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Pharmacognostical and pharmacological evaluation bark of *Lavandula stoechas* for anti-diabetic potential

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Abstract

In the present study the bark of *Lavandula stoechas* were evaluated for their toxicity studies and for antidiabetic activity as they have been reported to have antidiabetic activity in the traditional system of medicine.

Different model systems like alloxan, streptozotocin viruses, insulin antibodies, hormones like dexamethasone, adrenaline and dithizone are available to screen the anti-diabetic activity of a given substance. In the present study chemicals like alloxan and streptozotocin were used to produce marked diabetic effects in animals. Alloxan diabetic model resembles type-I diabetes (IDDM) without significant insulin resistance whereas streptozotocin induced diabetic animals inhibit reduced response to insulin in hepatic and peripheral tissues. Further rats treated with streptozotocin display many of the features seen in human with uncontrolled *diabetes mellitus*.

The literature screened in the process of the proposed work indicates that the bark of *Lavandula stoechas* contain classes of chemical constituents which have shown antidiabetic activity. Plant taken for proposed work has no scientific claims for antidiabetic activity. Phytochemical and pharmacological investigations of the selected plant may yield useful information and material for better management for preventing diabetes. It can be concluded that the traditional formulation of *Lavandula stoechas* have a significant anti-diabetic potential. However, further work needs to be done for isolating the main constituents responsible for this activity and for elucidating the mechanism of action of the antidiabetic activity of these three plant extracts.

Keywords:*Lavandula stoechas*, Anti-diabetic, Phytochemical Analysis, Alloxan, Streptozotocin.

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1. Introduction

1.1 Herbal remedies for *diabetes mellitus*

Herbal medications have been used for the treatment of variety of ailments; a huge number of populations in the world is entirely dependent on traditional medicines. A number of medicinal plants and their formulations are used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices. In India, indigenous remedies have been used in the treatment of diabetes mellitus since the time of *Charaka* and *Shusrutha*. From the ethnobotanical information, about 800 plants which may possess anti-diabetic potential have been found. Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents. This practice may be due to its fewer side effects compare to the synthetic hypoglycemic agents and because of their safety, effectiveness, and availability. Although various synthetic drugs were developed to treat diabetes but still very a smaller number of drugs is available for the treatment of diabetes. There are about 200 pure compounds from plant sources reported to show blood glucose lowering effect. The compounds may be alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, peptides and amino acids,

lipids, phenolics, glycopeptides a diridoids. Many anti-diabetic products of herbal origin are now available in the market. More than 1200 species of plants have been screened for activity on the basis of ethnomedicinal uses [1].

1.2 Need and Scope of Alternative Remedies

Regardless of the type of diabetes, patients are required to control their blood glucose with medications and/or by adhering to an exercise program and a dietary plan. Insulin therapy by injection is given to those with type-1 DM and also to some patients with type-2 DM when oral hypoglycaemic drugs fail to lower blood glucose. Due to modernization of lifestyle, non- insulin dependent diabetes mellitus is becoming a major health problem in developing countries. Patients with type-2 DM are usually placed on are stricted diet and are in-structed to exercise, the purpose of which primarily is weight control. If diet and exercise fail to control blood glucose at the desired level, oral anti-diabetic medication is prescribed [2].

Oral anti-diabetic agents exert their effects by various mechanisms: (1) stimulation of beta cells in the pancreas to produce more insulin (sulfonylureas and meglitinides), (2) increasing the sensitivity of muscles and other tissues to insulin (thiazolidinediones), (3) decreasing gluconeogenesis by the liver (biguanides), and (4) delaying the absorption of carbohydrates from the gastrointestinal tract (alpha-glucosidase inhibitors). These treatments have their own drawbacks, ranging from the developing of resistance and adverse effects to lack of responsiveness in large segment of patient's population. Sulfonylureas lose effectiveness for 44% of patients within six years. Also, these treatments are associated with side effects or even toxic effects (e.g., thiazolidinediones may cause liver toxicity; sulphonyl ureas might worsen heart disease, lower the glucose below the nor-mal range and increase the body weight gain; bloating, flatulence, diarrhea and abdominal discomfort and pain are the major complaints with glucosidase inhibitors). According to literature, two -thirds of medications prescribed for use in children have not been proven safe or effective for this patient population. Moreover, none of these glucose-lowering agents adequately controls the hyperlipidemia that frequently met with the disease [3-4].

Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Medicinal plants useful in diabetes were reviewed recently. The hypoglycemic effect of some herbal extracts has been confirmed in animal and human models of Type-2 diabetes. There are reports of using herbal extracts for the treatment of diabetes mellitus in humans. Adverse effects are indeed a cause of concern; however, available evidence suggests that herbal medicines are relatively safe. [5-6]

The potential role of the medicinal plants as hypoglycemic agents has been reviewed by several authors. Many Indian medicinal plants are reported to be useful in diabetes.

Different model systems are available to screen the antidiabetic activity of a given substance. In the in- vivo models, chemicals like alloxan and streptozotocin are used to produce marked diabetic effects in animals. Alloxan diabetic model resembles Type I diabetes (IDDM) without significant insulin resistance whereas streptozotocin induced diabetic animals' exhibit reduced response to insulin in hepatic and peripheral tissues. Further, rats treated with streptozotocin display many of the features seen in humans with uncontrolled diabetes mellitus [7].

Although there are no definitive preventive measures that can be taken against diabetes at this time, except for identifying persons at high risk and encouraging appropriate management. Research into the causes and control of this disease continues to provide the possibility of new cures. The therapy of diabetes will surely be altered dramatically over the next few decades.

Research continues on the islet cell transplantation techniques, including research that uses stem cells derived from pancreatic ducts. There has been laboratory success with transplantation of islet cells derived from stem cells in mice, but not yet in humans [8-10]

Looking further into the future, researchers are studying the use of gene therapies to correct the genetic defects that are the original cause of diabetes. As successful gene therapy will affect a true cure of the disease, it seems likely that researchers will continue to pursue its development, despite many hurdles. At this point, however, gene therapy for treatment of diabetes appears far away in the future [11-12].

Medicinal plants have been used for diabetes safely and with reasonable success. Despite the great strides that have been made in understanding and management of diabetes mellitus, serious complications continue to confront patients and physicians. The graph of diabetes related mortality is raising unabated. Therefore, search for new anti-diabetic drugs continue [13].

Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Emphasis is on managing short- term as well as long-term diabetes- related problems.

There is an important role for patient education, nutritional support, self-glucose monitoring, as well as long-term glycaemic control. A scrupulous control is needed to help reduce the risk of long-term complications. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications must be implemented to control blood pressure and cholesterol by exercising more, smoking cessation, and consuming an appropriate diet [14].

Conventional treatment of diabetes in allopathic medicinal system revolves around the use of following drugs.

a) Insulins: Rapid acting, short acting, Intermediate acting, longacting

b) Sulfonylureas First generation, Second generation

c) Biguanides

d) Meglitinide/Phenylalanine analogues

e) Thiazolidinediones

f) Glucosidase Inhibitors

g) Novel drugs in diabetes

a) Insulins

Secretion of insulin from beta cells of pancreas, which has an overall effect to favour storage of fuel. Insulin also facilitates glucose transport across cell membrane & glycogen synthesis from glucose in liver, muscle and fat by stimulating the enzyme glycogen synthetase. It inhibits gluconeogenesis from protein in liver and lipolysis in adipose tissue and favours triglyceride synthesis [15].

