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GC-MS analysis, preliminary phyto-chemical screening, physico-chemical analysis and anti-diabetic activity of ethanol extract

GC-MS analysis, preliminary phytochemical screening, physico-chemical analysis and anti-diabetic activity of ethanol extract of *Jasminum cuspidatum* leaves

Singumsetty Vinay, Shaik Karimulla and Devarajan Saravanan

Ratnam Institute of Pharmacy, Pidathapur, Nellore, Andhra Pradesh 524 346, India.

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Abstract

The purpose of the present study was investigating the GC-MS analysis, preliminary phytochemical screening, physicochemical analysis and anti-diabetic activity of ethanol extract of the leaves of *Jasminum cuspidatum*. The anti-diabetic activity was investigated in streptozotocin-induced diabetes rats with treatment of ethanol extract at the dose level of 200 and 400 mg/kg, which was compared with glibenclamide at a dose level of 4 mg/kg and the parameter measured being the blood glucose, total cholesterol, HDL, LDL, VLDL, triglycerides, and total protein. An oral glucose tolerance test (OGTT) was also investigated in experimental rats. The GC-MS analysis revealed that the ethanol extract contained seven phytoconstituents. Preliminary phytochemical screening showed the presence of triterpenoids, flavonoids, glycosides and steroids. The result of *in vivo* anti-diabetic activity revealed that the ethanol extract of *J. cuspidatum* showed significant anti-diabetic activity.

Introduction

Jasminum cuspidatum is belonging to the family of the Oleaceae. It's normally called Urumikol in Malayalam, and Oosi Malli in Tamil. It's widely distributed in Kerala, Karnataka. Tamil Nadu in all districts (Wealth of India, 2003). It's rigid, dense shrub, stem hardly subsucculent, glabrous. Leaves are opposite, simple, 4-6 cm long and 2-3 cm broad, lanceolate, ovate, acute, shining green, coriaceous, with reticulating nerves. Nerves from the base and from midrib, distinct below. Petiole is short, and 2-3 mm long. Flowers fragrant, in few-flowered dense, sessile cymes. The calyx teeth linear, rigid erect, 4-6 mm long. Corolla white tube, corolla tube 1.5-2.5 cm long. Lobes are 1.5-1.7 cm long, oblong, acute, shorter than the tube. Berry is 8 x 7 mm, ovoid, glabrous (Hentry et al., 1987).

J. cuspidatum is widely cultivated in gardens and simply found in forests throughout tropical Asia and heat temperate regions in Europe and Africa. Flowers and

leaves of genus *J. cuspidatum* are well recognized for utile uses. For example, the flowers are utilized as ancient medicines in Asia to treat several diseases as well as breast cancer, diarrhea, toothache, conjunctivitis, fever, asthma, abscess, abdominal pain, dermatitis, and uterine bleeding. In China, the leaf parts are used for the treatment of dysentery, quadriplegia gall, and bellyache (Kunhachan et al., 2012; Gunasekaran et al., 2012). Therefore, the current study was aimed to investigate the GC-MS analysis, preliminary phytochemical screening, physicochemical analysis and anti-diabetic activity of ethanol extract of the leaves of *J. cuspidatum*.

Materials and Methods

Collection and authentication of plant material: *J. cuspidatum* plant leaves were collected from Talakona forest near to Tirupati, Andhra Pradesh, India in the month of January and were authenticated by Dr. P.



Jayaraman, Director, Institute of Herbal Botany, West Tambaram, Chennai, and Tamil Nadu. A voucher specimen (JAS) has been kept in the Herbarium of the Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India.

Drugs and chemicals: Streptozotocin (STZ) was purchased from sigma Aldrich Chemicals, Germany. Glucose, Citric acid and sodium citrate were purchased from M/S Hi media Ltd, Bombay. Glibenclamide was obtained as gift samples from Accent Pharma, India. All other chemicals and reagents used in the study were of analytical grade.

Extraction procedure: The collected plant leaves were washed with tap water and dried under room temperature for 3 days. Approximately about 200 g of leaves were pulverized to a powder in a mechanical grinder. The dry powder of the leaves of *J. cuspidatum* (100 g) was extracted with ethanol (500 mL, 46 hours) at a temperature between 60-65°C by using a soxhlet extractor. The extract was filtered in hot condition and concentrated in vacuum under reduced pressure and dried in desiccators.

