EVALUATION OF HYPOGLYCEMIC ACTIVITY OF *PROSOPIS JULIFLORA* ON ALLOXAN INDUCED DIABETIC RAT MODEL

Ukande M. D.[a*], Krishna Murthy[b], Shete R. V.[b] and Solunkhe R. S.[c]

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ABSTRACT

The present study aimed to evaluate the hypoglycemic activity of *Prosopis juliflora* leaf extract on Alloxan-induced diabetic rats. Preliminary phytochemical investigation of *P. juliflora* leaf extract (PJLE) was done according to standard tests. The standardization of PJLE was done by HPTLC analysis. PJLE (100,200,400 mg/kg) was administered to all animals group-wise with standard glibenclamide, Normal saline solution for the normal control group for 21 days orally. After 21 days, one animal from each group were sacrificed. Blood samples were collected for biochemical analysis and pancreas tissue was subjected to histopathological examination. Rats treated with PJLE showed significant changes in blood glucose level, body weight, organ weight and biochemical parameters. There was also a significant improvement in the histopathological scores in PJLE treated groups when compared with a diabetic control group. This study evaluates the effectiveness of PJLE as a therapeutic agent in hypoglycemic activity by alloxan-induced diabetic rat model in dose-dependent manner.

Keywords: *Prosopis juliflora*, Phytoconstituents, Alloxan, Glibenclamide, Hypoglycemic activity, HPTLC, Quercetin.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia. It is associated with abnormalities in carbohydrate, fat, and protein metabolism and results in chronic complications including microvascular, macrovascular, and neuropathic diseases[1].

Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics, environmental factors, and lifestyle choices. Depending on the etiology of diabetes mellitus, factors contributing to hyperglycemia may include decreased insulin secretion, decreased glucose utilization, and increased glucose production. The World Health Organization (WHO) found that more than 180 million people worldwide have diabetes[2].

The ancient and modern medicinal uses of the "mesquite" plant (Babul), *Prosopis juliflora* (Sw.) DC (Leguminosae) in different countries worldwide have been surveyed. The different medicinal uses are grouped separately with their pharmacological action, whether real or presumed, within the various medical specialties. The results show extensive use of this plant throughout the world and suggest that further research should be carried out to determine the active principles present in the various parts of the plant. Leaves are rich in essential alkaloids and amino acids but low in sulfur-containing amino acids. Tannins, flavonoids and polyphenols are present in leaves of *P. juliflora.* The alkaloids juliflorine, juliprosine, juliprosopine, julifloricine and julifloridine have been isolated from *P. juliflora*. Diketone, prosopidione and cytotoxin patulitrin have been isolated from *P. juliflora* leaves. The antifungal, antibacterial and general antimicrobial activity of plant extracts of *P. juliflora* are well established. Cytotoxic effects are also observed with extracts of *P.juliflora* and alkaloids were reported to cause hemolysis of rat and human erythrocytes[3].

MATERIALS AND METHODS

MATERIALS

Collection and Authentication of *P. juliflora* Leaves

The leaves of *Prosopis juliflora* were collected from the Saswad region of Maharashtra in the month of December 2018 and was authenticated by Authenticated from outfields of Botanical Survey of India, A voucher specimen (No. RDCOP PG/PJ/18-19/01/MU) has been deposited in the Pharmacology Department of RDCOP, Bhor.

[a] Department of Pharmacognosy, [b]Department of Pharmacology, [c] Department of Pharmaceutics, Rajgad Dnyanpeeth's College of Pharmacy, Bhor. Dist-Pune, 412 206, Maharashtra, India

* For correspondence: E-mail: maniisha20595@gmail.com

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Animals

Wistar rats of either sex (150-250 g) for activity and female Wistar rats (for toxicity study) were procured from the LACSMI Biofarm Pvt. Ltd, Alephata Pune. (CPCSEA No: 1277) were used for the study.

Housing Conditions

Animals were maintained at a temperature of 25±1° C and relative humidity of 45 to 55 % under 12 h light, 12 h dark cycle. The animals had free access to standard pellet rodent diet (Purchased from Nutrivet Life Science Pvt. Ltd., Pune Maharashtra, India) and tap water *ad libitum.*

Institutional Animal Ethics Committee (IAEC) Approval

The experimental design and research plan along with animal handling and disposal procedure were approved from Institutional Animal Ethics Committee of Rajgad Dnyanpeeth's College of Pharmacy, Bhor. IAEC approval No: 615/PO/Re/S/02/CPCSEA dated 4th Nov 2016.

