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ANTI-DIABETIC ACTIVITY OF METHANOLIC (SEED) EXTRACT OF *PROSOPIS JULIFLORA (SW.)* DC IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

Prosopis juliflora could be a commercially important genus, that has been used since precedent days, notably for medicative functions. Traditionally, Paste, gum and smoke from woody stems, leaves and pods are applied for anticancer, antidiabetic, anti-inflammatory and antimicrobial purposes. The present study was carried out to investigate the antihyperglycemic properties of the methanolic seed extract of *Prosopis juliflora* in Streptozotocin (50 mg/kg intraperitoneal) induced diabetic rats for 28 days. The streptozocin induced diabetic wistar rats were fed with methanolic extract of *Prosopis juliflora* seeds at the increasing dosage of 200, 400 and 600 mg/kg. The positive control group was receiving GlIbenclamide 5mg/kg/day, per oral for 28days . Treatment of streptozocin induced diabetic wistar rats with the extract caused a significant reduction in the blood glucose level. Therefore for mechanistic evaluation we also check the inhibitory potential of methanolic extract of *Prosopis juliflora* against α-glucosidase, an important enzyme for diabetes management. The results indicated that methanolic extract at the concentration of 100 µg/mL inhibit the α-glucosidase activity**.** Furthermore we also check the toxicity of methanolic extract of an and we found that the extract at all the three tested doses (500, 1000, 1500) did not alter the lipid profile, liver function parameter and kidney function parameter in comparison to vehicle control. The dose of 600mg/kg showed maximum significant decrease as compared to other two doses. This result suggests that the methanolic extract of *Prosopis juliflora* possess antidiabetic effect on streptozocin induced diabetic Wistar rats.

K*ey words*: Hyperglycemia, methanolic extract, Streptozotocin.

Introduction

Diabetes mellitus (DM) may be a disorder ensuing from a defect in hormone secretion, hormone action, or both. Insulin deficiency successively ends up in chronic symptoms with disturbances of carbohydrate, fat and protein metabolism. It is a significant public health problem nowadays (Bastaki, 2005). The prevalence of diabetes is rising rapidly worldwide due to increased food consumption, decreased physical activity and widespread embrace of a western lifestyle (Elizabeth, 2013). Worldwide, around 150 million people are suffering from diabetes. The International Diabetes Federation (IDF) estimates the whole range of individuals in Asian country with polygenic disorder to be around fifty-eight million in 2010, rising to eighty-seven million by 2030. India is asserted as "Diabetes capital of the world." According to the World Health Organization (WHO) criteria, the prevalence of known diabetes was 5.6% and 2.7% among urban and rural areas, respectively (Ramachandran *et al*., 2010).

The uses of medicinal plants for the treatment of diverse diseases are actively practiced from ancient periods until today, wherever seasoning medicine is competitive with counterfeit medicine. There is a growing interest in herbal remedies due to the side effects associated with the existing therapeutic agents (Bhakuni *et al*., 1969). Systemic investigations on medicinal plants should, therefore, be carried out to identify new bioactive substances, which could be used as active therapeutic agents. *Prosopis juliflora* (Sw.) (Leguminosae), commonly known as mesquite is a shrub or small tree native to Mexico, South America and the Caribbean (Pasiecnik, 2001). Prosopis comprises 44 species distributed mainly in arid, semi-arid, tropical and subtropical countries (Astudillo *et al*., 2000). Many plants of the genus Prosopis

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are known to have medicinal properties and are used in folk medicine as astringents, in rheumatism and as remedies against scorpion stings and snake bites (Wassel *et al*., 1972). The members of the Prosopis species are abundantin phenols, piperidine alkaloids, flavonol glycosides, hydroxycinnamic acids, juliprosopine and mesquitol (Ahmad *et al.,* 1989 and Sirmah *et al.*, 2009). Some Prosopis species have antidermatophytic (Khan *et al*., 1989), antibacterial (Ahmad *et al*., 1986), antifungal (Ahmad *et al.*, 1989 and Tapia *et al.*, 2000), hemolytic (Kanthasamy *et al*., 1989), anti-inflammatory (Ahmad *et al.*, 1989), antihypercholesterolemic (Narasimhacharya A.V.R.L. *et al.*, 2010), antitumour (Batatinha, 1997) and antioxidant (Sirmah *et al.*, 2009) properties. As *P. juliflora* has ancient medicative values that haven't been absolutely accomplished, this study was carried out to investigate the antidiabetic potential of P. juliflora seed using *in vitro*, *in vivo* model systems. The seed extracts were prepared using Soxhletion followed by *in vitro* α-glucosidase activity. The *in vivo* potential was checked by using STZ- induced rat model. Further the acute toxicity of methanol extract was observed in rat.