i) Highly Purified Insulin Preparations

Pork insulin, being more homologous to human insulin, is less immunogenic and is used. Gel filtration reduces proinsulin content to 50-200 ppm, but pancreatic peptides and insulin derivatives remain; the preparation is called "single pork insulin". It has significant immunogenicity. Further purification by ion-exchange chromatography removes most contaminants and reduces proinsulin to< 10 ppm. These preparations are termed ―Highly purified or Mono component (MC) insulins. Immunogenicity of pork MC insulins is similar to that of human insulins. Moreover, MC insulins are more stable, cause less insulin resistance or injection site lipodystrophy [17]

ii) Human insulins

In the 1980s, the human insulin (having the same amino acid sequence as human insulin) was produced by recombinant DNA technology in *Escherichia coli* – proinsulin recombinant bacterial (prb) and in yeast-precursor yeast recombinant (pyr) or by enzymatic modification of porcine insulin. Human insulin is more water soluble as well as hydrophobic than porcine or bovine insulin. It has a slightly more rapid s.c absorption, earlier and more defined peak and slightly shorter duration of action. [18].

b) Sulfonylureas

First generation Second generation

- Tolbutamide Glibenclamide
- Chlorpropamide (Glyburide)
- Glipizide
- Gliclazide
- Glimepiride

c) Biguanides

- Metformin

d)Meglitinde/Phenyl Alanine Analogues

- Repaglinide, Nateglinide

e) Thiazolidinediones

- Rosiglitazone, Pioglitazone

f) Glucosidase Inhibitors

- Acarbose, Miglitol

g) Miscellaneous

This is aimed at lowering hyper-triglyceridaemia and oxidative stress and biologists are hunting novel targets like: -

- Protein tyrosine phosphatase-IB(PTP-IB) and glycogensynthasekinase-3 (GSK-3)
- Inhibitors of glyconeogenesis like pyruvate dehydrogenase kinase (PDH), lipolysis
- Fat oxidation including carnitine palmitoyl transferase (CPT) I and II inhibitors
- β-3 adrenoreceptor agonists.

Mangroves have long been a source of astonishment for the layman and of interest for scientist. For many people living in the Indo-West Pacific and Americas-East Atlantic regions, the word mangrove will be a familiar one. The term mangrove is also used to designate halophytic (salt loving) and salt resistant marine tidal forests comprising of trees, shrubs, palms, epiphytes, ground ferns and grasses, which are associated in stands or groves. Mangroves are usually found only in tropical climates, as they need consistently warm conditions for development and survival[19-20].

Mangrove leaf fall contributes more than 70% of total litter production in many mangrove forests of the world. A substantial amount of the leaf fall is exported from man grove eco systems by tidal waters, either fresh or in various stages of decomposition. It is through the decomposition process that nutrients and other organic compounds such as lipids are released to estuarine waters and sediments via tidal transport. The lipids and nutrients are vital for the functioning of the estuarine communities e.g. crabs and fish[21].

Lavandula stoechas (Rhizophoraceae) is mostly widely distributed in the tropical and subtropical coastlines of China. According to a previous study, phenolics are important components in the leaf extract of *L. stoechas* and show excellent antioxidant activities. The hypocotyls of *L. stoechas* also have high phenolics levels. Therefore, the *L. stoechas* hypocotyls may be a good candidate for further development as an antioxidant remedy.

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect[22].

2. Materials and Methods

2.1 Collection identification and authentication of the selected plant

The season of plant material collection plays an important role in determining the quality of drug. Organoleptic characters, morphological characters, and microscopical examination would help in identifying crude drug. For identification of unknown drugs herbariums and leading botanical gardens are of great help. Generally, three methods are employed in the extraction of plant materials as (1) Maceration (2) Percolation (3) Soxhlet Extraction. Maceration and percolation may be employed in extraction of thermo labile constituents. Soxhlet extraction is rapid and continuous and may be employed in extraction of sparingly soluble constituents due to repeated extraction, which cannot be done by either maceration or percolation methods. The main advantage of extraction using a soxhlet apparatus is that it is an automatic, continuous and saves solvent by recycling it over the sample. Due to these various advantages offered by soxhlet extraction, this method was selected for present study.