GC-MS analysis: The GC-MS analysis of ethanol extract was investigated by Perkin Elmer GC-MS (Model Perkin Elmer Clarus 600, USA) equipped with a VF-5 MS fused silica capillary column (30 m x 0.25 mm i.d, film thickness 0.25 µm). Pure helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Mass transfer line and injector temperatures were set at 250°C. The oven temperature was programmed at 60°C for 2 min, then increased to 300°C for 6.0 min at the rate of 10°C/min. The samples were injected in split mode as 10:1 (Saravanan et al., 2013).

Identification of phytochemicals: Interpretation of Mass-Spectrum GC-MS was carried out with reference to the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

Physicochemical and phytochemical analysis: Physicochemical values such as percentage of ash value and extractive value were determined according to the well established method and procedure (Indian Pharmacopoeia, 1996; WHO, 1998). Preliminary phytochemical screening was carried out by using standard procedure (Kokate, 1986; Harborn, 1998).

Animals: Swiss albino rats of either sex weighing 150-250 g were acclimatized to the experimental room at temperature 23 ± 2°C, controlled humidity conditions (50-55%) and 12 hours light and dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. The study conducted was approved by the

Institutional Animal Ethical Committee of Ratnam Institute of Pharmacy (Reg. No. 1558/PO/a/11/CPCSEA), Pidathapolur, Andhra Pradesh, according to prescribed guidelines of CPCSEA, Government of India.

Acute toxicity studies: Acute toxicity study of ethanol extract of *J. cuspidatum* was carried out in Swiss albino rats of either sex (150-250 g) according to the OECD (Organization for Economic Cooperation and Development) guidelines No. 423. Extract at different doses up to 2,000 mg/kg p.o. was administered and the animals were observed for behavioral changes, toxicity, and mortality up to 48 hours (OECD guidelines, 2000).

Experimental models

Oral glucose tolerance test: Fasted rats were divided into four groups comprising of six rats in each group. Group I served as normal control and received citrate buffer. Group II received standard drug glibenclamide (4 mg/kg). Group III and IV received an ethanol extract at doses of 200 and 400 mg/kg respectively. After 30 min of extract and standard drug administration, the rats of all groups were orally treated with glucose solution (2 g/kg). Blood samples were collected from the rat's tail vein just prior to glucose administration and in 30, 60, 90, and 120 min after glucose administration (Sellamuthu et al., 2009) and blood glucose level was measured immediately by using auto analyzer (Transasia Erba-ERBA CHEM-7).

Experimental induction of diabetes: The animals were fasted for 12 hours prior to the induction of diabetes. The rats were injected intraperitoneally with streptozotocin dissolved in ice cold citrate buffer (pH 4.3) at a dose of 60 mg/kg body weight. 5% glucose solution was administered orally for 24 hours to prevent mortality due to initial hypoglycemia induced by streptozotocin. After 72 hours of streptozotocin injection, fasting blood glucose level was tested using auto analyzer. Rats showing fasting blood glucose more than 200 mg/dL were considered diabetic and used for further study.

Experimental design: The rats were randomized into five groups (I-V) comprising of six animals in each group after the induction of streptozotocin diabetes. Group I served as a normal control and rats were received citrate buffer. Group II served as a diabetic control, and rats were received a citrate buffer. Group III, received a glibenclamide (4 mg/kg/day, p.o.). Group IV, received an ethanol extract (200 mg/kg/day, p.o.). Group V, received an ethanol extract (400 mg/kg/day, p.o.). After administration, the blood sample was collected from the retro-orbital plexus of each rat under mild anesthesia on 0, 1, 10 and 15th day and serum glucose was estimated by auto analyzer. On the 15th day of the study, blood was collected for biochemical estimations such as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and total

protein by auto analyze-ERBA CHEM-7 (Shete et al., 2013).

