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Research Article

Antihyperglycemic Efficacy of *Pongamia pinnata* (L.) Pierre Against Alloxan Induced Diabetic Rats and its Correlation with Phytochemical Screening

¹S. Morajkar Abhay, ²B. Sharma Bharat and ³R. Kharat Kiran

¹Department of Zoology, VPM's B. N. Bandodkar College, University of Mumbai, India

²Department of Zoology, KET's V. G. Vaze College, University of Mumbai, India

³Department of Biotechnology, Deogiri Biotechnology Research Laboratory, Aurangabad, India

Abstract

Background and Objective: *Pongamia pinnata* (L.) and its various parts have been used as a traditional medicine in the treatment and prevention of several kinds of ailments in many countries such as for treatment of piles, skin diseases, wounds healing and antidiabetic. This study was aimed at evaluating *in vivo* effects of *P. pinnata* on glycemic markers, the structural integrity of the pancreas and phytochemicals responsible for hypoglycemic effect and pathophysiology of the pancreas. **Materials and Methods:** Experimental induction of diabetes was carried out by alloxan monohydrate followed by oral administration of *P. pinnata* extracts. Blood was collected from the retro-orbital plexus to assess the changes in Blood Sugar Fasting (BSF), Glycosylated haemoglobin (HbA1c) and Mean Blood Glucose (MBG) levels. In the end, pancreas were prepared for histopathological studies. Chemical fingerprinting and LC-MS characterization was carried out to know the compound responsible for glycemic index reversal and pancreatic altered histopathology. **Results:** Treatment with alcoholic extract of *P. pinnata* produces a significant reduction in levels from 339.81-99.41 mg dL⁻¹ in BSF, 13.63-8.4% in HbA1c and 269.97-125.67 mg dL⁻¹ in MBG. *Pongamia pinnata* enhanced the histo-architectural changes in the exocrine and endocrine part of the pancreas but was distinct with PPAlcExt extract. Photochemistry reveals 43 common compounds from both the extract belongs to a different class of phytochemicals. **Conclusion:** The result accentuates that bioactive phytochemicals derived from plants can be an alternative source of natural drugs that can be used to treat diabetes.

Key words: BSF, HbA1c, pancreas histopathology, chemical fingerprint, LC-MS

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Corresponding Author: S. Morajkar Abhay, Department of Zoology, VPM's B. N. Bandodkar College, University of Mumbai, India

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a clinical condition characterized by hyperglycemia in which a raised amount of glucose circulates in the blood. It is a serious, metabolic disorder long-term condition with a major impact on lives worldwide¹. Hyperglycemia is the main pathogenic factor underlying the development of secondary complications of diabetes. The use of plants, their medicinal functions have developed various synthetic drugs like metformin, from guanidine and galegine of *Galega officinalis* (French lilac) which were Potent hypoglycemic compounds. In the early stage of DM, inflammatory cytokine load penetrate pancreatic cells leads to infiltration. These cytokines produce excess Nitric Oxide (NO) in the islet cells eventually inhibit mitochondrial metabolism, modification of mRNA and DNA leading to the demise of β -cell and impaired insulin secretion². Alloxan is a known diabetogenic agent that exerts its toxic effect by generating reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells³. Renewed interest in phototherapy in diabetes is identifying a large number of bioactive plant constituents with wide-ranging effects on animal and human glucose and lipid metabolism⁴. The polyphenols derived from natural sources are sources that improve insulin secretion and lower the other glycemic indices in humans, as well as in animal models. These indicate their roles in glucose homeostasis, mediated by their effects on several organs like the pancreas, liver, intestine and insulin-sensitive peripheral spaces⁵.

Indian traditional medicinal systems like Ayurveda, Siddha and Unani have a very rich history of their effectiveness for diabetes but only a small number of these have received scientific and medical evaluation to assess their efficacy. One such Indigenous plant *Pongamia pinnata* (L.) Pierre was evaluated for its ethnopharmacological action to treat and prevent diabetes. *Pongamia pinnata* commonly known as Karanj (Hindi), it is a fast-growing deciduous tree up to 20 m tall that is thought to have originated in India and is found throughout Asia, Indonesia and into northern Australia. Ethnomedically, this plant is used in the diverse treatment of tumours, piles, skin diseases, wounds and ulcers^{6,7}. Oil has been used in bronchitis, chronic fever, hypertension, whooping cough in Indian ayurvedic herbal medicine and cosmetic preparations⁸. In china phytochemicals isolated from *P. pinnata* were used as natural therapeutic agents for neurodegenerative diseases⁹. A pharmacological study done by previous authors reported that some parts of the plant showed that this plant has antidiabetic activity^{10,6}. The antidiabetic activity of *Glycyrrhiza glabra* is greatly

appreciated in Indian Ayurveda as the plant belongs to the same family Fabaceae and used in the top ayurvedic product "diabecon" manufactured by Himalaya¹¹. Considering this unique fact, this study was designed to evaluate the therapeutic efficacy of *P. pinnata* in the prevention of glycemic index and on histoprotective ability on the pancreas.

