Antidiabetic effect of aqueous extract of *Withania coagulans* flower in Poloxamer-407 induced type 2 diabetic rats

Sudhanshu Kumar Bharti*1, Amit Kumar2, Neeraj Kumar Sharma1, Supriya Krishnan3, Ashok Kumar Gupta1,2 and Shree Ram Padamdeo1

1Department of Biochemistry, Patna University, Patna, Bihar, India.
2School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi, India.
3Department of Psychology, Banaras Hindu University, Varanasi, India.
4Department of Biotechnology, National Institute of Pharmaceutical Education and Research, EPIP Campus, Hajipur, Bihar, India.

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*Withania coagulans* (Indian cheese maker, Paneer dodi) is an important medicinal plant. In view of its varied therapeutic potential, it has also been the subject of numerous pharmacological investigations. The effect of aqueous extract of *W. coagulans* on insulin sensitivity in Poloxamer-407 (PX-407) induced type 2 diabetes mellitus (T2DM) rats was evaluated. The extract of *W. coagulans* (150 and 200 mg/kg body weight) was administered orally once a day for 5 weeks after the animals were confirmed diabetic (that is, 75 days after PX-407 injection). A group of citrate control rats (Group I) were also maintained. A significant increase in blood glucose, glycosylated haemoglobin (HbA1c), and serum insulin levels were observed in control rats. Treatment with *W. coagulans* extract reduced the elevated levels of blood glucose, HbA1c, and insulin in T2DM rats. In oral glucose tolerance test, we found a significant improvement in glucose tolerance in the rats treated with *W. coagulans* extract. The insulin sensitivity, that is, both peripheral and hepatic insulin resistance was assessed. *W. coagulans* extract treatment significantly improved insulin sensitivity index (KI TT) that was decreased in control rats. There was considerable rise in homeostasis model assessment of insulin resistance (HOMA-R) in control rats, whereas *W. coagulans* extract treatment significantly prevented the rise in HOMA-R in T2DM-treated rats. Our data suggest that aqueous extract of *W. coagulans* extract (200 mg/kg body weight) normalizes hyperglycemia in T2DM rats by improving insulin sensitivity.

Key words: Antidiabetic, type 2 diabetes, insulin resistance, hyperglycemia, glucose tolerance.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is possibly the world's fastest growing metabolic disorder that results from defects in insulin secretion (Kahn, 2001) on one side, and insulin resistance on the other side (Polonsky et al., 1996). About 90% diabetic patients are of T2DM with insulin resistance and are playing a key role in the development of disease (National Diabetes Statistics, 2011). The progression of T2DM begins with an impairment of glucose tolerance (Zimmet and Thomas, 2003) and is often associated with a state of insulin resistance (Robertson and Harmon, 2006). In recent years, there has been global upsurge in the clinical use of drugs from herbal sources. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders, including diabetes (Koehn and Carter, 2005). Management of T2DM without any side effect is still a challenge to the

*Corresponding author. E-mail: sudhanshu_bharti@rediffmail.com. Tel: +91-84-34364951.
medical system. Many oral hypoglycemic agents are available along with insulin for the treatment of diabetes (Holman and Turner, 1991). But these synthetic agents can produce serious side effects; furthermore, they are not suitable for use during pregnancy (Gilman et al., 1985; Rao et al., 1997; Valiathan, 1998). This leads to an increase in demand for natural products having antidiabetic activity with fewer side effects and are relatively economical as compared to oral hypoglycemic agents. It is assumed that herbal medicine can be effective alternative to oral hypoglycemic agents in the treatment of T2DM, where pancreatic islets are not totally destroyed (Koehn and Carter, 2005).

Withania coagulans Dunal (family: Solanaceae), commonly known as Indian cheese maker or Paneer dodi is widely used in Ayurvedic system of medicine for over 3,000 years in India (Indian Pharmacopoeia, 1985). The adaptogenic, hepatoprotective, anti-inflammatory, anti-hyperglycemic, hypolipidaemic (cardio-protective), antioxidant, antimicrobial, cardiovascular, central nervous system depressant, immunomodulating, anti-platelet (wound healing), antitumour, and cytotoxic activities of W. coagulans have been documented by Maurya et al. (2010), Ojha and Arya (2009) and Prasad et al. (2010). The aqueous extract of the fruits of W. coagulans has been shown to exert antiangiogenic (Mirjali et al., 2009) and antidiabetic (Jaiswal et al., 2010) activities. According to Jaleel et al. (2008) Triadimefon a triazole derivative plant growth regulator isolated from Withania somnifera can be used to enhance the antioxidant potential like superoxide dismutase, peroxidase, polyphenol oxidase, and catalase activities. Anwar et al. (2008) found improved insulin sensitivity index, that is, reduction in elevated blood glucose levels, glycated hemoglobin (HbA1c) and insulin in T2DM rats treated with W. somnifera extract. Hoda et al. (2010) found that aqueous extract of W. coagulans showed highly significant decrease (p < 0.01) in the blood glucose (52%), triglyceride, total cholesterol, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) level and highly significant increase (p < 0.01) in high density lipoprotein (HDL) level. They also observed anti hyperglycemic effect slightly superior (6%) to metformin.