Histochemical study: At the end of pharmacological activity, the animals were sacrificed under anesthesia and pancreas was removed, cleaned, washed with ice-cold normal saline, and fixed overnight in 10% formalin solution. Pancreas sections were made by microtome, dehydrated in graduated ethanol (50-100%), cleared in xylene and embedded in paraffin. The pancreas sections (4-5 μm) were stained with hematoxylin and eosin dye and examined with a photomicroscope (Bancroft et al., 2002).

Statistical analysis: The data are expressed as the mean \pm SEM. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's test for the multiple comparisons using prism Graphpad version 5.0. The p values less than 0.001 were considered statistically significant.

Results

The GC-MS analysis of phytoconstituents in the ethanol extract of the leaves of *J. cuspidatum* revealed the presence of seven phytoconstituents (Figure 1). The identification of the phytoconstituents in the ethanol extract by GC-MS was confirmed based on the peak area, retention time, and molecular formula. The major

phytoconstituents in ethanol extract as n-hexadecanoic acid (27.4%), ethyl-14-methyl hexadecanoate (3.3%), phytol (2.9), (R)-(-)-14-methyl-8-hexadecyn-1-ol (18.9%), octadecanoic acid (8.3%), 2,6,10,14,18,22-tetracosahexaene-2,6,10,15,19,23-hexamethyl-(All E) (36.0%), and 2H-1-benzopyran-6-ol-3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl) (3.2%).

Preliminary phytochemical screening of ethanol extract mainly revealed the presence of triterpenoids, flavonoids, glycosides, sterols, steroids, phenols, carbohydrates, and saponins.

Physicochemical analysis of leaf powder viz. ash value and extractive value are presented in Table I. The moisture content was 8.6%w/w. The total ash, water soluble ash, acid insoluble ash, and sulfated ash values were found to be 9.1%w/w, 10.2%w/w, 3.1%w/w, and 11.1%w/w respectively on the basis of dry weight, whereas extractive values such as petroleum soluble, chloroform soluble, acetone soluble, ethanol soluble, and ethyl acetate soluble were found to be 3.1%w/w, 3.8%w/w, 11.8%w/w, 18.1%w/w, and 10.1%w/w respectively.

It indicates that the extract was found to be safe at the dose of 2,000 mg/kg. Hence, 1/10th and 1/5th of doses were selected for the present anti-diabetic activity (Mundugaru et al., 2014).