In the present investigation, the aqueous and alcoholic extracts of *P. pinnata* were screen for possible secondary metabolite by chemical fingerprinting followed by characterization to find lead components to study its potential hypoglycemic ability and pancreatic cell injuries by histopathological approach. It will help the scientific community to predict the prospective impact of available phytochemical for new drug development against diabetes mellitus.

MATERIALS AND METHODS

Study area: The study was carried out from August, 2014 to April, 2017.

Plant collection and identification: Stem branches of *P. pinnata* were collected from the local areas from Mulund (GPS Coordinates 19.162990°N, 72.957544°E) Mumbai, India. The plant was identified and authenticated by Blatter Herbarium, St. Xavier's College, Mumbai (Specimen Number shah 183 of G. L. Shah).

Preparation of plant extract: The plant stem branches were dried under the mild sun and branches were ground into a fine powder using a pulverizer. Fifty gram of powder crude extract was prepared in 250 mL distilled water as well as ethanol using the Soxhlet apparatus for 6-7 cycles¹². Each time the extracts were dried using a vacuum evaporator and stored at 4°C to maintain standard quality throughout the study.

Chemical fingerprinting and quantification of *P. pinnata* extracts

Chemicals: The standard chemicals like toluene, ethyl acetate, diethylamine, formic acid, glacial acetic acid, methanol, chloroform, n-hexane, the water of HPLC grade solvents were purchased from Merck (Germany).

Preparation of solutions for HPTLC: Hydroalcoholic solution was prepared, where the aqueous powdered extract was diluted in methanol i.e. 500 mg in 5 mL methanol and alcoholic powdered extract was diluted in ethanol to get distinct separation of compounds.

HPTLC equipment and application of spots: HPTLC analysis was performed with Linomat V (Camag, Muttenz, Switzerland) (auto sprayer) connected to a nitrogen cylinder. The software-controlled version allows stepping up to HPTLC. Then sample applied on the TLC silica gel plate was processed through the developing chamber. Various bands of volume applied were 1, 2, 3 and 5 μ L. The solvent was removed from the spot by air-drying. The position of the origin was marked.

Observation under TLC visualizer: TLC visualizer provides illumination with direct and/or transmitted white light as well as with direct UV 254 nm and UV 366 nm light. The images were captured by an integrated powerful 12-bit camera with a highly linear CCD chip with the whole process conveniently controlled by the TLC software.

Derivatization and evaluation: Derivatization was achieved by dipping (immersing) chromatogram into suitable solvents which are required for the particular class of compounds whenever necessary by immersing a TLC plate into the derivatizing reagent a very homogenous reagent transfer was achieved using the Chromatogram Immersion Device. The CAMAG TLC Scanner was used for the Evaluation of Chromatogram and Win CATS Option "Image Comparison Viewer" used to compare image tracks of samples¹³.

Characterization of *P. pinnata* extracts

Liquid chromatography-mass spectrometry analysis: Liquid Chromatography-Mass Spectrometry (LC-MS) analysis of extract was performed using Dual AJSESI. The mobile phase consisted of 100% water, A containing 0.1% formic acid in water and 100% Acetonitrile, B containing 90% Acetonitrile+10% water+0.1% Formic acid. The analysis followed a linear gradient program. Initial conditions were solvent A 95%: A 5%, 0-25 min, changed to solvent A 5%: B 95%, 25-26 min and went back to solvent A 95%: B 5%, 26-30 min. The flow rate was set to 0.2 mL min⁻¹, the injection volume was 3 μ L and the column used was C18 (Zorbax Eclipse).

Animals: About 5-6 weeks male Swiss albino rats weighing 200-300 g were maintained at the animal facility of Pharmacy College, Dombivali, India. The animals were housed in polypropylene cages under conditions of humidity (45 $\pm$ 3%), temperature (24 $\pm$ 3°C) and 12/12 hrs light and dark cycle respectively. Swiss albino rats fed with a standard diet and water (*ad libitum*). All the protocols used in the study were approved by the Institutional Animal Ethical Committee (CPCSEA No:704) of Pharmacy College, Dombivali, Mumbai.

Induction of diabetes and dose preparation: Diabetes was induced by a single intraperitoneal (I.P) injection of 145 mg kg⁻¹ Alloxan monohydrated dissolved in 0.9% sterile saline (i.e. 29 mg in 1 mL saline, the mixture was vortexed and stored in the dark container by sealing the tube with aluminum foil.) in all the experimental rats except in NC group. PPAqExt was administered with a dose of 28 mg kg⁻¹ (i.e., for 200 g rat 4.5 mg mL⁻¹ D/W) and PPAlcExt was administered with a dose of 28 mg kg⁻¹ (i.e., for 200 g rat 4.5 mg in 0.1 mL DMSO + 0.9 mL D/W). Standard drug dosage Glycomate 250 mg tablets were used at a dose of 80 mg kg⁻¹. (Extrapolation of human dose to animal dose for 200 g rat 0.23 mg powder dissolves in 1 mL D/w)¹⁴.