However, the reports on the effect of W. coagulans extract on hyperinsulinemia, glucose intolerance, and insulin sensitivity are scanty in the literature. Therefore, the present study was conceived to investigate the effect of W. coagulans extract on hyperinsulinemia, glucose intolerance, and insulin sensitivity in T2DM model of rats.

MATERIALS AND METHODS

Experimental animals

Healthy albino Wistar rats were housed under good hygienic conditions and allowed to acclimatize for 15 days under controlled condition of illumination (a 12 h light/dark cycle) and temperature of 20 to 25°C. They were maintained on standard pellet diet (Lipton rat feed Ltd., Pune, India) and water ad libitum throughout the experimental period. The experimental study was approved by the Institutional Animal Ethics Committee of Jamia Hamdard, New Delhi, India.

Drugs and chemicals

The root extract of W. coagulans in powdered form was purchased from Natural Remedies, Bangalore, India. Poloxamer-407 (PX-407) (PX-407) was procured from Sigma Chemical Co., St. Louis, MO, USA. The enzyme-linked immunosorbent assay (ELISA) kit for insulin assay was purchased from Merodia (Uppsala, Sweden). Hyperlipidemic diet supplement like olive oil, cholic acid, and cholesterol were purchased from Zeel Pharmaceuticals (Mumbai, Maharashtra, India). All the other chemicals used for the experiment were of analytical grade.

Induction of diabetes

The animal model for the current study was based on multiple administration of freshly prepared PX-407. The PX-407 extract was dissolved in injectable distilled water and administered at a dose of 10 mg/kg body weight (in 1 mLM of cold citrate buffer at pH of 4.5) to an adult rat once a day for five week. For induction of diabetes, initially the normal rats were kept 24 h without food and water. The weights of normal rats were taken. After making the stock solution; 11 ml stock solution was taken in a beaker and 50.2 mg of PX-407 dissolved in it. Of this solution, 0.5 ml was injected to each rat intraperitoneally once a day for five week by insulin syringe; afterward, food and water was supplied. Rats with fasting blood glucose level of 200 mg/dl or higher were considered to be diabetic and were used in the study.

Experimental design

The rats were divided into five groups comprising six animals in each group as follows: Group I: Citrate control rats received citrate buffer (0.1 ml/10 g, intraperitoneally); Group II: PX-407 induced T2DM rats received PX-407 in multiple dose (10 mg/kg, intraperitoneally); Group III: PX-407 induced T2DM rats received W. coagulans extract (200 mg/kg, per orally); Group IV: PX-407 induced T2DM rats received W. coagulans extract (150 mg/kg, per orally); and Group V: W. coagulans extract- treated rats received W. coagulans extract (200 mg/kg, per orally).

W. coagulans extract (150 and 200 mg/kg) was dissolved in water and given until the end of the study (5 weeks) to groups III, IV, and V animals. During experiment, blood samples were collected by nicking the tip of tail for biochemical estimations.

Determination of blood glucose

Blood glucose level was estimated by glucose oxidase method (Braham and Trinder, 1972) using a commercial diagnostic kit from Span diagnostic Limited, Surat, India.

Determination of HbA1c level

HbA1c level was estimated according to the method of Bannon (1982) using a commercial diagnostic kit from Monozyme India Limited, Secunderabad, India.