Thirty minutes after glucose administration, the peak

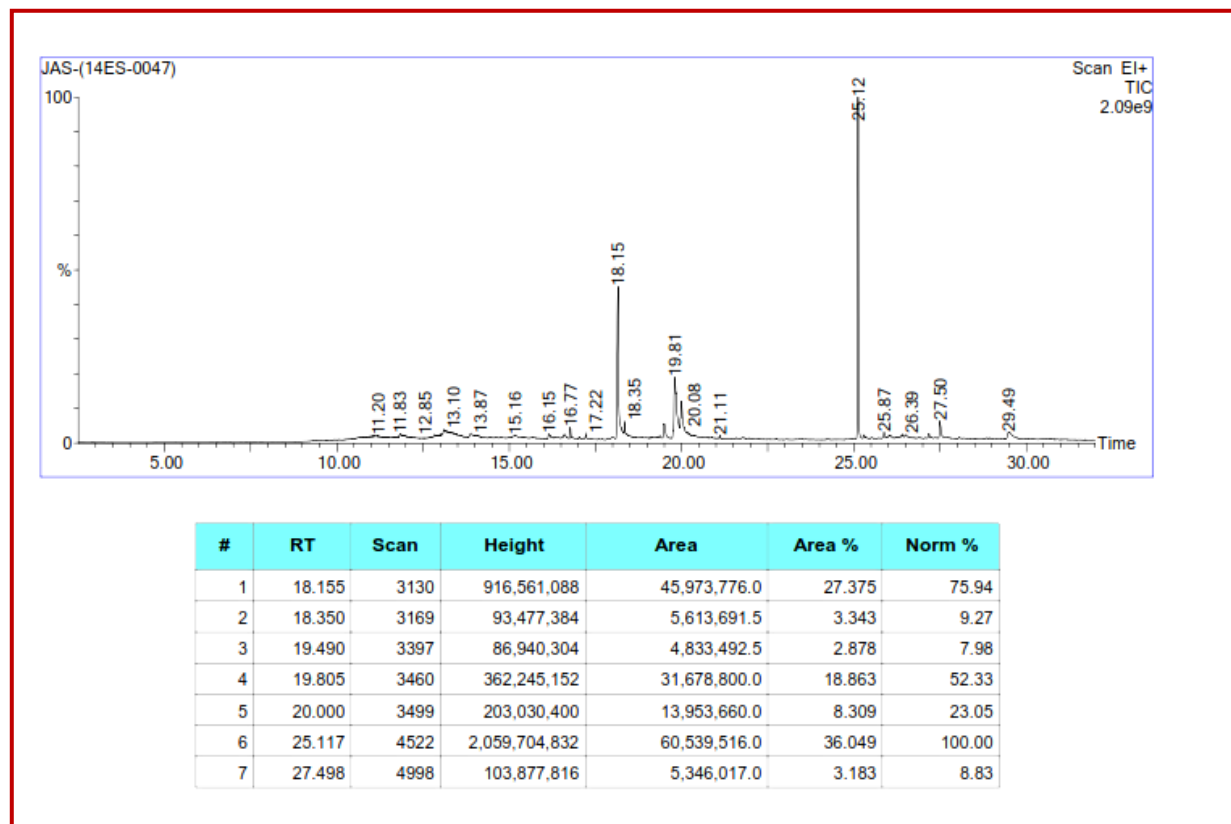


Figure 1: GC-MS spectrum of ethanol extract of leaves of *J. cuspidatum*

Table I		
Physicochemical parameters of powdered leaf of <i>J. cuspidatum</i>		
SL. No	Parameters	Values (%)
1	Moisture content	8.6 ± 0.1
2	Total ash	9.1 ± 0.0
3	Water soluble ash	10.2 ± 0.1
4	Acid insoluble ash	3.1 ± 0.0
5	Sulphated ash	11.1 ± 0.0
6	Petroleum ether soluble extractive	3.1 ± 0.0
7	Chloroform soluble extractive	3.8 ± 0.0
8	Acetone soluble extractive	11.8 ± 0.0
9	Ethanol soluble extractive	18.1 ± 0.1
10	Ethylacetate soluble extractive	10.1 ± 0.1

blood glucose level was increased rapidly from the fasting value and then subsequently decreased at 60 min (Figure 2). The ethanol extract of the leaves of *J. cuspidatum* exhibited remarkable blood glucose lowering effect at 60 min.

Streptozotocin induced diabetic rats were treated with ethanol extract of the leaves of *J. cuspidatum* at oral doses of 200 and 400 mg/kg of body weight for 15 days. Treatment with ethanol extract at a dose of 200 mg/kg showed the significant ($p < 0.001$) decreased blood glucose level after 10th day onwards and treatment with ethanol extract at a dose of 400 mg/kg showed the significant ($p < 0.001$) decreased blood glucose level after 5th day onwards compared to diabetic control. Treatment with glibenclamide 4 mg/kg showed the significant ($p < 0.001$) decreased blood glucose level after

5th day onwards and thereafter (Table II). Ethanol extract at a dose level of 200 mg/kg showed the less significant improvement in the body weight on the 5th day of the treatment and showed the significant improvement of body weight on the 10th day ($p < 0.001$) onwards. An oral dose of ethanol extract 400 mg/kg shows significant improvement in the body weight of streptozotocin induced diabetic rats on the 5th day of treatment ($p < 0.001$) onwards. Glibenclamide (4 mg/kg) produced significant improvement in body weight of streptozotocin induced diabetic rats (Figure 3).

Furthermore, lipid profile have been estimated and compared to untreated, treated, and control groups. The TC, LDL, VLDL, and TG were significantly ($p < 0.001$) increased in rats treated with streptozotocin (60 mg/kg) and HDL cholesterol, total protein levels were significantly ($p < 0.001$) decreased in rats treated with streptozotocin (60 mg/kg). The total cholesterol, LDL cholesterol, VLDL cholesterol, and triglyceride levels were significantly decreased in rats treated with ethanol extract at a dose of 200 and 400 mg/kg and glibenclamide at a dose of 4 mg/kg, when compared to diabetic control. The HDL cholesterol and total protein content levels were significantly increased in rats treated with ethanol extract at a dose of 200 and 400 mg/kg and glibenclamide at a dose of 4 mg/kg, when compared to diabetic control (Table III).