Chemicals and Drugs

All the chemicals used in the study were of analytical grade. The alloxan monohydrate was purchased from Explicit Chemicals Pvt. Ltd, MIDC Kurkumbh Pune, Maharashtra, and glibenclamide tablet (manufacturerd by Sanofi India Limited) batch number 7NA0047 from local chemist from Tal-Bhor, Dist-Pune, Maharashtra.

Preparation of Hydroalcoholic Extract of *P. juliflora* Leaves

The leaves were shade dried and powdered by using a grinder. The hydroalcoholic extract of *P. juliflora* leaf was prepared by the soxhlet extraction of the powdered material in 50 % V/V ethanol for 3 days. Then it was filtered and concentrated on a water bath at 40-45^{0}C to obtain brownish-black semisolid mass, the final semisolid mass of PJLE was stored in desiccator till it was used for further studies[1,4].

Preliminary Phytochemical Analysis of the PJLE

The PJLE was subjected to qualitative chemical analysis for the presence of various phytoconstituents like alkaloids, carbohydrates, phytosterols, saponins, tannins, proteins, amino acids and flavonoids by using standard phytochemical tests[4,5].

Determination of Quantitative Standards for Standardization of Extract

Total phenolic content

Total phenolic content of PJLE was determined as per the method described by Pradeep Pal[6,7].

Preparation of methanolic extract 1g of *P. juliflora* leaf extract was dissolved separately in 10 mL methanol & allowed to stand for 1 h.

Standardization of *P. juliflora* Leaf Extract

Hydroalcoholic extract of *P. juliflora* leaf extract showed the presence of alkaloids & flavonoids. Hence, methanol fractions were used for HPTLC studies to detect the quercetin.

Preparation of standard solution.

A stock solution was prepared by dissolving 10 mg of quercetin in 10 mL methanol i.e 1 mg/mL.

Sample preparation.

0.1 g of the PJLE extract was dissolved in 10 mL methanol to yield test sample i.e 100 mg in 10 mL (10 mg/mL)

Instrumentation and Chromatographic Conditions

TLC plates (10 cm X 10 cm aluminium backed plates coated with silica gel GF254) were used for HPTLC analysis. The standard solution (quercetin) and test solution (PJLE) were applied on plate, maintaining a distance of 10 mm from the bottom & corner of the plate with help of CAMAG Linomat 5 sample applicator with a 10 µl syringe. Toluene: ethyl acetate: formic acid (5:1.5:1 V/V/V) was used as a mobile phase in a CAMAG glass twin through the chamber. The chamber was saturated with the mobile phase for 20 min and the plate was dried in an oven after complete development then scanned at 254 nm and 366 nm by a CAMAG TLC scanner with WINCAT software. Calibration curves of standard solution & test solution were prepared in HPTLC grade methanol. 30 µL volume of solution was spotted on the TLC plate to obtain a concentration of 100-500 ng per band of quercetin, respectively[8].

Acute Toxicity Study

The acute toxicity study of PJLE was carried out as per OECD guidelines No. 423 for acute oral toxicity[9].

Effects of Hydroalcoholic Extract of *P. juliflora* Leaves in Diabetes Mellitus

Alloxan Induced Diabetes Mellitus in Wistar Rats

Wistar rats of either sex (150-250g.) were used for this study. The rats were randomly divided into six groups each consisting of six animals and groups 2 to 6 were

injected with alloxan monohydrate (150 mg/kg, i.p.) After 6 h of alloxan administration animals were treated with 20% D-glucose (2 mL/kg, p.o.) and continued with 5% D-glucose for 24 h in drinking water[10].

After 72 h, serum glucose levels were measured and animals with serum glucose level above 150-200 mg/dL were selected for the further study. Then rats were randomly distributed in groups 2, 3, 4, 5, 6 and treated as bellow for 21 days[11,12,13].

Table I: Treatment schedule in alloxan monohydrate induced diabetes mellitus in Wistar rat

Groups	Treatments
Normal	Vehicle treated (Distilled water, 5 mL/kg, and p.o.)
Diabetic control	Alloxan monohydrate, (150 mg/kg, i.p.)
Glib10	Alloxan monohydrate (150mg/kg, i.p.) +Glibenclamide (10 mg/kg /day, p.o.)
PJLE 100	Alloxan monohydrate (150mg/kg, i.p.) + PJLE (100 mg/kg/day p.o.)
PJLE 200	Alloxan monohydrate (150mg/kg, i.p.) + PJLE (200 mg/kg/day p.o.)
PJLE 400	Alloxan monohydrate (150mg/kg, i.p.) + PJLE (400 mg/kg/day p.o.)