Determination of insulin level

Plasma insulin level was estimated quantitatively by ELISA method.
Table 1. Effect of Withania coagulans extract on blood glucose and glycosylated haemoglobin levels in experimental rat groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
<th>Glycosylated haemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Citrate control</td>
<td>96.98 ± 3.01</td>
<td>5.680 ± 0.064</td>
</tr>
<tr>
<td>II</td>
<td>PX-407 (10 mg/kg, intraperitoneally)</td>
<td>325.06 ± 10.87</td>
<td>12.28 ± 0.324</td>
</tr>
<tr>
<td>III</td>
<td>PX-407 + W. coagulans extract (150 mg/kg, per orally)</td>
<td>150.91 ± 4.09</td>
<td>9.29 ± 0.117</td>
</tr>
<tr>
<td>IV</td>
<td>PX-407 + W. coagulans extract (200 mg/kg, per orally)</td>
<td>120.98 ± 1.78</td>
<td>6.94 ± 0.068</td>
</tr>
<tr>
<td>V</td>
<td>Only W. coagulans extract (200 mg/kg, per orally)</td>
<td>98.58 ± 1.17</td>
<td>5.68 ± 0.156</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± SE.; n = 6 in each group. ¹p ≤ 0.001, compared to the corresponding value for citrate control animals (group I). ²p ≤ 0.001, compared to the corresponding value for T2DM control animals (group II).

For this purpose, insulin ELISA kit was used.

**RESULTS**

Effect of *W. coagulans* extract on hyperglycemia and HbA₁c levels in experimental rat groups

Intraperitoneal administration of PX-407 to overnight fasted normal rats (Group II, III, and IV) caused marked elevations (p ≤ 0.001) in serum glucose and HbA₁c levels (Table 1) after 72 h. On its own, *W. coagulans* extract treatment (Group V) did not register any significant change in the blood glucose level as well as HbA₁c level when compared to citrate control rats (Group I). Regular oral administration of *W. coagulans* extract to rats of Groups III and IV at two doses (150 and 200 mg/kg) antagonized the remarkable alterations in blood glucose level in a dose-dependent manner.

Effect of *W. coagulans* extract on insulin level in experimental rat groups

The effect of *W. coagulans* extract on insulin level in experimental rats is as shown in Figure 1. Hyperinsulinemia was observed in T2DM control rats (Group II) when compared with citrate control rats (Group I). Treatment with *W. coagulans* extract significantly (p ≤ 0.001) reduces the elevated levels of insulin (Groups III and IV) when compared to T2DM control rats. On its own, *W. coagulans* extract treatment (Group V) did not induce any significant change in the level of insulin.

Effect of *W. coagulans* extract on OGTT in experimental rat groups

The blood glucose levels of experimental rats after oral administration of glucose (2 gm/kg) is as shown in Table 2. In T2DM control rats (Group II), the peak increase in blood glucose level was observed after 1 h and remained high over next 1 h. Treatment with *W. coagulans* extract for two doses (150 and 200 mg/kg) rats (Groups III and IV) showed significant (p ≤ 0.001) decrease in blood glucose level at 1 and 2 h when compared with T2DM control rats. Only *W. coagulans* extract treatment (Group V) did not register any significant change in the blood levels.
Effect of *W. coagulans* extract on insulin sensitivity in experimental rats

The level of $K_{ITT}$ (an index of peripheral insulin resistance) and the level of HOMA-R (an index of hepatic insulin resistance) is as shown in Figures 2 and 3, respectively. T2DM control rats (Group II) showed significant decrease in $K_{ITT}$ with significant increase in HOMA-R level when compared with citrate control rats (Group I). Treatment with *W. coagulans* extract significantly ($p \leq 0.001$) increased the level of $K_{ITT}$ and prevented increase in HOMA-R level in T2DM rats (Groups III and IV) when compared with control rats (Group II). There was no significant change in the levels of $K_{ITT}$ and HOMA-R in only *W. coagulans* extract-treated rats (Group V) and citrate control rats (Group I).

DISCUSSION

*Diabetes mellitus* is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. Treatment that is inadequate or instituted too late predisposes the affected individual not only to the basic metabolic disturbances but also to a number of serious complications of diabetes. PX-407 inductions to neonatal rats manifest hyperglycemia, an impaired response to the glucose tolerance test (Portha et al., 1979) and loss of $\beta$-cell sensitivity to glucose (Giroix et al., 1983). Therefore, PX-407 induced diabetes has been described as a useful experimental model to study the activity of antihyperglycemic agents (Szkudelski, 2001; Yamagishi et al., 2001). Hyper-insulinemia has generally been considered a marker of insulin resistance, that is, a decrease in the effect of insulin to stimulate glucose uptake at a given serum insulin concentration (Tenenbaum et al., 2003). Hence, in addition to glycaemic control, management of hyper-insulinemia is also essential for controlling insulin resistance and limiting the complications of T2DM.