Histology of the pancreas sections of the normal control rats showed the normal pancreatic beta cell (Figure 4A-E). The pancreas sections of streptozotocin (60 mg/kg) treated rats showed the complete damage of pancreatic beta cell due to the induction of streptozotocin when

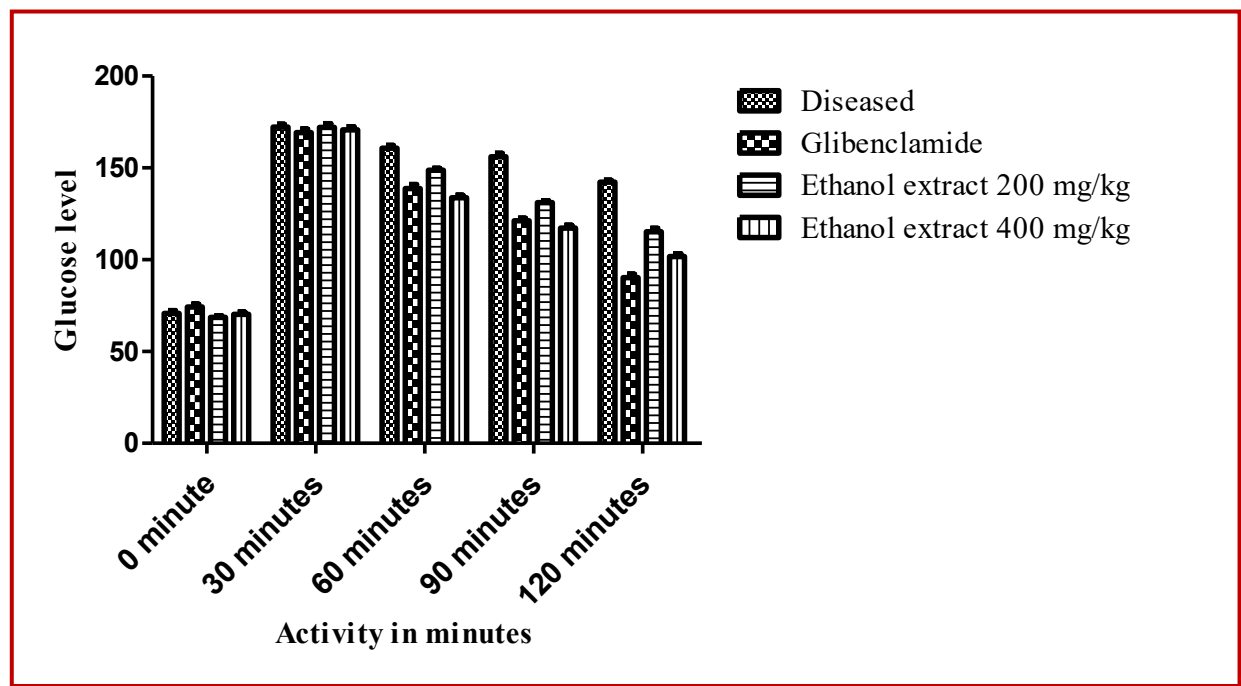


Figure 2: Effect of ethanol extract on blood glucose level in oral glucose tolerance test in normal rats