Experimental design: Animals were divided into 6 groups (6 rats/group) as follows:

Group I : (Normal Control)+Food+D/W. (NC)

Group II : Alloxan (145 mg kg⁻¹, i.p)
Untreated+Food+D/W.(DC)

Group III : DC+(*P. pinnata* aqueous extract) PPAqExt 28 mg kg b.wt. (TD1)

Group IV : DC+(*P. pinnata* alcoholic extract) PPAlcExt 28 mg kg b.wt. (TD2)

Group V : DC+Metformin 80 mg kg b.wt (SD)

Group VI : DC+DMSO 0.1mL/300 kg b.wt+D/w. (VC)

Experimental induction of diabetes in 5 groups of rats was carried out Except for the NC group. After 72 hrs, Alloxan-induced rats with elevated Blood Sugar Fasting (BSF) levels ($\geq$ 250 mg dL⁻¹) were included in the study (Day 0). Plant extracts, standard drug and DMSO treatment were started on the 7th day of the Alloxan treatment (i.e., Day 1) as a single dose in the morning and was continued for 3 months with respective groups.

Body weight and urine examination: The animals are weighed using a digital weighing balance of Wensar daily before and after the induction of diabetes, till the 90th day (i.e., up to the day of dissection). Urines are collected over a clean petri dish then urine transferred to a plain BD vacutainer with a micropipette to determine volume. The urines were immediately stored in the freezer at 4°C for the subsequent measurement of the urinary protein and Glucose concentration by strips method.

Collection of blood samples: Animals fasted overnight before the 30th, 60th, 90th day and blood was collected from every rat with retro-orbital plexus technique¹⁵. For serum separation,

blood samples were allowed to clot for 30 min, followed by centrifugation at 3000 r min^{-1} for 10 min at 4°C ¹⁶. On the 90th day after blood collection animals were sacrificed by cervical dislocation to collect the pancreas for histopathology. Each time BSF, Glycosylated Hemoglobin (HBA1c) and Mean Blood Glucose (MBG) were evaluated monthly from serum.

Biochemical investigation: Blood Sugar Fasting (BSF) level was measured by the glucose oxidase/peroxidase (GOD/POD) method. The absorbance was measured at 520 nm using a fully automated Biochemical analyzer (Mispa Nano) and the fasting level in blood was specified as mg dL^{-1} ¹⁷. Quantitative measurement of the Glycosylated Haemoglobin (HBA1c) component was measured by the standard protocol of the High-performance Liquid Chromatography method¹⁸. The Mean blood glucose was measured by the relationship analysis with the HBA1c method¹⁹.

Histopathological investigation: At the end of the study, animals were sacrificed by cervical I decapitation under anaesthesia to collect the pancreas for histopathology. A pancreas was cut into two to three pieces of approximately 5-6 mm^3 sizes and fixed in a 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 μm thickness of pancreas tissue were cut and stained with hematoxylin-eosin. The thin sections of the liver were made into permanent slides and examined under a high-resolution light microscope²⁰.

Statistical analysis: The results are expressed as $\text{Mean} \pm \text{SD}$ and data were analyzed through a two-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The groups with significant mean differences at ($p < 0.05$) ($p < 0.01$) ($p < 0.001$) are represented on the graph wherever required.

RESULTS

Chemical fingerprinting of *P. pinnata*: High-performance thin layer chromatography was carried out for evaluation of various phytoconstituents from alcoholic as well as aqueous extracts of *P. pinnata*. It is evident from Table 1 that a maximum of 12 alkaloids, 10 flavonoids, 09 glycosides, 06 saponins, 09 sterols, 10 tannins and 11 triterpenoids peaks were observed from alcoholic extracts whereas 02 alkaloids, 09 flavonoids, 10 glycosides, 04 saponins, 10 sterols, 07 tannins and 10 triterpenoids peaks were observed from Aqueous extracts. Both the extracts showed the Best result of the Thin Layer. The analysis results all together show 67 compounds in AlcExt, 52 Compounds in AqExt and 43 compounds were found to be common which is present in both.

Characterization of PPAqExt and PPAlcExt: Compounds identified by LC-MS analysis in PPAqExt and compounds reported against acquisition time was shown in Table 2 and Fig. 1, respectively. LC-MS analysis of PPAlcExt revealed the presence of 13 compounds and the acquisition graph reveals the 7,4' Dimethoxy isoflavone as a lead compound with 16.61 as retention value.

Compounds identified by LC-MS analysis in PPAqExt and compounds reported against acquisition time was shown in Table 3 and Fig. 2, respectively. LC-MS analysis of PPAlcExt

Table 1: Phytochemicals from alcoholic and aqueous extract

Class of compounds	AlcExt	AqExt	Common
Alkaloids	12	02	01
Flavonoids	10	09	06
Glycosides	09	10	08
Saponins	06	04	03
Sterols	09	10	08
Tannins	10	07	07
Triterpenoids	11	10	10
Total	67	52	43

