ANTI-DIABETIC ACTIVITY OF METHANOLIC (SEED) EXTRACT OF
PROSOPIS JULIFLORA (SW.) DC IN STREPTOZOTOCCIN 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.

Key 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 abundantly phenols, piperidine alkaloids, flavonoids, glycosides, hydroxycinnamic acids, jujuprosopine 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 Soxhletation 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.

#### 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 until further 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 intraperitoneal 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>
<thead>
<tr>
<th>S. No.</th>
<th>Dose (mg/kg)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Bilirubin (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>287.56±16.25</td>
<td>15.12±1.73</td>
<td>0.372±0.035</td>
<td>104.17±7.21</td>
</tr>
<tr>
<td>2</td>
<td>500mg</td>
<td>309.58±24.12</td>
<td>17.48±1.32</td>
<td>0.426±0.073</td>
<td>94.35±5.25</td>
</tr>
<tr>
<td>3</td>
<td>1000mg</td>
<td>314.68±28.52</td>
<td>16.97±1.39</td>
<td>0.455±0.034</td>
<td>98.32±7.99</td>
</tr>
<tr>
<td>4</td>
<td>1500mg</td>
<td>316.21±23.82</td>
<td>14.95±2.86</td>
<td>0.411±0.023</td>
<td>99.12±8.13</td>
</tr>
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Table 2: Effect of the methanolic extract of P. juliflora on Lipid profile parameters.
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 retro-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*-Nitrophenyl alpha-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
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/A) × 100% (A represents the OD value of DMSO control and A represents that 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 days respectively. 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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