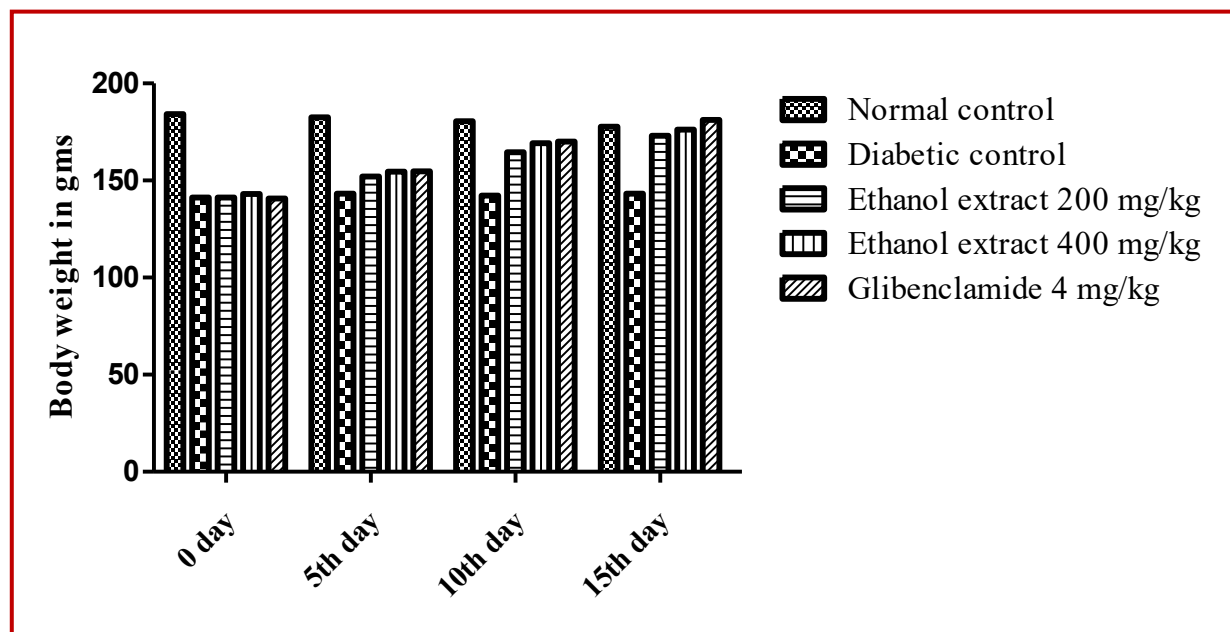


Figure 3: Effect of ethanol extract of leaves of *J. cuspidatum* on body weight in streptozotocin-induced diabetes in rats

Table II

Effect of ethanol extract of leaves of *J. cuspidatum* on glucose level in streptozotocin-induced diabetic rats

Groups	Blood glucose level (mg/dL)			
	0 day	5 th day	10 th day	15 th day
Normal control	77.0 ± 1.7	77.7 ± 1.8	78.8 ± 1.4	79.5 ± 1.6
Diabetic control	299.5 ± 3.6 ^a	303.3 ± 3.0 ^a	304.5 ± 1.9 ^a	313.3 ± 2.2 ^a
Glibenclamide (4 mg/kg)	302.0 ± 3.0	244.8 ± 3.9 ^b	185.3 ± 3.9 ^b	117.5 ± 2.0 ^b
Ethanol extract (200 mg/kg)	300.2 ± 2.8	226.8 ± 2.6 ^b	239.2 ± 3.2 ^b	206.8 ± 2.7 ^b
Ethanol extract (400 mg/kg)	305.7 ± 2.6	260.7 ± 2.1 ^b	215.3 ± 2.7 ^b	173.7 ± 2.2 ^b

All values are expressed as mean ± SEM for six animals; ^ap<0.001 compared to normal control; ^bp<0.001 compared to diabetic control

Table III

Effects of ethanol extract on lipid profile in streptozotocin-induced diabetic rats

Groups	Lipid profile (mg/dL)					
	TC	HDL	LDL	VLDL	TG	Total protein
Normal control	117.5 ± 3.8	42.1 ± 2.9	49.0 ± 4.6	29.5 ± 0.6	143.4 ± 3.5	6.5 ± 0.2
Diabetic control	168.4 ± 3.0 ^a	28.6 ± 1.7 ^a	103.2 ± 5.0 ^a	38.5 ± 1.0 ^a	186.4 ± 3.5 ^a	3.6 ± 0.1 ^a
Glibenclamide (4 mg/kg)	120.4 ± 3.1 ^b	38.2 ± 2.5 ^b	56.5 ± 4.6 ^b	31.4 ± 0.9 ^b	152.9 ± 3.0 ^b	6.0 ± 0.3 ^b
Ethanol extract (200 mg/kg)	126.1 ± 3.8 ^b	35.4 ± 3.1 ^b	84.8 ± 3.2 ^b	34.9 ± 0.7 ^b	169.2 ± 3.4 ^b	5.1 ± 0.5 ^b
Ethanol extract (400 mg/kg)	140.8 ± 4.4 ^b	38.6 ± 2.5 ^b	56.5 ± 3.1 ^b	33.6 ± 0.7 ^b	151.9 ± 3.2 ^b	5.3 ± 0.7 ^b