The barks of *Lavandula stoechas* W. & A. were collected in the month of March from the local market of Bhopal (MP). These were identified and authenticated by Dr. S. N. Dwivedi (HOD) and voucher specimens were deposited in the herbarium of the Department of Pharmacy, RKDF College of Pharmacy, SRK University, Bhopal (M.P.). The fruits were washed, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

2.2 Traditional formulation of *Lavandula stoechas* (TFKR)

It was prepared by maceration using *Lavandula stoechas* bark (50mg), dried ginger (50mg) and rose water (qs to make 100 ml). The bark mixed with dried ginger or long peper & rose water, is said to be cure for diabeties.

2.3 Isolation of extract

The barks were washed, shade dried and pulverized into moderately coarse powder using hand grinder. Powdered barks were weighed and packed in soxhlet apparatus. The powdered bark was defatted with petroleum ether (40°- 60° C) for about 09 hrs.& complete defatting was censured by placing a drop from the thimble on a filter paper which did not exhibit any oily spot. The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted material was subjected to extraction by methanol and water as solvent. The process was carried out for about different timings for different solvents. The liquid extracts were collected in a tarred conical flask. The solvent was removed by distillation. Last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated.

2.4 Preliminary phytochemical investigations of extracts

Qualitative chemical tests of aqueous, ethanolic and petroleum ether extracts of conducted to identify the various phytoconstituents.

2.5 Animal and exposure conditions

Swiss albino mice weighing 30-35 gms of either sex were taken. The animals (rats and mice) were obtained from Bhopal, Madhya Pradesh. The animals were fed with rat feed and water ad libitum. They were housed in clean propylene cages, under identical conditions of food, water, temperature and degree of nursing care. Male and female animals were kept in separate cages. They were exposed to 12-hours, light-dark cycle and the relative humidity was in the range of 61-76% and temperature range was15-25ºC. All procedures were performed according to CPCSEA guidelines after proper approval from the Institutional Animal and Ethics Committee at, Faculty of Pharmacy, Bhopal, Madhya Pradesh. All the chemicals used were of the analytical grade obtained from standard companies and distilled water was used during all the experiments.

2.6 Pharmacological studies

Methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) were evaluated for antidiabetic activity in mice. Acute oral toxicity studies for 72 hours and

sub-acute toxicity studies for 14 days were also conducted on the plant extracts using albino mice. Plant extracts were suspended in 2%gum acacia and administered orally to rats and mice. In anti-diabetic studies, alloxan and streptozotocin were used to induce diabetes and were dissolved in normal saline. Glibenclamide was used as a standard antidiabetic drug and was given orally to rats. In all the experiments, an oral feeding needles no 21 was used to administer the doses of extracts, glibenclamide and the vehicle.

2.7 Toxicity Studies

Acute Toxicity Studies (72 hours)

Subacute Toxicity Study (14 days)

2.8 Anti-diabetic Studies

These studies were conducted using two models

Alloxan (10day study)

Streptozotocin (15-day study)

2.9 Acute Oral Toxicity Study (72 hours)

Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300, and 2000 mg/kg (exceptionally, an additional fixed dose of 5000 mg/kg may be considered). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death are described in detail in a separate OECD Guidance Document. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose, or when deaths occur at the lowest dose.

Methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) were evaluated for its acute oral toxicity in mice. The animals were divided into five groups of two mice each weighing about 20-25 g. The acute oral toxicity was conducted in three sets of experiments.