Materials and Methods

Plant Material

The seed of *Prosopis juliflora* was procured in the month of December 2016 from Kisan Seed company, Lucknow India. The plant material was taxonomically identified by the National Botanical Research Institute (N.B.R.I.) Lucknow (Uttar Pradesh), India and the Voucher Specimen (LWG-77) were retained in Department for future reference.

Table 1: Qualitative analysis of phytochemicals present in the methanol extracts of *P. juliflora.*

S. No	Test	Observation	Inference
1	Alkaloids	Reddish-brown precipitate	+ve
2	Flavonoids	Yellow color	+ve
3	Terpenoids	Reddish-brown precipitate	+ve
4	Saponins	Froth formation	-ve
5	Steroids	Reddish brown color	+ve
6	Glycosides	No blue color develop	-ve

Table 2: Effect of the methanolic extract of *P. juliflora* on Lipid profile parameters.

S. No.	Dose	Triglyceride (mg/dl)	HDL (mg/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)
1	Control	287.56± 16.25	15.12±1.73	0.372± 0.035	104.17± 7.21
2	500mg	309.58 ± 24.12	17.48 ± 1.32	0.426±0.073	94.35± 5.25
3	1000mg	314.68 ± 28.52	16.97± 1.39	0.455± 0.034	98.32± 7.99
4	1500mg	316.21± 23.82	14.95± 2.86	0.411± 0.023	99.12±8.13

Preparation of Extract

The plants parts were shade dried, powdered and extracted individually with methanol by hot continuous using Soxhlet apparatus. The extract was filtered concentrated to dryness under vacuum on a rotary evaporator to give the dried residues of extract. Then the extracts were kept in a vacuum desiccator for complete removal of the solvent. The extracts were stored at 4°C in airtight glass vials untilfurther use.

Phytochemical analysis

The methanolic extract of *Prosopis juliflora* was screened for the presence of various phytochemical constituents such as alkaloids, flavonoids, saponins, glycosides, proteins, steroids and tannins according to the previously described method (Edeoga *et al*., 2005; Sofowora 1993).

Animal care and monitoring

The study was carried out on mixed sex of Wistar albino rats (150–200 g). Animals were obtained from CDRI, Lucknow U.P., India. They were housed at a temperature of 24 ± 2°C and relative humidity of 50% maintained on 12 h. light/dark cycle and allowed food and water *ad libitum*. Permission for the use of animal and animal protocol was obtained from the Institutional Animal Ethical Committee (IAEC) (No. 1585/PO/E/5/11/CPCSEA) as per the requirement of the Committee for Control and Supervision on Animals (CPCSEA), New Delhi.

Anti-diabetic activity

Experimental Induction of diabetes

After fasting for 18 h. 40 rats were injected by intraperitoneally with a single dose of 50 mg/kg streptozotocin after dissolving it in freshly prepared ice cold citrate buffer (pH 4.5). After the injection they had free access to feed and water and were given 5% glucose solution to drink over night to counter the hypoglycemic shock (Akbarzadeh *et al*., 2007). The development of diabetes was confirmed after 48 h. of the Streptozotocin injection. The animal having fasting blood glucose levels more than 200mg/kg was selected for the experimentation. Out of 40 animals three died before grouping and one was omitted from the study because of mild hyperglycemia. Remaining 36 diabetic animals were divided into 6 groups each having 6 rats.

Experimental protocol

Control (n=6): received distilled water *ad libitum* for the period of 28 days

Group 2: Diabetic rats (HFD: High Fat Diet, n=6): administered distilled water *ad*

Table 3: Effect of the methanolic extract of *P. juliflora* on Liver function parameters of rats.