Blood samples of each animal from respective treatment groups were collected from the tail vein on 0, 7, 14 and 21 days of the treatment for the estimation of biochemical parameters. Then, the animals were sacrificed for the estimation of physical and biochemical parameters and histopathological study[14].

Following parameters were evaluated in the above paradigms

A) Physical parameters change in body weight and liver, kidney, pancreas weight.
B) Histopathology of pancreas
C) Serum biochemical parameters

Total Protein (TP), total cholesterol (TC), triglycerides level (TG), SGOT (serum-glutamate-oxaloacetate transferase), SGPT (serum glutamate pyruvate transferase), lactate dehydrate level (LDH), alkaline phosphatase (ALP), total bilirubin (TB), creatinine, direct bilirubin (TB) and blood urea nitrogen (BUN).

Histopathological studies

After completion of the experimental study, from each group, one animal was sacrificed for histopathological study. The tissues of the pancreas isolated from all experimental groups, after isolation, was washed immediately with saline solution & then fixed in 10% buffered formalin (pH 7.4) for 24 h. Then organs were dehydrated by using a graded series of ethanol and fixed in paraffin wax. Sections of 5 µm were cut by using a microtome (RM2255 rotary microtome, USA) then mounted on glass slides. After mounting, tissue was stained with hematoxylin and eosin, and examined under a light microscope. Then photographs were taken at 10X magnification[14,15].

Statistical Analysis

The values were expressed as mean±SEM (n=6). The statistical significance was assessed using Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's test and $p<0.05$ and $p<0.01$ were considered as statistically significant[16].

RESULTS

Preliminary Phytochemical Investigation

The preliminary phytochemical screening of the hydroalcoholic extract of *P. juliflora* leaf revealed the presence of alkaloids, carbohydrates, tannins, proteins, amino acids and flavonoids.

Estimate Quantitative Standards with Standardization of Extract

Estimation of Total Phenolic Content by UV-Spectroscopic Method

The total phenolic contents in the test sample were calculated using the following regression equation: $Y= mx + c$

where, x-concentration of extract, Y- absorbance

Table II: Total Phenolic Content of PJLE

Test Sample	Total Phenolic Content as mg GAE/g
PJLE	0.9009 mg

Data expressed as Gallic Acid Equivalent mg per g of the extract of *P. juliflora* leaf.

Acute Toxicity Study

No mortality and no signs of any toxicity were evident after the administration of a limit dose of 2000 mg/kg of PJLE in an acute oral toxicity test. Hence,

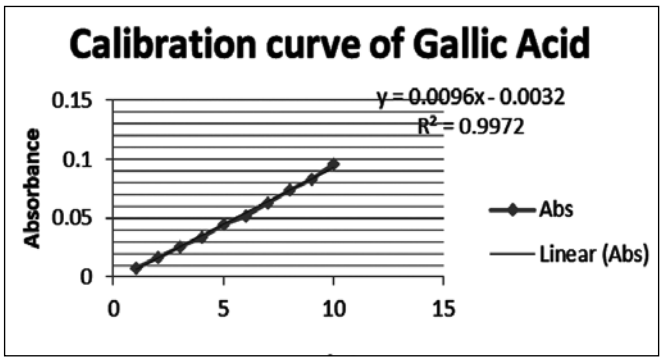

Fig. 1: Calibration Curve of Gallic Acid

Standardization of Extract by HPTLC

as per guidelines, next upper dose 5000 mg/kg was tested for toxicity study. Administration of PJLE 5000 mg/kg showed signs of toxicity such as salivation, convulsions, excitement and diarrhoea during 24 h of observation period and mortality was observed within 24 h of the observation period. Hence, for oral administration, the doses selected were 100, 200 and 400 mg/kg.

