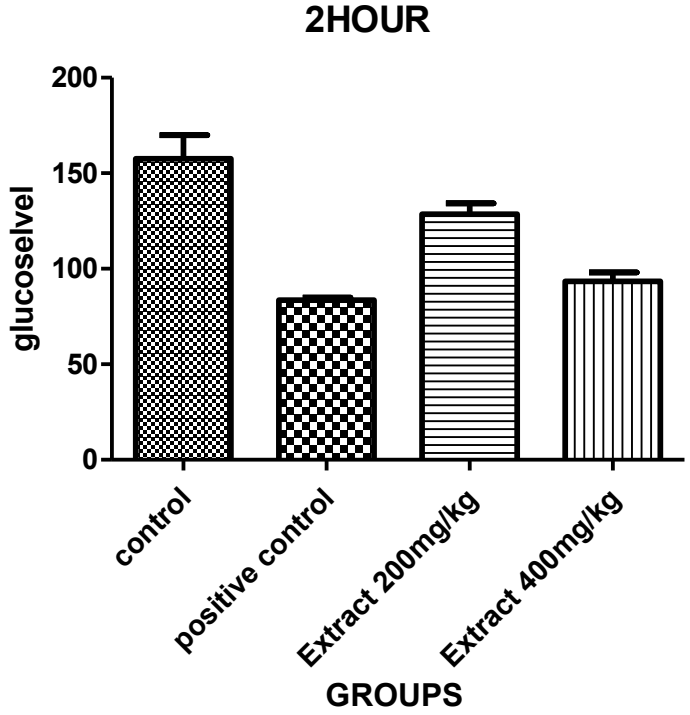
treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (5.1±0.07↓ & 11±1.08↓), ($P<0.001$) **& ($P<0.0001$) *** when compared to control and positive control.

Table-Ii, Oral Glucose Tolerance Test

TREATMENT	DOSE mg/kg	**Blood Glucose Level** (mg/dl)						
		0 min	0.5hr	1 hr	1.5hr	2 hr	2.5hr	3hr
Control Carboxymethyl Cellulose(Cmc)	0.5 %	68.00±2.429	142.5±6.292	187.5±9.465	172.5±12.25	157.5±12.38	153.5±12.83	130.2±13.31
Positive Control Glibenclamide	2	69.00±0.6325	104.2±7.323***	110.5±6.980***	93.67±1.308***	83.67±1.308***	77.17±4.070***	74.33±2.940***
Aqueous Ethanolic Extract Of *Berberis Aristata*	200	68.80±2.245	128.3±6.009	147.3±2.404*	138.5±5.667*	128.5±5.667*	113.8±6.760**	108.8±6.107**
Aqueous Methanolic Extract Of *Berberis Aristata*	400	68.00±2.429	115.0±6.191**	121.2±6.188**	103.3±4.766***a	93.33±4.766*** a	86.67±3.602***a	83.67±2.906***a

The glucose levels were analyzed by using glucometer and all values are expressed as Mean±SEM (n=6), Group 2 was compared with group 1, Groups — 3,4 were compared with group 2; *p<0.05, **p<0.01,p<0.001*** evaluated by one way, ANOVA followed by Dunnet 't' test.


1HOUR
250
200
150
100
50
0
glucoselvel
control
positive control
hepridin
nargin
GROUPS


2HOUR
200
150
100
50
0
glucoselvel
control
positive control
Extract 200mg/kg
Extract 400mg/kg
GROUPS

Oral Glucose Tolerance Test (OGTT) results have been expressed on Table No:II. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased (74.5±3.863↑, 35.2±6.6905↑, 59.5±3.764↑ & 65.58±3.762↑). The blood glucose levels were significantly decreased for , aqueous ethanolic extract of *Berberis aristata*200 & 400 mg/kg (59.5±3.764↓& 65.58±3.762↓) ↓), (P<0.001) **& (P<0.0001) ** when compared to control and positive control at 1hour and each and every ½ hour blood glucose levels(200 mg/kg : 8.8±3.26,10±0, 6.66±1.164 & 3±0.696, 400 mg/kg:17.9±1.422,9.97±0,14.7±1.093&5±0.696) (P<0.05)*, (P<0.001)**& (P<0.0001)*** were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produce the equipotent activity.