Table 2: Components present in PPAqExt identified by LC-MS analysis

Retention time	Compound name	Chemical formula
1.687	D-1-piperidine-2-carboxylic acid	$\text{C}_6\text{H}_9\text{NO}_2$
1.777	Ethosuximide	$\text{C}_7\text{H}_9\text{NO}_3$
11.754	Amylose	$\text{C}_{14}\text{H}_{26}\text{O}_{11}$
12.91	Dihydro-deoxy-streptomycin	$\text{C}_{21}\text{H}_{41}\text{N}_7\text{O}_{11}$
12.91	Leukotriene	$\text{C}_{28}\text{H}_{44}\text{N}_2\text{O}_8\text{S}$
13.69	Pteryxin	$\text{C}_{21}\text{H}_{22}\text{O}_7$
15.06	2-Naphthalenepropanol, 6-methoxy-a-methyl, hydrogen sulfate	$\text{C}_{15}\text{H}_{18}\text{O}_5\text{S}$
15.60	Pachyrrhizin	$\text{C}_{19}\text{H}_{12}\text{O}_6$
16.14	Eupatorin	$\text{C}_{18}\text{H}_{16}\text{O}_7$
16.61	7,4' Dimethoxy isoflavone	$\text{C}_{17}\text{H}_{14}\text{O}_4$
17.77	Idarubicinol aglycone	$\text{C}_{20}\text{H}_{18}\text{O}_7$
19.26	Mefloquine	$\text{C}_{17}\text{H}_{16}\text{F}_6\text{N}_2\text{O}$
19.95	Estradiol-17 beta 3-sulfate	$\text{C}_{18}\text{H}_{24}\text{O}_5\text{S}$