Evaluation of effect of PJLE in alloxan induced diabetes mellitus in Wistar rat

Administration of alloxan (150 mg/kg, i.p.) produced histopathological injury to pancreatic tissue as evident

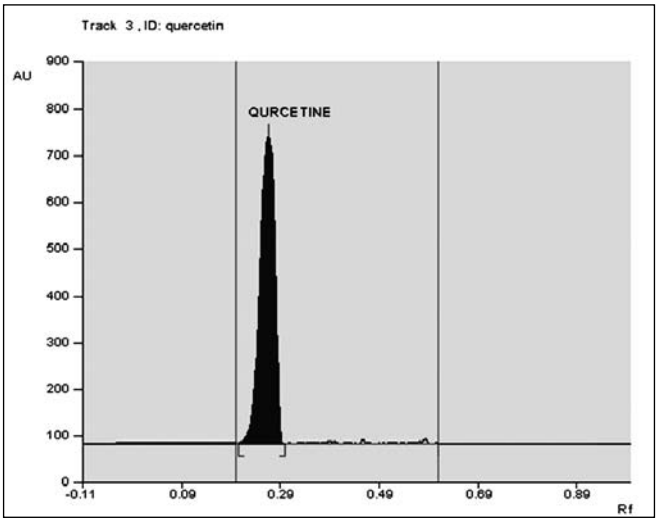

Fig. 2: Standard: Quercetin

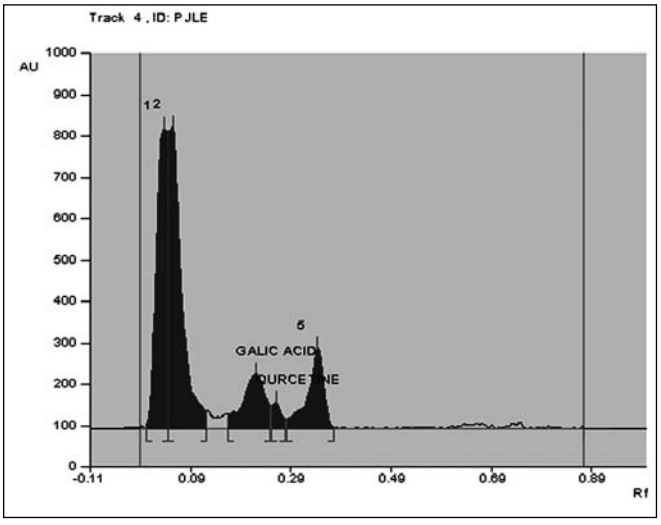

Fig. 3: Quercetin detected in PJLE

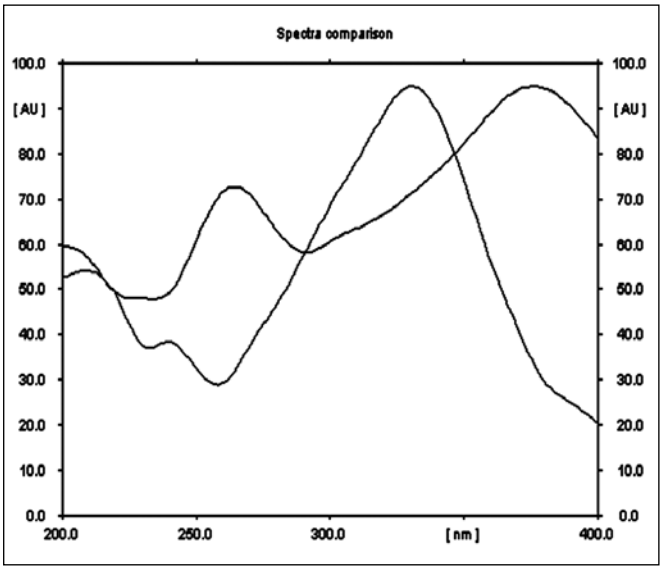

Fig. 4: R_f values: Quercetin 0.27 and PJLE 0.22

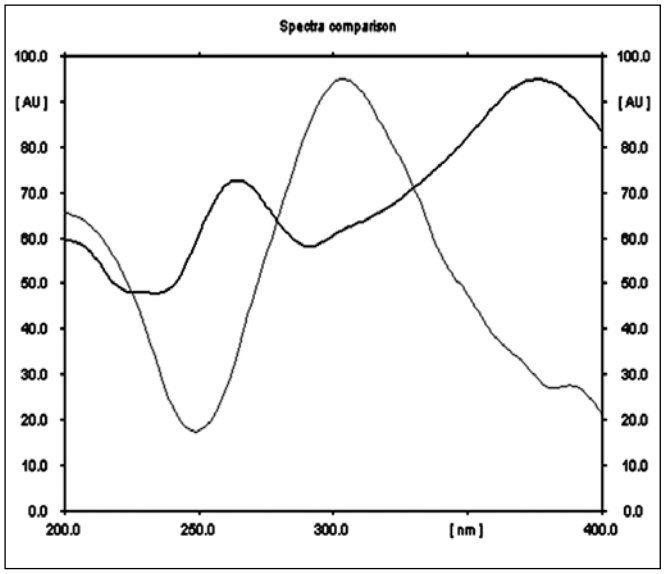

Fig. 5: R_f values: Quercetin 0.27 & PJLE 0.26

Table III: Effect of PJLE on change in body weight in alloxan induced diabetes mellitus