In the first experiment, methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) respectively were studied for their acute oral toxicity effects in mice. For this total twenty mice were taken and in each set of experiment, ten mice were divided into five groups of two mice each and the groups were as follows: -

Group I ----- Normal Control. (2% gum acacia)

Group II ----- KRM (500 mg / kg b.w)

Group III ---- KRM (1000 mg / kg b.w)

Group IV ---- KRM (1500 mg / kg b.w)

Group V ---- KRM (2000 mg / kg b.w)

The mice were acclimatized for a period of 7 days before the start of treatment. Methanolic extract of KRM in four dose levels was given orally in single doses to mice of Groups II, III, IV, V while Group I received only the vehicle.(2% gum acacia). The extracts were administered in 2% gum acacia. The animals were observed for mortality and general behavior periodically, for 48 hr. to 72 hr. The behavior of the animals was observed daily for 1 hr. in the forenoon (10 to 11.am) The animals were observed continuously for the initial 4 hr. and intermittently for the next six hr. and then again after 24, 48 and 72 hrs. following administration of different doses of KRM extract.

The same procedure was followed for carrying the acute oral toxicity study of aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) in which again ten mice were divided into five groups of two mice each.

Group I ----- Normal Control. (2% gum acacia)

Group II ----- KRA (500 mg / kg b.w)

Group III ---- KRA (1000 mg / kg b.w)

Group IV ---- KRA (1500 mg / kg b.w)

Group V ---- KRA (2000 mg / kg b.w)

2.10 Following parameters were observed during acute oral toxicity study

Grooming was considered in mice if the animal cleared the fur and skin of itself oranother animal *Hyper active* if there was any abnormal or excessive activity and the animal was unable to relax *Sedated* if the animal was calm and composed without any stress *Having respiratory arrest*, if there was raising of head *Having convulsions* if there was tremor in the tail or paddling of the feet *Motor activity*, increased or decreased, *Mortality*, if any.

2.11 Subacute Toxicity study (14 days)

The methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) were administered orally once daily to mice. Before initiation of experiment, the mice were acclimatized for a period of seven days. To study subacute toxicity, animals of either sex (20-25 g body weight) were divided into five groups of six mice each. The treatment was given as per the following protocol.

Group I- Normal Control (2% aqueous gum acacia)

Group II KRM (200 mg/kg b.w)

Group III KRM (400 mg/kg b.w)

Group IV KRA (200 mg/kg b.w)

Group V KRA (400 mg/kg b.w)

The treatment was continued for 14 days. During this period, mice of control group received only 2% gum acacia. After 14 days, animals were fasted overnight and blood was collected by cardiac puncture. The blood samples were taken for haemoglobin and white blood corpuscles estimation. The blood was allowed to clot for one hour and serum was separated by centrifuging and evaluated for different biochemical parameters. The results obtained were subjected to ANOVA followed by students t test, $p>0.05$was

considered as nonsignificant, p< 0.05 – significant, p< 0.01- highly significant and p<0.001 as very highly significant.

After taking the blood samples, the animals were sacrificed. Liver and kidney were excised from the animals, preserved in 10% formalin and sent for histopathological studies. The following biochemical parameters were evaluated in the subacute toxicity studies.

a) Serum Glucose Levels

b) Kidney Function Tests

i. Serum Urea Levels

ii. Serum Creatinine Levels

c) Liver Function Tests

i. Serum Bilirubin Levels

ii. Serum Glutamate Oxaloacetate Transaminase (SGOT)

iii. Serum Glutamate Pyruvate Transaminase (SGPT)

iv. Serum Total Proteins

v. Serum Albumin

vi. Serum Alkaline Phosphatase

d) Blood Function Tests

i. Hemoglobin Value

ii. WBC Count

2.12 Antidiabetic Study

Study of Methanolic extracts of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) against alloxan induced diabetes (10 days study)

In this study, methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) were evaluated for anti-diabetic activity against alloxan induced diabetes mellitus in rats. Rats were divided into 8 groups consisting of 6 rats in each group. The rats were acclimatized for a period of 7 days before starting the experiment. After overnight fasting, hyperglycaemia was induced by administering a single dose of alloxan monohydrate supplied by S.D Fine-Chemical Ltd. Mumbai, India (120 mg/kg b.w) prepared in sterile saline to all the groups except group I which served as normal control. During this period, the animals were given free access to water. After 5 days of alloxan administration, fasting blood glucose levels of rats were checked by glucose trips. The animals having blood glucose levels > 250 mg/dl were separated and selected for further studies and then re-grouping of these hyperglycemic rats was done as per the following protocol, for studying the anti-diabetic activity of different extracts.