S. No.	Dose	SGOT(U/L)	SGPT(U/L)	ALKP(U/L)
1	Control	40.15 ± 3.77	91.12± 3.68	98.72± 8.45
2	500mg	43.35± 2.89	93.23± 5.33	100.25 ±6.34
3	1000mg	47.12± 4.23	96.77± 8.43	103.66 ± 8. 55
4	1500mg	49.26 ± 6.98	101.51 ± 6.12	108.12± 8.98

Table 4: Effect of the methanolic extract of *P. juliflora* on Kidney profile parameters of rats.

S. No.	Dose	Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)	Uric Acid (mg/dl)
1	Control	1.54± 0.027	14.12± 1.11	2.98± 0.018
2	500mg	1.41± 0.021	14.35± 1.23	3.11 ± 0.024
3	1000mg	1.66 ± 0.029	14.72± 0.98	3.24 ± 0.028
4	1500mg	1.74± 0.068	15.09 ± 2.26	3.39± 0.033

Table 5: α-glucosidase inhibition by the methanolic extract of *P. juliflora.*

S. No.	Plant name	% Inhibition at 100 µg/mL phytoextract/standard[a]
		MeOH
3	*Methanol extract*	53.76 ± 1.18
Inhibitor (Acarbose)[b]		59.27 ± 2.64

[a]Values are presented as mean ± SEM of three different experiments in triplicates. [b]Standard used for this study

Table 6: Effect of methanolic extract of *P. Juliflora* (PJ) on body weight of HFD-STZ induced diabetic rats after a prolonged treatment

Groups	Bodyweight (g)	
	Initial	Final
Normal Control	163.28±8.40	172.38±9.85
Diabetic Control	148.05±9.70	142.07±12.72
Diabetic + PJ1	154.6±11.72	139.01±10.32
Diabetic + PJ2	152.2±13.50	137.07±15.80
Diabetic + PJ3	159.07±11.52	128.57±13.50
Diabetic + Glibenclamide	143.4±10.56	157.45±13.89

Values are expressed as means ± SD (n=6 rats).

libitum for period of 28 days.

Group 3: *P. juliflora* treated group:

Diabetic rats were administered methanolic extract of *P. Juliflora* (PJ) at different doses of 200, 400 and 600 mg/kg/day[14] and further divided into subgroups. Diabetic animals were treated with *P. juliflora* orally for 28 days.

Group 3a: Rats treated with 200mg/kg/d (PJ1) of methanolic extract of *P. juliflora* seed.

Group 3b: Rats treated with 400mg/kg/d (PJ2) of methanolic extract of *P. juliflora* seed.

Group 3c: Rats treated with 600mg/kg/d (PJ3) of methanolic extract of *P. juliflora* seed.

Group 4: Glibenclamide treated groups:

Diabetic rats were administered Glibenclamide 5 mg/kg/day, p.o., for 28 days.

Measurement of Bodyweight & Blood Glucose Level

All the groups of animals received the treatment for 28 days. The body weight and blood glucose level were measured at about every 1, 7, 14, 21 and 28 days interval. Blood samples were collected one hr. after the drug administration to determine the blood glucose level by electronic glucometer. Blood samples were obtained from retro-orbital plexus under light ether anaesthesia using in capillary tubes (Micro Hemocrit capillary, Mucaps) into Eppendorf tubes containing EDTA and serum was separated within 30 min after collection using centrifuge at 2000 rpm for 2min.

Acute toxicity Study

Different doses of the methanolic extract of *P. juliflora* (500, 1,000, 1,500, mg/kg) were injected intraperitoneal (i.p.) to rat (20–25 g) divided into separate groups, each consisting of six animals. The animals were observed for 7 days after administration of the extract for any acute toxicity symptoms, *e.g.*, behavioral symptoms. After seven days, the blood was collected from retero-orbital plexus. The serum was separated for biochemical analysis. In the acute toxicity study, methanolic extracts up to the dose of 500, 1000 and 1500 mg kg^{-1} of body weight did not exhibit any toxic symptoms.