Groups	Change in body weight (g)		
	7 Day	14 Day	21 Day
Normal	4.166± 0.445	8.500 ±1.945	14.333± 3.062
Diabetic control	-12.500± 1.235#	-17.010± 1.688#	-24.833± 2.341##
Glib10	10.666± 5.094	30.010± 5.673*	45.333± 5.914**
PJLE 100	13.500± 2.874	20.500± 3.073	27.833± 3.996*
PJLE 200	11.833± 3.894	22.833± 3.926	33.667± 3.728*
PJLE 400	9.833± 1.149	24.833± 2.691	40.666± 2.861**

Table IV: Effect of PJLE on blood glucose level in alloxan induced diabetes mellitus

Groups	Blood Glucose Level (mg/dL)			
	0 Day	7 Day	14 Day	21 Day
Normal	89.83± 3.41	87.17± 3.98	88.83± 3.429	88.17± 4.527
Diabetic control	201.5± 16.68	241.8± 31.95##	260.5± 26.209##	340.5± 28.51##
Glib10	174.67± 14.72	140.33± 14.37*	116.83± 14.99**	98.17± 6.074**
PJLE 100	251.17± 37.821	195.33± 19.82	182± 19.11	159.67± 9.5627*
PJLE 200	211.67± 23.431	179.33± 8.754	143.33± 6.829*	124± 4.830**
PJLE 400	170.33± 19.724	154.67± 7.44*	136.83± 6.529*	108.83± 5.59**

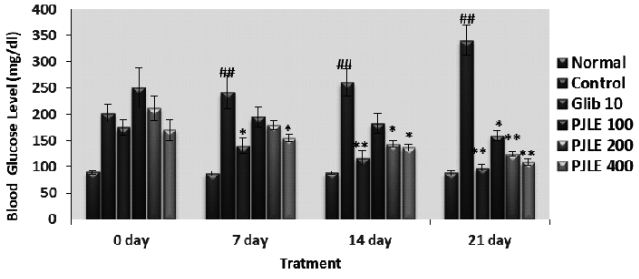

Fig. 6: Effect of PJLE on Blood Glucose Level in Alloxan Induced Diabetes Mellitus

from necrosis of islets, infiltration of Mucinous Non-neoplastic Cyst (MNC), reduction in the size of islets and acinar cell degeneration as compared with the normal group. Administration of glibenclamide (10 mg/kg, p.o.) showed a reduction in histopathological injury induced by alloxan as evident from the decrease in necrosis of islets, infiltration of MNC, reduction in the size of islets and acinar cell degeneration as compared with diabetic control. Administration of PJLE (100 mg/kg, p.o.) did not show a reduction in histopathological injury induced by alloxan as evident from the increase in necrosis of islets, infiltration of MNC, reduction in the size of islets and acinar cell degeneration as compared with diabetic control. Administration of PJLE (200 mg/kg, p.o.) showed a reduction in histopathological injury induced by alloxan as evident from the decrease in necrosis of islets, infiltration of MNC, reduction in the size of islets and acinar cell degeneration as compared with diabetic control. The effect was more prominent on reduction in the size of islets. Administration of PJLE (400 mg/kg, p.o.) showed a reduction in histopathological injury induced by alloxan, as evident from the decrease in necrosis of islets, infiltration of MNC, reduction in the size of islets and acinar cell degeneration as compared with diabetic control. The effect was more prominent on the infiltration of MNC, reduction in the size of islets and acinar cell degeneration.

DISCUSSION

Diabetes mellitus is a chronic disease which is characterized by hyperglycemia. Hyperglycemia caused due to deficiency of insulin or due to the presence of resistance to the insulin action at cellular level[17].

Diabetes requires long term control treatment without any side effects. A major problem in the treatment of diabetes is dependent a on medication for lifetime. The available synthetic drugs for the treatment of diabetes mellitus cause disturbances in body system which leads to side effects and imbalance in metabolic system[18].

Present drugs available for the treatment of diabetes mellitus showed side effects like nausea, diarrhoea, skin rashes, weight gain, infections of the respiratory tract, liver damage, hypertension and neuropathy[19].