All values are expressed as mean ± SEM for six animals; ^ap<0.001 compared to normal control; ^bp <0.001 compared to diabetic control

compared to normal control rats. The pancreatic sections of ethanol extract treated rats showed an increased the pancreatic beta cell count and remodeling of the structure of the pancreas when compared to glibenclamide-treated and control group's rats. The histopa-

thological changes are exhibit-ted in Figure 4A-E. The ethanol extract of the leaves of *J. cuspidatum* at both doses (200 and 400 mg/kg) restored the damaged beta cell morphology to normal, when compared to normal control group rats and glibenclamide treated rats.

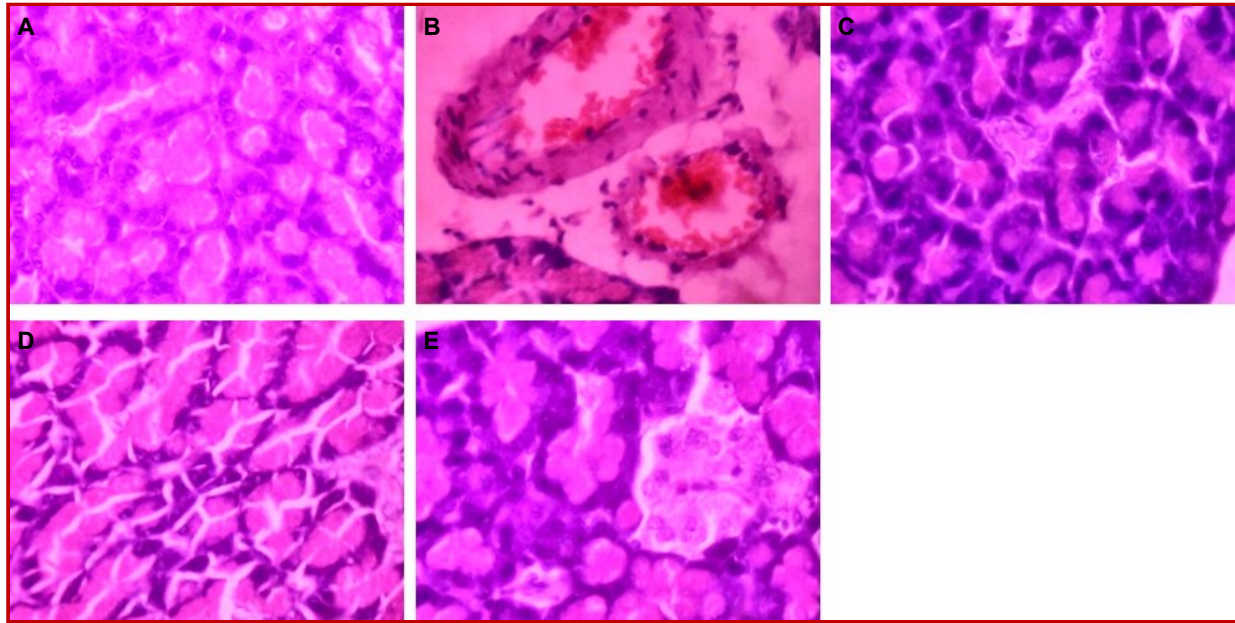


Figure 4: Histopathological pictures of pancreas for different animal groups. Normal control (A); Diabetic control (B); Glibenclamide treated (C); Ethanol extract (200 mg/kg) treated (D); Ethanol extract (400 mg/kg) treated (E)

Discussion

Gas chromatography-mass spectroscopy (GC-MS) analysis of plant extracts is becoming a very valuable tool to detect the presence of various phytoconstituents before aiming large scale purification and to minimize replicative phytochemical studies. The GC-MS analyses of ethanol extract majorly contain sterol and fatty acid esters. These phytoconstituents are responsible for various pharmacological actions like antibacterial, anti-inflammatory, anthelmintic activities for sterol and anti-arthritis, pesticide, antitumor, cancer preventive, anti-histaminic, hepatoprotective, hypocholesterolemic and anti-inflammatory activities for fatty acid ester (Sellamuthu et al., 2009).