8. DISCUSSION

Invitro study is on the principle of Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller. Sequential extraction was done according to increasing polarity order (Petrolium ether,Ethyl acetate and Ethanol). Each extracts were tested for α-amylase inhibition to get the extraction with minimum IC_{50} value. As per the above mechanism all the extract have concentration dependent affinity towards the inhibition of α-amylase. Finally ethanolic extract was observed as more active extract. LC-MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. Even with a very sophisticated MS instrument, HPLC is still useful to remove the interferences from the sample that would impact the ionization. Closely related to LC-MS are some other techniques, like flow injection/MS, CE or CEC/MS, capillary LC or Nano LC-MS In all cases, there is the need for an interface that will eliminate the solvent and generate gas phase ions, then transferred to the optics of the mass spectrometer. Most instruments now atmospheric pressure ionization (API) technique where solvent elimination and ionization steps are combined in the source and take place at atmospheric pressure. When electron impact ionization (EI) is the choice, the solvent elimination and ionization steps are separate. Present study I have been used LC-MS for isolate and characterize the antidiabetic active compounds from extract. LC-MS for most active extract was taken and bioinformatics was collected by PASS. PASS is the computer generated program which provides the simultaneous prediction of several hundreds of biological activity types for any drug-like compounds. In this bio informatics software prediction is based on the analysis of structure-activity relationships of (SAR) the training set including more than 30000 known biologically active compounds .The most active antidiabetic constituent in the ethanolic extract of Berberis aristatawas confirmed as *Quercetin* and other antidiabetic active constituent was confirmed as *Kaempferol.* In this present study Acute toxicity study was carried out in rats. The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic

class method is a step wise procedure with three animals of a single sex per step. Average two to three steps may be necessary. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of EEKAGA was 2000 mg/kg body weight p.o as most of the crude extracts posses LD_{50} value more than 2000 mg/kg p.o and also found to be the maximum safe dose. Observe for signs for toxicity and were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study.The principle involved in the alloxan induced diabetes mellitus in rats, Alloxan, a beta cytotoxic, diabetes induced chemical but wide variety of animal species by damaging the insulin secreting cells of the pancreas. Literature sources indicate that the alloxan induced rats are hyperglycaemic. The treatment of lower doses of alloxan (100mg/kg b.w.) produced partial destruction of pancreatic β-cells even though the animals become permanently diabetic. Thus these animals have surviving β-cells and regeneration is possible. It is well known that the sulfonylurea's act by directly stimulating the β-cells of the islets of Langerhans to release more insulin and these compounds are active in mild alloxan induced diabetes. *Invivo* anti diabetic screening was performed for the confirmation of above mechanism of action was undergone the ethanolic extract of Berberis aristatain biological system (Wich was already resulted for α-amylase inhibitory activity by *Invitro* and constituents analysis by LCMS methods). At the end of the the ethanolic extract of *Berberis Aristata.*(200 mg/kg p.o, 400 mg/kg p.o.) showed statistically significant decrease in blood glucose levels. So the ethanolic extract of *Berberis aristata* showed antidiabetic activity. This work will be useful for further diabetes mellitus and it's related diseases research worker to develop new entity for the treatment of diabetes mellitus.

9. CONCLUSION

Extraction of leaf was done by sequential extraction method The leaves *of Berberis aristata* using the solvent with increasing polarity order (petroleum ether, ethyl acetate and ethanol) and the active extract was tested by *invitro* antidiabetic screening method. The *invitro* antidiabetic study have been performed based on the α-amylase inhibition assay. Each extracts were tested for α-amylase inhibition and the extract with minimum IC_{50} have been undergone phyto chemical screening. The preliminary phytochemical tests was performed to identify the active phytochemicals present in the ethanolic extract of *Berberis aristata* and showed the presence of Phenols, Flavanoids, Alkaloids, Glycosides, Saponins and Terpenoids. LCMS analysis and PASS (bioinformatic software) was performed four compounds were found out *Kaempferol, Rhamnetin, Rhamnoxanthin, Quercetin and luteolin*. The antidiabetic constituents were found to be *Quercetin* (more active) and *Kaempferol* (less active). Finally the *invivo* antidiabetic activity of Ethanolic extract of *Berberis aristata* leaf was tested by using alloxan induced diabetic rat. Acute toxicity study was carried out in rats. The procedure was followed by OECD 423(Acute toxicity class method).$1/10^{th}$ (200mg/kg)and $1/5^{th}$ (400mg/kg)of the maximum safe dose (2000mg/kg)were selected for further study. Fasting blood sample were drawn from retino orbital puncture of rats at weekly intervals till the end of the study 1,7 and 14 days. On these days fasting blood glucose were collected and and analysed for glucose. At the end of the study (14^{th} day)the ethanolic extract of *Berberis aristata* leaf (200mg/kg p.o and 400 mg/kg p.o) treated diabetic groups showed statistically significant decrease in blood glucose similar to the standard drug glibenclamide (2mg/kg), which indicated block the alfa amyalase activity and antagonize the alloxan action.The present study suggested that the isolation of active constituents from ethanolic extract of *Berberis aristata* leaf and characterize the compounds by using preliminary phytochemical studies and LCMS instrument used to isolate the compounds like *Quercetin and Kaemferol* were confirmed by confirmatory chemical tests.

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