Due to these side effects, there is a need to discover alternative therapies for the treatment of DM. Since ancient times, People have an interest in herbal medicine for the treatment of various diseases. Many herbal medicines have been used in the treatment of diabetes mellitus. Several studies have been carried out searching for

Table V: Effect of PJLE on SGOT and SGPT in Alloxan Induced Diabetes Mellitus.

Parameter	Normal	Diabetic Control	Glib 10	PJLE 100	PJLE 200	PJLE 400
SGOT (U/L)	46.308± 3.604	90.635± 6.374##	50.156± 3.38**	82.662± 6.695	68.968± 5.15*	54.806± 6.278**
SGPT (U/L)	35.494± 1.683	62.091± 2.116 ##	39.491± 1.989**	58.378± 2.453	53.99± 3.467	43.208± 3.238**
Total Bilirubin (mg/dL)	0.886± 0.090	1.423± 0.164 ##	0.91± 0.081**	1.096± 0.070*	0.955± 0.060**	0.93± 0.086**
Direct Bilirubin (mg/dL)	0.376± 0.033	0.910± 0.083 ##	0.494± 0.041**	0.887± 0.115	0.669± 0.04	0.461± 0.051**
Creatinine (mg/dL)	0.741± 0.049	1.551± 0.139 ##	0.751± 0.063**	1.12± 0.107*	1.097± 0.091*	0.881± 0.05**
Cholesterol (mg/dL)	91.878± 3.716	177.5± 19.028 ##	93.707± 8.448**	137.07± 12.337	121.91± 8.855*	97.09± 8.789**
Triglycerides (mg/dL)	149.1± 49.352	178.53± 19.833 ##	133.82± 16.251**	168.18± 19.675	154.58 ± 14.626*	145.3± 18.129**
Alkaline phosphate (IU/L)	102.73± 1.09	210.43± 17.272 ##	103.42± 9.561**	155.87± 13.941*	119.76± 11.982**	108.36± 12.915**
Lactate Dehydrate Level (IU/L)	693.9± 12.786	980.63± 154.57 ##	713.99± 98.502**	821.46± 105.84*	801.25± 96.362*	721.76± 94.726**
Total Protein (g/dL)	7.968± 0.343	6.572± 0.264 #	8.143± 0.339*	7.527± 0.248	8.073± 0.269*	8.235± 0.412*
Blood Urea Nitrogen (mg/dL)	15.244± 0.819	22.773± 1.312 ##	16.432± 0.358**	18.757± 1.434*	17.091± 0.784**	16.977± 0.562**