Screening of plant extracts for α-glucosidase inhibition *in vitro*

The alpha-glucosidase enzyme reaction was performed using PNPG (*p*-Nitrophenylalpha-D-glucopyranoside) as a substrate. Together with 25 µg/ml reduced glutathione in 0.067 M potassium phosphate buffer, 0.2 unit/ml of alpha-glucosidase was treated with 250 µg/mL phyto extracts/standard/compound for 10

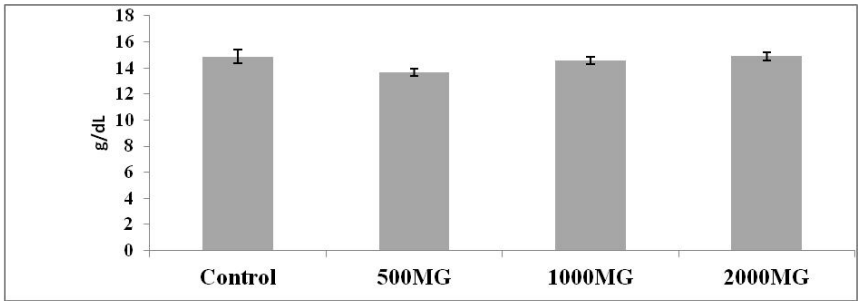

Fig. 1: Total Hemoglobin data

Table 7: Effect of Methanolic extract of *P. juliflora* and Glybenclamide on blood glucose.

Group No.	Groups	Blood Sugar Level				
		Long Term Study (Days)				
		1	7	14	21	28
1	Normal control	101 ± 1.849	104 ± 1.165	97 ± 1.361	95 ± 1.025	100 +_ 0.847
2	Diabetic control	329± 1.627###	324 ± 1.670*	321 ±2.208###	314 ± 2.091*	313+_2.107*
3	Methanolic extract (200 mg/kg)	335± 1.143*	317 ± 0.944	314±0.547	305± 2.405*	265+_2.490**
4	Methanolic extract (400 mg/kg)	338± 2.361*	257 ± 1.084**	184±1.067***	122± 1.392***	114+_0.477***
5	Methanolic extract (600 mg/kg)	342± 2.341*	246 ± 1.195***	179±1.077***	118± 1.401***	112+_0.398***
6	Glibenclamide(5 mg/kg)	321.8 ± 2.176	198.4 ± 3.49**	167.3± 2.77***	159.8± 0.24***	139+_0.956**'

min at 37°C, with the same volume of DMSO as a negative control and 250 µg/mL acarbose as a positive control. The final volume of the reaction solution was 0.2 ml. After then, 5µl of PNPG (0.116 M) was added to initiate the enzyme reaction. OD values of p-nitrophenol released from PNPG were detected at 400 nm after incubating the mixture at 37°C for 10 min in Spectra Max M2 spectrophotometer. Enzymatic inhibitory activity = (1-A_x/A_0) × 100% (A_0 represents the OD value of DMSO control and A_x represents those of samples being tested). Acarbose will be uses as standard with concentration of 1 mg/ml (M. mei Si *et al.*, 2010).

Discussion

The use of herbal drugs as complementary approaches in existing medications for the treatment of diabetes and its complications is growing worldwide and many plants in different countries are known to have antidiabetic effects (Hasani-Ranjbar *et al.*, 2008). The ancient Indian literature reports more than 800 plants with antidiabetic properties while ethnopharmacological surveys indicate that more than 1200 plants can be used for hypoglycemic activity (Mishra *et al.*, 2010). The present investigation was aimed to evaluate the hypoglycemic effects of methanolic extracts of *P. juliflora* on STZ-induced diabetes-mediated metabolic alterations in rats. The anti-diabetic potential of the extract and the reference drug in streptozotocin induced diabetes model in rats is shows that after streptozotocin administration, blood sugar in rats reached to a peak value and various doses of methanolic extract of *P. juliflora* produced a significant inhibition in the blood sugar at the end of 3, 7, 14, 21 and 28 dayas rspectivily. Maximum percent inhibition of sugar exhibited with 600 mg/kg of methanolic extract of *P. juliflora* and the effect was comparable to that of the standard drug. It has been reported that STZ administration produces partial destruction of pancreatic β-cells with permanent diabetes conditions (Aybar. *et al*., 2001). The results of preliminary phytochemical studies showed the presence of alkaloids, triterpenes, steroids, polyphenols and carbohydrates in methanolic extract of *P. juliflora*. Among them polyphenolics are the most reported phytoconstituents showing a wide range of pharmacological effects including antidiabetic activity (Saraf *et al*., 2007 and Bhattacharya, 2011). The presence of polyphenols or other phytoconstituents may be responsible for the promising antidiabetic activity of methanolic extract of *P. juliflora*.