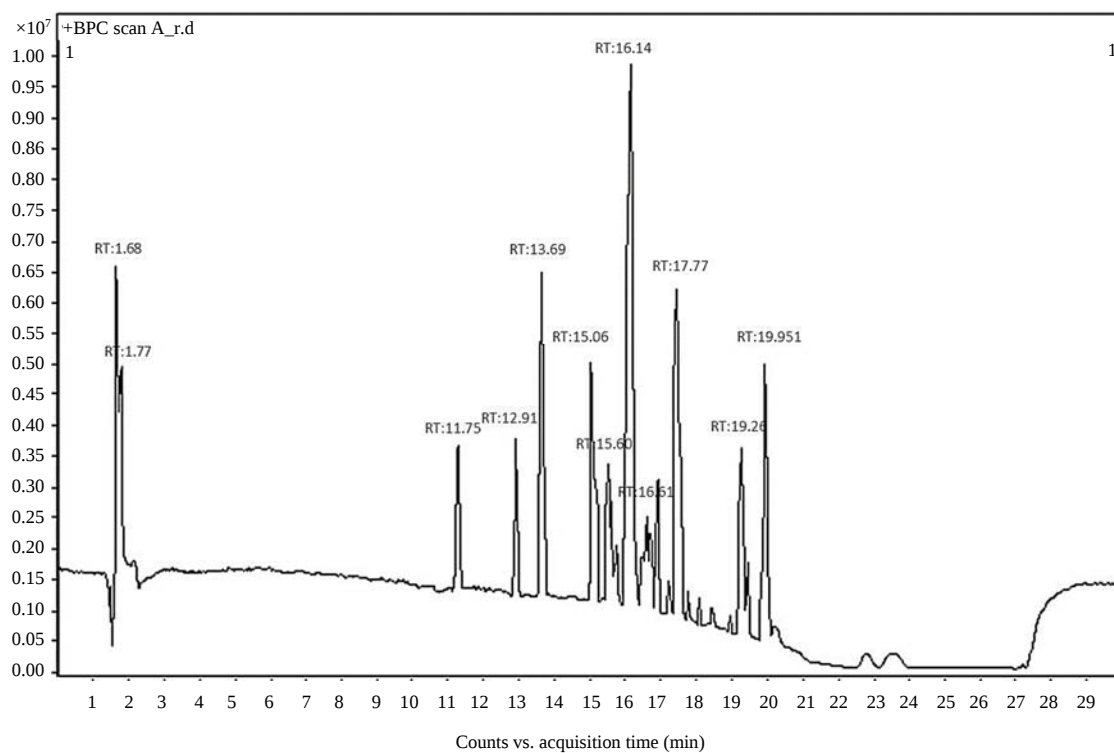


Fig. 1: LC-MS analysis chromatogram of PPAqExt

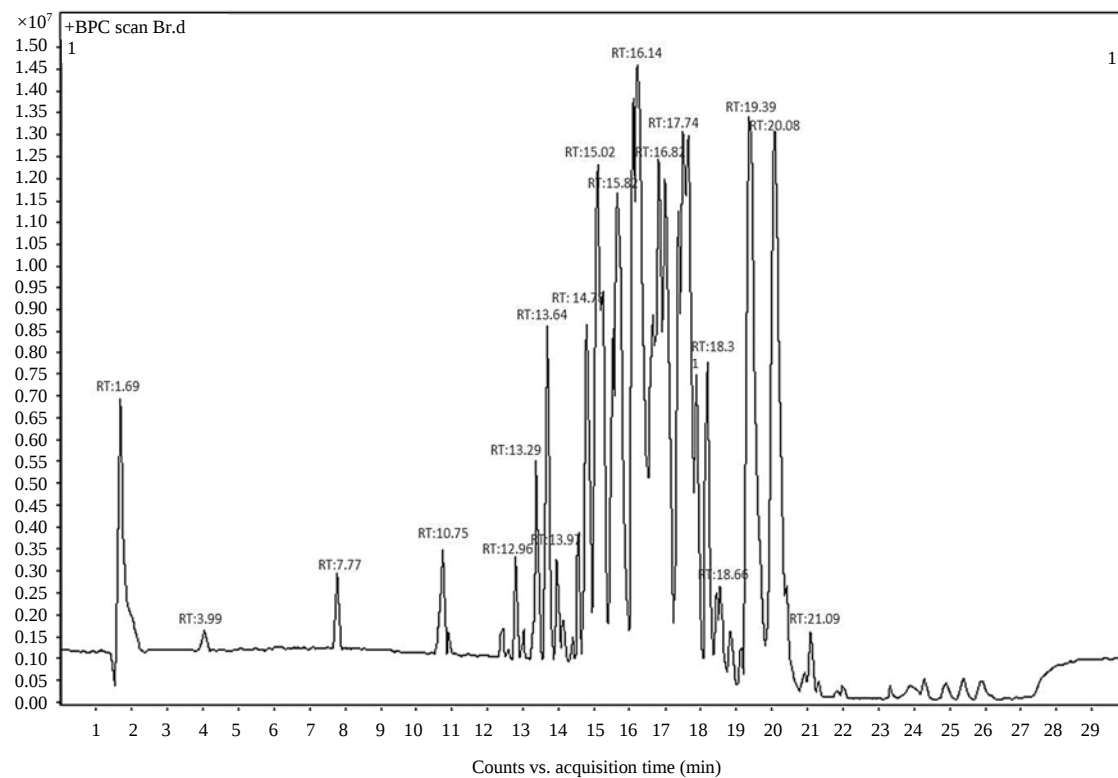


Fig. 2: LC-MS analysis chromatogram of PPAIcExt

Table 3: Components present in PPAlcExt identified by LC-MS analysis

Retention time	Compound name	Chemical formula
1.699	3-Dehydrocarnitine	C ₇ H ₁₄ NO ₃
3.991	Ecgonine	C ₉ H ₁₅ NO ₃
7.77	Lupanyl acid	C ₁₄ H ₂₄ N ₂ O ₂
10.753	Lycorine	C ₁₆ H ₁₇ NO ₄
12.962	Methyl 7-Desoxypurpurogallin-7-carboxylate trimethyl ether	C ₁₆ H ₁₆ O ₆
13.29	Rosmarinic acid	C ₁₈ H ₁₆ O ₈
13.64	Marmesin	C ₁₄ H ₁₄ O ₄
13.971	Karanjin	C ₁₇ H ₁₀ O ₄
15.022	Sulindac	C ₂₀ H ₁₇ FO ₃ S
15.82	Alpha-Toxicarol	C ₂₃ H ₂₂ O ₇
16.14	Idarubicinol aglycone	C ₂₀ H ₁₈ O ₇
16.827	Sulindac sulfide	C ₂₀ H ₁₇ FO ₂ S
16.927	7-O-Methylsterigmatocystin	C ₁₅ H ₁₈ O ₅ S
17.74	Deoxypodophyllotoxin	C ₂₂ H ₂₂ O ₇
17.88	Idarubicinol aglycone	C ₂₀ H ₁₈ O ₇
18.31	2-ethoxycarbonyl-2-ethoxyoxaloyloxy dihydrochrysin dimethylether	C ₂₄ H ₂₄ O ₉
18.66	Dihydrorotenone	C ₂₃ H ₂₄ O ₆
19.39	Methyl Robustone	C ₂₂ H ₁₈ O ₆
20.08	Estradiol-17beta 3-sulfate	C ₁₈ H ₂₄ O ₅ S

Table 4: Differences in body weight, food intake and water intake during the study period

Experimental groups	Body weight (g)	Food intake (g)	Water intake (mL)
NC	91.48±23.81	1.22±0.8	1.17±1.77
DC	16.58±7.21	10.22±1.73	84.17±22.85
TD1	25.7±9.43*	7.26±1.0*	73.25±1.89*
TD2	38.26±14.51**	5.13±1.15**	30.55±10.06**
SD	59.63±28.79***	2.48±0.38***	9.06±0.26***
VC	14.04±2.94	13.64±0.88	99.28±16.21

Data are expressed as Mean ± SEM, n = 18. ANOVA followed by multiple comparison two-tail "t" test. ***p<0.001: Highly significant as compared treatment with disease control, **p<0.01: Highly significant as compared treatment with disease control, *p<0.05: Significant as compared treatment with disease control

Table 5: Effects of PPExts on urinary parameters in experimental animals

Experimental groups	Normal	DC	TD1	TD2	SD	VC
Physical examination						
Quantity (mL)	0.5	1.5	0.7	0.6	0.3	1.0
Appearance	Clear	Hazy	Clear	Clear	Clear	Hazy
Reaction (PH)	6.5	8.5	7.5	7.0	6.5	6.5
Specific gravity	1.010	1.005	1.015	1.005	1.020	1.020
Chemical examination						
Proteins	Absent	+1	Trace	Trace	Absent	Trace
Glucose	Absent	+4	+1	+1	Absent	+3

+1: 0.3 g L⁻¹ of proteins, +1: 5.6 mmol L⁻¹, +3: 28 mmol L⁻¹, +4: 56 mmol L⁻¹ glucose

revealed the presence of 19 compounds and the acquisition graph reveals the Marmesin and Karanjin as a lead compound with 13.64 and 13.971 as retention time, respectively.