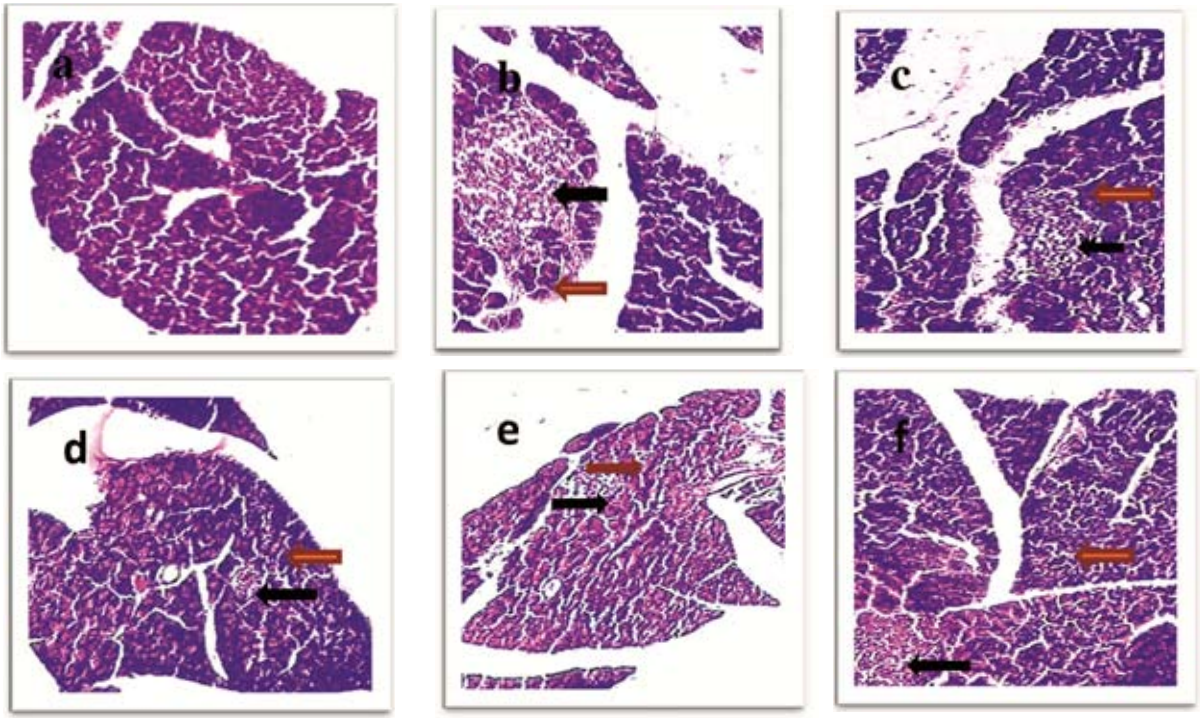

Fig. 7: Histopathological Observation of Pancreas Tissue in Alloxan Induced Diabetes Wistar rats

a- Normal pancreas, b- Diabetic control, c- Glib 10, d- PJLE 100, e- PJLE 200, f- PJLE 400. Black arrow- Necrosis of the islet. Orange arrow- Cellular infiltration. Stain- H and E.

Table VI: Effect of PJLE on weight of kidney, liver, and pancreas in alloxan induced diabetes mellitus

Groups	Liver weight (g)	Kidney weight (g)	Pancreas weight (g)
Normal	6.123	0.692	0.775
Diabetic control	7.942	0.798	1.066
Glib10	6.436	0.729	0.799
PJLE 100	6.892	0.781	1.002
PJLE 200	7.937	0.769	0.98
PJLE 400	6.435	0.859	0.809

suitable herbal drugs that would be a good antidiabetic agent. Recently, there is increasing in demand by patients to use natural drugs for the treatment of chronic diseases like diabetes mellitus due to safety, effectiveness and low cost[20].

Various plants have been investigated and have exhibited significant antidiabetic activity which has been scientifically reported to be effective in diabetes mellitus, like *Aegle marmelos, Cinnamomum tamala*, *Eugenia jambolana*, *Terminalia pallida* and *Syzygium cumini*[10,21,22,23].

Prosopis juliflora is a medicinally important herb of Indian origin. The hexane extract of *P.juliflora* pods was used in the treatment of DM[24].

Hypoglycemic effect of *P. juliflora is* implicated due to the presence of flavonoids, alkaloids, terpenes, tannins, amino acids and proteins. Tannins and flavonoids showed antioxidant activity. Antioxidant causes resistance to oxidative stress by scavenging free radicals inhibiting lipid peroxidation. The antioxidant activity of *P. juliflora* may be due to the presence of tannins and flavonoids[25].

This revealed that the leaves of *P. juliflora* would be a good antidiabetic agent to assess the therapeutic efficiency of diabetes mellitus. Hence the present study was undertaken to evaluate the effect of 50% ethanolic extract of leaves of *P. juliflora* in alloxan induced diabetic rat model.

Phytochemical investigation of PJLE revealed the presence of alkaloids, flavonoids, tannins, amino acids and proteins. The total phenolic content was detected by using folin-Ciocateu reagent by UV-Visible Spectrophotometer. The total phenolic content found in PJLE extract was 0.900 mg[6,7].

The standardization of extract was done by HPTLC. In this study; several solvent systems were used for individual estimation of the phenolics and flavonoids. From the solvent system investigations, mobile phase consisting of toluene: ethyl acetate: formic acid in the ratio of 5:1.5:1 V/V/V demonstrated the best resolution between other peaks of extract. The identification of Quercetin was confirmed by the bands from the test sample and standards. The test samples were scanned at 254 nm and 366 nm[8].

In the toxicity study of PJLE, no mortality was found at limit dose but toxicity was found at the upper dose. So the selected dose was 100 mg/kg, 200mg/kg, 400 mg/kg which is safe for further study. Alloxan induced diabetes model is a frequently used model. For evaluation of the hypoglycemic activity of PJLE, the same model was employed[1,21,26].

In this model, diabetes was induced in rats using Alloxan. Alloxan produces hyperglycemia by the selective cytotoxic effect on pancreatic β-cells and one of the intracellular phenomenons for its cytotoxicity is through the generation of free radicals, which has been demonstrated both *in vivo* and *in vitro*[27,28].

Diabetic animals showed a significant increase in serum glucose level due to overproduction of glucose through excessive hepatic glycogenolysis. Gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus and produces the hyperglycemic condition. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant decrease in serum glucose level as compared to diabetic control. To obtain the maximum effect of PJLE on the diabetic rats, the extract was administered orally daily once, at a dose of 100, 200, 400 mg/kg for 21 days, the period which produced a significant reduction in serum glucose level of diabetic animals and this effect was potent after repeated oral administration. These results confirm the previous findings that the effectiveness of the drugs depends, probably, on the cumulative effect of active principles[29].