Group I- Normal Control (2% of gum acacia.)

Group II- Diabetic Control (Alloxan monohydrate and 2% gum acacia)

Group III- Alloxan monohydrate + Glibenclamide (10 mg/kg,).

Group IV- Alloxan monohydrate + TFKR (10 ml/kg b.w)

Group V- Alloxan monohydrate + KRM (100 mg/kg b.w)

Group VI Alloxan monohydrate + KRM (200mg/kg b.w)

Group VII Alloxan monohydrate + KRA (100 mg/kg b.w)

Group VIII Alloxan monohydrate + KRA (200 mg/kg b.w)

The treatment was started from the same day except normal control and diabetic control groups for a period of 10 days orally. During this period, animals in all groups had free access to standard diet and water. Blood glucose levels were estimated on 1st,4th, 7th and 10th day of the treatment. Besides this during this study the body weight of the rats were recorded on 1st, 4th, 7th and 10th day of the treatment. On the 11thday, blood samples were collected from overnight fasted rats by cardiac puncture. The animals were anaethesized by mild ether anaesthesia before cardiac puncture. Blood was collected and allowed to stand for one hour, serum was separated bycentrifuging and evaluated for different biochemical parameters.

Study of Methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) against Streptozotocin (STZ) induced diabetes (15 days study)

In this study, methanolic extract of barks of *Lavandula stoechas* W. &A. (KRM) and aqueous extract of *Lavandula stoechas* W. &A. barks (KRA) were evaluated for anti-diabetic activity against streptozotocin induced diabetes mellitus in rats. Rats were divided into 8 groups consisting of six rats each. The rats were acclimatized for a period of 7 days before starting the experiment. After an overnight fasting, hyperglycaemia was induced by administering a single dose of streptozotocin (50 mg/kg b.w) to all rats excepting group I which served as normal control. Streptozotocin was freshly dissolved in 0.1 M citrate buffer (pH=4.5) and injected intraperitoneally within 15min of dissolution in a vehicle volume of 0.4 mL with 1 mL of tuberculin syringe fitted with 24-gauge needle. Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. During this period, the animals were given free access to water. After 3rd day of STZ administration, fasting blood glucose levels of rats were checked by glucose trips. The animals having blood glucose levels > 250 mg/dl were separated and selected for further studies and then re-grouping of these hyperglycemic rats was done as per the following protocol, for studying the anti-diabetic activity of different extracts. Rats were given the following treatment in this study.

Group I Normal Control (2% of gum acacia).

Group II Diabetic Control. Received STZ (50 mg/kg b.w single dose i.p.)

Group III STZ + Glibenclamide (3 mg/kg,)

Group IV STZ + + TFKR (10 ml/kg b.w)

3. Results and Discussion

Diabetes mellitus is a metabolic disorder characterized by resistance in the action of insulin, insufficient insulin secretion or both. It is becoming one of the most common diseases of the world. Type II diabetes in young has increased 30-fold over the last 20 years concomitant with increase in obesity. Studies have revealed that all incidences of diabetes in this young age group is 2.5% and alarmingly 25% of their young adults have abnormalities of blood glucose [23].

In *Diabetes mellitus*, besides hyperglycemia, cardiovascular disease (CVD) is a major cause of death in the world and is mainly due to atherosclerosis (hardening of the arteries). Abnormal blood lipids are risk factors for CVD. So the prevention of cardiovascular disease in diabetic patients is necessary. It has also been seen that the liver function tests which include serum amino transferases i.e. ALT, AST, Alkaline phosphatases (AP) and Bilirubin are raised in diabetes. Prevention of liver injury in type-II diabetes due to insulin resistance is necessary. The treatment for diabetes mellitus would be a drug that not only controls the glycemic level but also prevents the development of atherosclerosis and other complications of diabetics. New drugs and new drug delivery systems for insulin have also been introduced [24].