Conclusion

The phytoconstituents present in this plant are tannins, phenolics, flavonoids, alkaloids, terpenes and steroids. This plant shows various pharmacological activities such as anti-bacterial, antifungal, anticancer, antioxidant and antidiabetic any many more. The present study suggests that the presence of flavonoids, phenolic compounds, alkaloids and other secondary metabolites are responsible for its pharmacological activities there for, the plant plays a vital role in maintenance of the human health and wellbeing.

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Refernces

Ahmad, A., K.A. Khan, V.U. Ahmad and S. Qazi (1986). Antibacterial activity of juliflorine isolated from *Prosopis juliflora*. *Planta. Med.*, **4:** 185.

Ahmad, A., A.K. Khursheed, Q. Sabiha and A. Viqaruddin (1989). Antifungal activity of some hydrosoluble *Prosopis juliflora* alkaloids. *Fitoterapia.*, **60:** 86-89.

Akbarzadeh, A., D. Norouzian, M.R. Mehrabi, Jamshidi, A. Farhangi,A.A. Verdi, S.M.A. Mofidian and B.L. Rad (2007). Induction of Diabetes by Streptozotocin in rats. Indian *Journal of Clinical Biochemistry*., **22(2):** 60- 64.

Astudillo, L., S.H. Guillermo, P.H. Juan and C. Manuel (2000). Proximate composition and biological activity of Chilean *Prosopis* species. *J. Sci. Food Agric.,* **80:** 567-573.

Aybar, M.J., A.N. Sαnchez Riera, A. Grau and S.S. Sαnchez (2001). Hypoglycemic effect of the water extract of *Smallantus sonchifolius* (yacon) leaves in normal and diabetic rats. *J. Ethno. Pharmacol*., **74:** 125-32.

Bastaki, S. (2005). Review diabetes mellitus and its treatment, *International Journal of Diabetes and Metabolism.*, **13:** 111-134. ISSN 1606- 7754.

Batatinha, M.J.M. (1997). Investigations on toxic influences of *Prosopis juliflora* D.C. (Algarobeira) on cell cultures as well as on ruminal fermentation in cattle (*in vitro*). Dissertation - Veterinary Medicine University, Hannover., 189.

Bhakuni, D.S., M.L. Dhar, M.M. Dhar, B.N. Dhawan and B.B. Mehrotra (1969). Screening of Indian plants for biological activity: part-II. *Indian J. Exp. Biol.,* **7:** 250-62.

Bhattacharya, S. (2011). Are we in the polyphenols era? Pharmacognosy Res., **3:** 147.

Chen, T.H., S.C. Chen, P. Chan, Y.L. Chu, H.Y. Yang and J.T. Cheng (2005). Mechanism of the polyglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. *Planta. Med*., **71:** 108-13.

Edeoga, H.O., D.E. Okwu and B.O. Mbaebie (2005). Phytochemical constit- uents of some Nigerian medicinal plants. *Afr. J. Biotechnol.,* **4:** 685-688.

Elizabeth, G.H. (2013). Hints of unique genetic effects for type 2 diabetes in India, *Diabetes journal.*, **62(5):** 1369-70. DOI: 10.2337/db12- 1836.

Elshater, A.E.A., M.M.A. Salman and M.M.A. Moussa (2009). Effect of Ginger Extract Consumption on levels of blood Glucose, Lipid Profile and Kidney Functions in Alloxan Induced-Diabetic Rats. *Egyptian Academy Journal of Biological Sciences*., **2(1):** 153-162.