Diabetic animals showed a significant decrease in body weight and produce weight loss in hyperglycemic condition. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant increase in body weight as compared to diabetic control. Diabetic animals showed a significant increase in SGOT, SGPT, total bilirubin, direct bilirubin and creatinine level in hyperglycemic condition. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant decrease in SGOT, SGPT, total bilirubin, direct bilirubin and creatinine level as compared to diabetic control[30].

Diabetes induced rats showed a significant increase in cholesterol and triglycerides levels in hyperglycemic

condition. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant decrease in cholesterol and triglycerides level as compared to diabetic control. Diabetes mellitus is usually associated with abnormal levels of serum lipids and such an increase causes the risk factor for coronary heart diseases[31,32].

Animals showed a significant decrease in total protein and Blood Urea Nitrogen level in the hyperglycemic condition of rats. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant increase in total protein and Blood Urea Nitrogen level as compared to diabetic control[33].

In diabetic animals, there is a decrease in serum protein level and increase in serum creatinine level. This is due to an increase in the catabolism of protein in diabetic condition. However PJLE treated groups show an increase in serum protein level and decrease in serum creatinine level by preventing the catabolism of protein. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant decrease in blood urea nitrogen level as compared to diabetic control[34].

Diabetes induced animals showed a significant increase in ALP and LDH level in hyperglycemic condition. Administration of PJLE (200, 400 mg/kg, p.o.) showed a significant decrease in ALP and LDH level as compared to diabetic control[10, 35].

The ethanolic extract of *P. juliflora* root is reported to possess free radical scavenging activity[36].

Given above, it can be proposed that the mechanism of hypoglycemic activity of PJLE may be its protective role in quenching free radicals (which destroy pancreatic cells) and thus a beneficial role in the survival of pancreatic β-cells. From the results of blood glucose level and other biochemical parameters, 200mg/kg and 400mg/kg are the two effective doses. But 400 mg/kg is the more effective dose showed in the histopathological results of the pancreas.

So, higher doses of the extract might have higher doses of hyperglycemic compounds. Thus, a dose PJLE 400mg/kg was found to be effective in diabetic animals.

Administration of alloxan produced histopathological injury to pancreatic tissue as evident from necrosis of islets, infiltration of MNC, reduction in the size of islets and acinar cell degeneration as compared with normal group. Administration of glibenclamide (10mg/kg) showed a reduction in histological injury as compared to diabetic control. Administration of PJLE (100 mg/kg) did not show histological changes while PJLE 200 and 400 mg/kg showed histological changes as compare to diabetic control group. The effect was more prominent on the infiltration of MNC, reduction in the size of islets and acinar cell degeneration.

CONCLUSION

PJLE was studied for preliminary phytochemical screening that revealed the presence of Alkaloids, flavonoids, tannins and amino acids. The standardization of extract was done by HPTLC which detected the quercetin i.e identified the flavonoid content in the extract. The results of the present study revealed that hypoglycemic effect showed by PJLE may be due to increased glucose utilization or decreased insulin resistance or increased insulin secretion from β-cells. This hypoglycemic effect of PJLE probably decreased the catabolism of proteins and fat and decreased the loss of tissue proteins leading to decreased muscle wasting. These effects may be prevented from the decrease in body weight due to hyperglycemia. The decreased catabolism of proteins is probably responsible for the increase in serum protein and the decrease in the formation of nitrogenous waste substances such as BUN and serum creatinine. The decreased catabolism of fat/lipids is probably responsible for the decreased serum cholesterol and triglycerides levels. The decrease in weight of liver, kidney and pancreas after the treatment with PJLE may be due to the prevention of inflammation, necrosis and infiltration of MNC due to the oxidative stress induced by alloxan. The protection against the histopathological injury of pancreas, as evident from the decrease inflammation, necrosis and infiltration of MNC in pancreatic tissue, also revealed the antioxidant potential of PJLE. Furthermore, decreased ALP, LDH, bilirubin, SGOT and SGPT levels suggested that PJLE was effectively protected the liver injury due to the oxidative stress induced by alloxan. The effectiveness of the PJLE might be due to the presence of flavonoids, alkaloids and tannins or due to synergistic action of these phytoconstituents. However, the exact role of phytochemicals and their mechanism of action needs future investigations.

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