Physicochemical analysis of the drug is important parameters in detecting adulteration. The leaf powder contains 8.6% moisture; hence less possibility for growth of bacteria and fungi. Ash values are used to find out the quality, and purity of crude drugs. It indicates the presence of various impurities such as oxalate, carbonate, and silicate. The water soluble ash is used to estimate the amount of inorganic compounds present in the crude drug. Acid insoluble ash is used to calculate the quantity of silica present in the crude drug. The acid insoluble ash consists of silica, and indicates the contamination with earthy material. Water soluble ash is the water soluble portion of the total ash. The fewer amounts of ash values indicate that less amount of inorganic matter and silica present in the *J. cuspidatum*. The extractive values are useful to evaluate the type of phytoconstituents present in the crude drug and also help in determination of specific phytoconstituents soluble in particular solvents. The maximum

extractive value was found in ethanol solvent (18.1%) and minimum extractive value found in petroleum ether (3.1%) (Rakholiya et al., 2012).

Diabetes mellitus is a chronic disorders caused by insulin deficiency. The insulin deficiency produces an inadequate glucose control and leads to diabetes mellitus. Diabetes mellitus is a metabolic disorder of carbohydrate, protein, and fat metabolism. It's not a single disorder, but diabetes mellitus is a group of metabolic disorder characterized by chronic hyperglycemia, resulting from defects in insulin secretion and insulin action (Ikewuchi et al., 2011). The screening of anti-diabetic activity of medicinal plant extract is performed in experimental animal models after induction of diabetes by several methods. To induce diabetes in animals, streptozotocin is commonly used diabetic agent, which produces moderate diabetes (Ramachandran et al., 2013). Streptozotocin is a selective beta cytotoxin, induces chemical diabetes in many animal species by damaging the insulin secreting beta cells of the Islets of Langerhans of the pancreas, and the animals became permanently diabetic. It is well known that the anti-diabetic drug, glibenclamide directly act beta cells of the pancreas, and stimulating the beta cells of the Islets of Langerhans to release more insulin (Selvaraj et al., 2014).

In the present study, induction of diabetes showed significant increased blood glucose, TC, TG, LDL, VLDL, and significant decreased total protein, HDL, and body weight compared to normal control rats which confirm the induction of diabetes. It may be due to damage the insulin secreting beta cells of the Islets of Langerhans of the pancreas by streptozotocin. The

treatment with ethanol extract at doses of 200 and 400 mg/kg in streptozotocin-induced diabetic rats produced a significant decrease in blood glucose level, TC, TG, LDL, VLDL and significant increase in HDL, total protein, and body weight when compared to glibenclamide treated rats and diabetic control rats. It may be due to ethanol extract may increase the secretion of insulin from damaged beta cells of the pancreas; this increased secretion of insulin stimulates fatty acid biosynthesis and also the incorporation of fatty acids into TG in the liver and adipose tissue.

Histopathological findings of the pancreas showed that administration streptozotocin leads to damage of normal beta cells of the pancreas that were restored with glibenclamide. Similar effects were observed with ethanol extract showing improvement of beta cell in damaged pancreas.

The phytochemical screening of *J. cuspidatum* revealed the presence of triterpenoids, flavonoids, glycosides, sterols, steroids, phenols, carbohydrates, and saponins compounds. Many authors reported the sterols, flavonoids, polyphenols, and alkaloids as bioactive anti-diabetic principles. Hence, the anti-diabetic activity of the ethanol extract is probably due to the presence of various bioactive anti-diabetic principles and their synergistic properties (Sikarwar et al., 2010).

An ethanol extract of leaves of *J. cuspidatum* at two doses 200 and 400 mg/kg possess the anti-diabetic activity on streptozotocin-induced diabetic in rats when compared to diabetic control rats.

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Author Info

Devarajan Saravanan (Principal contact)
e-mail: devasaro@yahoo.co.in