Body profile analysis: A body profile includes three parameters viz. bodyweight, food intake and water intake. It is evident from Table 4 that increases in body weights of all the experimental animals except in the DC and VC groups. After the treatment recovery in the body weight of rats was observed. Polydipsia was observed in the DC and VC groups which were signed after the 1st week of Alloxan treatment with untreated control and continue up to the end of the treatment. After the treatment of TD1 and TD2 to the diabetic animals for 3 months, a decrease in the water intake of rats

was observed which is almost closer to a standard drug. A polyphagia was observed in DC and VC groups. Observations were significantly higher as compared to NC. After treatment with AqExt and AlcExt to the diabetic animals for 3 months, a significant decrease in the feeding of rats was observed in TD1 and TD2 as compared to the SD group.

Urine examination: Administration of Alloxan results in polyuria, glycosuria and proteinuria presented in Table 5. The magnitude of the effect was always higher for the DC group. PPExts treatment completely restored the polyuria, glycosuria and proteinuria in both groups (TD1, TD2). Protein value and glucose concentration in the urine of DC and VC groups are indicative of the diabetic status of animals. Whereas after

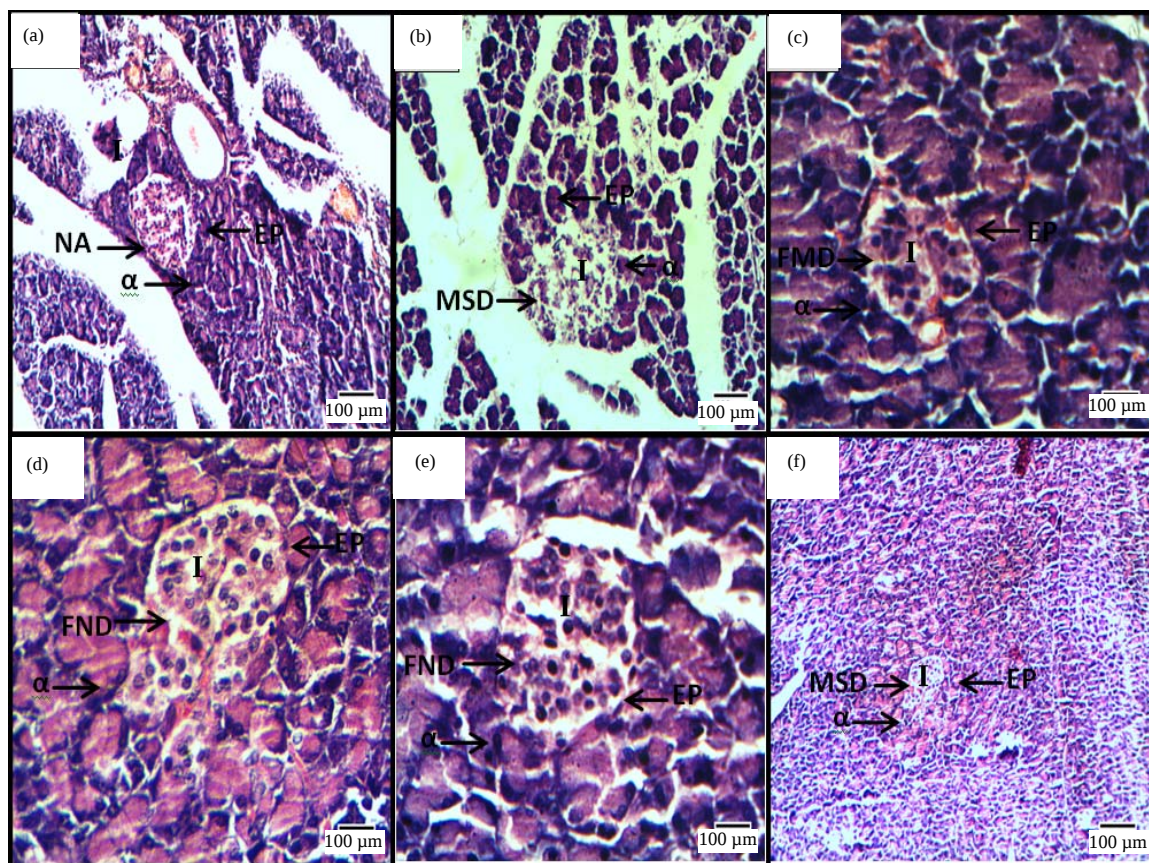


Fig. 3(a-f): Pancreatic histopathological sections of experimental animals stained with Haematoxylin-Eosin (H and E, 100×). (a) Normal structure of pancreas, (b) Multifocal severe degenerative changes in the pancreas, (c) Focal moderate degenerative changes in the pancreas, (d) Focal minimal degenerative changes in the pancreas, (e) Focal minimal degenerative changes in the pancreas and (f) Multifocal severe degenerative changes in the pancreas
NA: Normal appearance, I: Islets of langerhans, α: Alpha cells, EP: Exocrine part, MSD: Multifocal severe degeneration, FMD: Focal moderate minimal degeneration, FND: Focal minimal degeneration