Grodsky, G.M., G.H. Epstein, R. Fanska and J.H. Karam (1971). Pancreatic action of sulphonylureas. *Fed Proc*., **36:** 2719-28.

Hasani-Ranjbar S., B. Larijani and M. Abdollah (2008). A systematic review of Iranian medicinal plants useful in diabetes mellitus. *Arch. Med. Sci*., **4:** 285-92.

Kanthasamy, A., S. William and S. Govindasamy (1989). Hemolytic effects of *Prosopis juliflora* alkaloids. *Current Science.*, **58:** 142-144.

Khan, K.A., A.H. Farooqui, S.A. Rasool, V.U. Ahmad, S. Qazi and T.S. Haroon (1986). *In vitro* studies of antidermatophytic activity of juliflorine and its screening as carcinogen in a *Salmonella/Microsome* Test System. *Arzneimittel-Forsch./Drug Res.,* **36:**17.

M. mei Si *et al.*, 2010. "Insulin releasing and alpha-glucosidase inhibitory activity of ethyl acetate fraction of Acorus calamus in vitro and in vivo," *J. Ethnopharmacol.*, **128 (1):** 154-159.

Mishra, S.B., C.V. Rao and S.K. Ojha (2010). An analytical review of plants for anti diabetic activity with their Phytoconstituents and mechanism of action. *Int. J. Pharm. Sci. Res*., **1:** 1647-52.

Narasimhacharya, A.V.R.L., K.V. Nitesh and N.D. Tejal (2010). *Prosopis juliflora* as an antihypercholesterolemic agent. *J. Herbal Med. Toxicol.,* **4(1):** 31-34.

Pasiecnik, N.M., P. Felker, P.J.C. Harris, L.N. Harsh, G. Cruz, J.C. Tewari, K. Cadoret and L.J. Maldonado (2001). The *Prosopis juliflora-Prosopis pallida* Complex: A Monograph. *HDRA, Coventry*. UK., 172.

Ramachandran, A., R.C. Ma and C. Snehalatha (2010). Diabetes in Asia, The Lancet., 375(9712): 408-18. PMID: 19875164; DOI: 10.1016/S0140-6736(09),60937-5.

Randle, P.J., P.B. Garland, C.N. Hales and E.A. Newsholme (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.*, **1:** 785-9.

Saraf, S., M.S. Ashawat, S. Saraf and Flavonoids (2007). A nutritional protection against oxidative and UV induced cellular damages. *Pharmacogn. Rev.*, **1:** 30-40.

Sirmah, P., S. Dumarçay, E. Masson and P. Gerardin (2009). Unusual amount of (-)- mesquitol from the heartwood of *Prosopis. juliflora*. *Nat. Prod. Res.,* **23(2):** 183-189.

Sivajyothi, V., A. Dey, B. Jaykar and B. Rajkapoor (2008). Antihyperglycemic, antihyperlipidemic and antioxidant effect of *Phyllanthus rheedii* on streptozotocin induced diabetic rats. *Iran J. Pharm. Res*., **7:** 53-9.

Sofowora, E.A. (1993). Phytochemical assays. In: Medicinal plants and traditional medicine in Africa, 3rd edn. Spectrum Books Ltd, Nigeria., 150-153.

Tapia, A., G.E. Feresin, D. Bustos, L. Astudillo, C. Theoduloz and G.S. Hirschmann (2000). Biologically active alkaloids and a free radical scavenger from *Prosopis* species. *J. Ethnopharmacol.,* **71(1-2):** 241-246.

Tattersall, R. (1995). Targets of therapy for NIDDM. *Diabetes Res. Clin. Pract*., **8:** 49-55.

Wassel, A., M. Rizk and E.F. Abdel-Bary (1972). Phytochemical investigation of *Prosopis juliflora* D.C. - Flavonoids and Free sugars. *Qual. Plant. Mater. Veg.,* **1:** 119-121.

Yallow, R.S., H. Black, M. Villazan and S.A. Berson (1960). Comparison of plasma insulin levels following administration of tolbutamide and glucose. *Diabetes J*., **9:** 356-62.