The Indian indigenous drugs have great importance both from professional and economic point of view. A large number of plants have been reported to possess anti-diabetic activity e.g., *Aconitum napeilus, Aloe vera, Carumcarvi, Cichorium intybus, Allium cepa, Aralia cachemirica, Allium sativum, Momordica charantia,etc*.

In the present study the bark of *Lavandula stoechas* were evaluated for their toxicity studies and for anti-diabetic activity as they have been reported to have antidiabetic activity in the traditional system of medicine.

Different model systems like alloxan, streptozotocin viruses, and insulin antibodies, hormones like dexamethasone, adrenaline and dithizone are available to screen the anti-diabetic activity of a given substance in the present study chemicals like alloxan and streptozotocin were used to produce marked diabetic effects in animals. Alloxan diabetic model resembles type I diabetes (IDDM) without significant insulin resistance whereas streptozotocin induced diabetic animals inhibit reduced response to insulin in hepatic and peripheral tissues. Further rats treated with streptozotocin display many of the features seen in human with uncontrolled diabetes mellitus.

Table.1Physical Characteristics and Percentage Yield of Methanolic Extract of *Lavandula stoechas*

Extract	Colour	Odour	% Extractive Value
Methanolic	Darkbrown	Characteristic	8.3%

Table.2 Preliminary Phytochemical Screening of Methanolic and Aqueous Extract of *Lavandula stoechas*

S. No.	Phytoconstituents	Methanolic Extract	Aqueous Extract
1	Alkaloids	++	-
2	Saponins	+	++
3	Steroids	+	-
4	Phenoliccompounds	+	+
5	Tannins	+++	++
6	Flavonoids	+	++
7	Terpenoids	++	+
8	Glycosides	+	-
9	Protein&Aminoacids	+	+
10	Carbohydrate	+	+

+++Highly Present, ++ moderately Present, +faintly present, -absent

Table No. 3. Effect of Extracts of *Lavandula stoechas* on oral glucose tolerance test in non-diabetic rats

Group	Treatment	Blood Glucose Levels (mg/dl)				
		Day1	Day 7	Day 14	Day 21	Day28
Group-I	Control 1 % gum acacia	79.88±5.5	81.47± 1.7	74.85± 2.2	79.84±4.3	80.11±8.8
Group-II	Diabetic Control 1 % gum acacia	288.5*±4.7	302.5±6.6*	300.1±8.9*	307.5±5.5 *	298.7±3.6*
Group-III	Glibenclamide 5mg/kg	270.1± 3.23#	218.55± 9.9#	184.5± 4.4#	158.5±0.2 #	131.1 ±1.5#
Group-IV	KRM (100mg/kg) + CCl_4 (3mg/kg)	281.64± 4.4	264.85± 6.7	192.42±5.5	170.45±2.1	145.77 ±2.4
Group-V	KRM (200mg/kg) +CCl_4(3mg/kg)	290.58±1.88**	223.5±3.8**	177.8±4.9**	166.58±0.44**	131.44 ±3.8
Group-VI	KRM (400mg/kg+ CCl_4 (3mg/kg)	284.8± 5.5**	209.7± 4.7**	174.5±4.6**	167.88± 4.4**	129.92 ±2.2
Group-VII	KRM (100mg/kg) +CCl_4 (3mg/kg)	282.46± 4.4	263.45± 6.7	193.48±5.5	171.23±2.1	146.02 ±2.4
Group-VIII	KRM (200mg/kg) +CCl_4 (3mg/kg)	291.85±1.88**	224.7±3.8**	176.4±4.9**	167.14±0.44**	132.47 ±3.8
Group-IX	KRM (400mg/kg+ CCl_4(3mg/kg)	283.6± 5.5**	206.8± 4.7**	175.21±4.6**	167.89± 4.4**	128.15 ±2.2

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