Table 6: Average values of BSF, Glyco Hb and MBG of experimental groups

Experimental groups	BSF (mg dL ⁻¹)	HbA1C (%)	MBG (mg dL ⁻¹)
NC	72.5518.77	7.560.30	101.678.50
DC	339.818.59	13.631.37	269.6738.17
TD1	218.1124.85*	9.560.30**	15411.13**
TD2	99.4112.03**	8.40.65**	125.6721.38**
SD	86.3713.49**	7.00.80**	87.6722.47**
VC	332.198.32	13.40.43	264.3310.78

Data are expressed as Mean ± SEM, n = 18. ANOVA followed by multiple comparison two-tail "t" test. ***p<0.001: Highly significant as compared treatment with disease control, **p<0.01: Highly significant as compared treatment with disease control, *p<0.05: Significant as compared treatment with disease control

treatment with PPExts less amount of proteins and glucose values were observed this showed that significant recovery was observed as compared to DC and VC.

Glycemic index analysis: It is evident from Table 6 that the reduction in the BSF and MBG level by PPExts was observed. The average value of BSF in the diabetic control group was 339.81 ± 8.59 which is reduced in PPExts treated group to

218.11 ± 24.85 and 99.41 ± 12.03 mg dL⁻¹. Similarly, MBG levels were reduced from 269.68 (DC) to 154 (TD1), 125.67 (TD2), 87.67 (SD). Also Table 6 reveals that HbA1C levels were reduced from 13.63 (DC) to 9.5% (TD1), 8.4% (TD2), 7.0% (SD).

Histopathology of the experimental animal pancreas:

Figure 3a reveals the histoarchitecture of the pancreas of the NC group showing closely packed lobules of α cells and

pancreatic acini with preserved numerous β -cells in I, undamaged islets widely distributed throughout the endocrine pancreas. Figure 3b shows multifocal severe degenerative changes in pancreas and rat pancreas treated with *P. pinnata* i.e., Fig. 3c revealed focal moderate degenerative changes in the pancreas, whereas Fig. 3d showing more prominent recovery where the population of β -cell and the size of the I restored towards normal integrity the almost usual structure of I with exocrine and endocrine portions which are formed of pyramidal cells. Treatment with SD i.e., Fig. 3e group rats showed focal minimal degeneration in the islet of Langerhans rest of the cellular architecture and integrity of the cell was normal along with structure, which is similar to normal control group rats. Figure 3f of VC groups revealed pathological changes of both the exocrine and endocrine part of the pancreas represented by a marked decrease of β cells, shrunken in I with multifocal severe degenerative changes in the pancreas.

DISCUSSION

The ethnopharmacological approach to new drug discovery is based on the fact that there would be no side effects associated with regular use of certain plant material on the other mode several synthetic drugs have opposing and intolerable side effects. Investigation on pharmacognosy, pharmacological therapeutics has been carried out on Chinese and indigenous medicinal plants with remarkable achievements in botanical medicines like Quinghaosu, Artemisinin²¹. Numerous plant-derived molecules like alkaloids, rauwolfia, guggulsterones, Mucuna pruriens, picrosides, phyllanthus, steroidal lactones and glycosides have come out on Ayurvedic experimental basis for various purposes²².

Phytochemical screening by HPTLC analysis of aqueous as well as ethanolic extracts of stem of *P. pinnata* revealed the presence of various phytochemicals in significant concentration. There is a dearth of reports regarding the HPTLC profile of *P. pinnata*. It was observed that a maximum of 12 peaks in Alkaloids, 10 in Flavonoids, 10 in Glycosides, 06 in Saponins, 10 in Steroids, 10 in Tannins and 11 in Triterpenoids. The results are in agreement with the previous study of authors²³. Few authors reported less no of peaks in HPTLC analysis whereas it was reported, more peaks in extracts and the concentration of each peak is significantly higher²⁴. Phytoconstituents identified by LC-MS analysis that have been previously reported for their anti-inflammatory activities belong to flavonoids like 7,4'-Dimethoxy isoflavone²⁵,

Marmesin, Karanjin^{26,27} class of compounds. Both chemical fingerprinting and LS-MS analysis conclude that why our extracts are having a more hypoglycemic, histo-protective effect at a lower concentration as compared to standard drug.

In the present research, one such indigenous plant was investigated for its hypoglycemic potential. Treated with *P. pinnata* stem extract was effective in exerting protection against body weight loss. TD1 rats showed a 7.4% increased whereas TD2 it showed 15.7% altogether quite less than SD which was 21.71% when compared with DC. The results are following other study²⁸. Where they reported 11% (26.5 g kg⁻¹) loss in body weight in untreated rats and after treatment it increased to 3% (7.9 g kg⁻¹) as compared to normal control.

The metabolism of macromolecules tends to induce negative nitrogen balance, this results in increase appetite (polyphagia). The combination of polyphagia coupled with weight loss is paradoxical and always raises the suspicion of diabetes²⁹. Increase polyphagia was observed which one of the symptoms of DM is. In the DC rats, it was increased by 46.59% as compared to NC. After the administration of Treatments (TD1 and TD2), it was brought down to 9.06, 16.8% respectively and 25.8% for SD. To compensate for water loss through urination, polydipsia was witnessed in DM. A similar observation was observed in DC rats where water intake was 79% increased as compared to NC rats. Treatment with PPAqExt and PPAIcExt reduced the water intake and they were 9.32% in TD1, 45.21% in TD2 and 63.06% in SD respectively. Polyuria, Oliguria, Proteinuria and Glycosuria are symptoms associated with diabetic nephropathy. Decreased, altered and disturbed in the secretion of vasopressin followed by ADH hormone in diabetes causes less water to be reabsorbed and more urine to be formed. At later stages, Nephropathy can be observed where surplus proteins and glucose is excreted in the urine. In our analysis, the excessive load of proteins and sugars was observed in untreated controls with a high volume of urine which gives a clear indication of oliguria. The reversal in the symptoms was observed after the treatment which is almost similar to other authors²⁸.

In the present investigation, the diabetic rats showed a persistent rise in BSF to 339.81 ± 8.59 at 150 mg kg⁻¹ intraperitoneally. The subsequent fall in TD1 and TD2 were (218.11 +24.85) and (99.41+12.03) respectively. A similar noteworthy observation was reported by the author³⁰, several other authors also noticed hypoglycemic activity at the dose of 50 and 100 mg kg⁻¹ respectively³¹. Furthermore, another study³² reported that acute oral toxicity data showed no mortality in normal mice up to 5000 mg kg⁻¹. There is no significant deviation in the results between the activity of standard drug Metformin and TD2. The probable mechanism

was reported that secondary metabolites regenerate the damaged β cells in the Alloxan diabetic rats³³. Another possible mechanism of action of the extract could be correlated secondary metabolites where polyphenols like Flavonoids act on Insulin-Sensitivity *in vivo* by Suppression of Nuclear Factor κ B Activation³⁴.

The glycated haemoglobin (HbA1c) level, defined as the ratio between HbA1c concentration and total haemoglobin concentration, is a very useful diagnostic marker to understand the status of diabetes. Glycated haemoglobin is produced from the multistep condensation reaction of glucose with a haemoglobin amine moiety³⁵. Moreover, with these previously reported results, the current study showed a significant elevation in Glycosylated haemoglobin (HbA1c) and mean blood glucose levels in DC rats. After three months of treatment with PPAqExt and PPAIcExt, glycosylated hemoglobin levels were in the normal range for extract-treated controls. A significant reduction was achieved in both the extract and it was 9.5% in TD1 and 8.4% in TD2 whereas in DC it was 13.63%. Similar observations were also noted for extract-treated groups were improved from 13.5-8.5% with *M. charantia* extract (MCFE)³⁶, they observed the same effect on diabetic rats with *M. cymbalaria* fruit aqueous extract. The level of HbA1c is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the Mean Blood Glucose (MBG) concentration in the proceeding 6-8 weeks. MBG was taken at the end of three months of treatment. In the current investigation MBG values in the diabetic control group were noted to be 269.68 ± 38.17 whereas in test groups (TD1 and TD2) it was 155 ± 15.55 and 125 ± 21.38 which can be similar to other authors³⁷.

In the present study, histopathological examination of the pancreas of Alloxan induced diabetic rat's revealed the destruction of beta cells and structural changes in Islets of Langerhans. Due to Alloxan multifocal severe degenerative changes were observed in the exocrine as well as endocrine part of the pancreas with loss of β cells, after long treatment with PPExts rats pancreatic sections were reversal in exocrine and endocrine changes were observed which are minimal and mild. Islets of Langerhans showed improvement whereas there was the restoration of β cells population size and recovery in structural integrity³⁶. The pancreas of rats treated with standard drug, ethanol extract and n-hexane extract showed moderate expansion of cellular population and size of islet cells when compared with the untreated diabetic rats³⁸. Furthermore, several other studies also revealed that *H. Sabdariffa*'s treatment unusually improved the dimensions

of the pancreatic islets and the numerical concentration of β -cell-depleted by STZ diabetes³³. The extent of reversal and recovery was partial with aqueous extract and was distinct with alcoholic extract.

CONCLUSION

Pongamia pinnata stem extract is effective not only in preventing body weight loss but also in helping to reverse diabetic symptoms. Significant reduction is witnessed in all the glycemic indices which establish that *P. pinnata* has a promising effect against hyperglycemia in diabetic rats. PPAIcExt was efficient to maintain the cellular architecture and integrity of the pancreas. The extract produced hypoglycemia due to a variety of secondary metabolites and few lead components revealed in LC-MS analysis that enlighten the holistic approach of Ayurveda where overall health status comes into consideration that targets multiple pathways of recovery at the same time.

SIGNIFICANCE STATEMENT

This study discovered a significant reduction in glycemic indices which proves a promising antihyperglycemic effect with the restoration of cellular pathological changes that can be beneficial for mankind. These isolated lead compounds will help the researchers to rethink on natural antidiabetic drug discovery and development process once safety and efficacy data of preclinical studies have been conducted as most researchers were not able to explore the phytochemical mechanism. Thus a new formulated conventional drug approach can be established.

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