In our conducted experiment, T2DM control rats (Group II) exhibited persistent hyperglycemia. Treatment with (150 and 200 mg/kg) of *W. coagulans* extract to T2DM rats reduced the elevated blood glucose level thereby showing its antihyperglycemic activity in a dose-dependent manner. Rats treated with 150 mg/kg *W. coagulans* extract showed a maximum fall of 55% in blood glucose level, whereas fall of 63% was observed...
Table 2. Effect of *W. coagulans* extract on oral glucose tolerance test in experimental rat groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Citrate control</td>
<td>82.81 ± 1.66</td>
<td>112.19 ± 2.07</td>
<td>149.49 ± 2.92</td>
<td>124.14 ± 1.69</td>
<td>98.43 ± 1.28</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>PX-407; 10 mg/kg, intraperitoneally</td>
<td>257.02 ± 5.85</td>
<td>301.51 ± 7.71</td>
<td>328.78 ± 5.68</td>
<td>342.97 ± 9.79</td>
<td>318.17 ± 6.82</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>PX-407 + <em>W. coagulans</em> extract (150 mg/kg, per orally)</td>
<td>140.90 ± 1.67</td>
<td>165.79 ± 1.48</td>
<td>189.56 ± 2.59</td>
<td>166.78 ± 1.87</td>
<td>150.50 ± 1.38</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>PX-407 + <em>W. coagulans</em> extract (200 mg/kg, per orally)</td>
<td>119.26 ± 2.17</td>
<td>138.21 ± 2.52</td>
<td>173.88 ± 5.73</td>
<td>150.33 ± 1.78</td>
<td>128.19 ± 2.94</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Only <em>W. coagulans</em> extract (200 mg/kg, per orally)</td>
<td>85.35 ± 2.68</td>
<td>120.20 ± 3.28</td>
<td>156.06 ± 3.54</td>
<td>127.79 ± 2.34</td>
<td>92.95 ± 3.55</td>
<td></td>
</tr>
</tbody>
</table>

The data are expressed in mean ± SE; n = 6 in each group. ¹p ≤ 0.001 compared to the corresponding value for citrate control animals (group I). ²p ≤ 0.001, compared to the corresponding value for T2DM control animals (group II).

with the dose of 200 mg/kg. Earlier dried fruit extract of *W. coagulans* has been shown to have hypoglycaemic activity in type 1 diabetic rats (Hemlatha et al., 2004).

In diabetes, there is an increased glycosylation of a number of proteins, including haemoglobin and β-crystalline of lens (Alberti, 1982). Measurement of HbA₁c has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes (Goldstein, 1995). The HbA₁c level was found to increase in T2DM control rats (Group II) when compared with citrate control rats (Group I). The two doses of oral administration of *W. coagulans* extract (150 and 200 mg/kg) decreased the HbA₁c level by 26 and 44%, respectively in a dose-dependent manner. Some studies have shown that *W. coagulans* extract has antioxidant properties, prevents lipid peroxidation (Chaudhary et al., 2003), and inhibit oxidative reactions associated with protein glycation (Elgawish et al., 1996). Administration of *W. coagulans* extract to T2DM rats reduced the glycosylation of haemoglobin by virtue of its free radical scavenging property and thus decreased the level of HbA₁c. A decrease in blood glucose level might also contribute to decreased level of HbA₁c in *W. coagulans* extract-treated T2DM rats.

Hyperinsulinemia appears to be a compensatory mechanism that responds to increased level of circulating glucose and is often associated with the progression to insulin resistance (Goldstein, 2002). The β-cells normally compensate insulin resistance by secreting more amounts of insulin to maintain glucose homeostasis. Bonora et al. (1983) reported that hyperinsulinemia is associated with decreased hepatic insulin clearance and hypersecretion of β-cells in mild glucose intolerance obese subjects. Our results clearly showed the condition of hyperinsulinemia in T2DM control rats (Group II). The hyperinsulinemia in T2DM rats is either due to decreased hepatic clearance of insulin or by down-regulation of insulin receptors and desensitizing post receptor pathways resulting in decreased insulin binding and degradation (Lautamaki et al., 2006). Even people with diabetes who take oral medication or require insulin injections to control their blood glucose level can have higher than normal blood insulin level due to insulin resistance. Despite hyperinsulinemia, the glucose level was greater in T2DM control rats than T2DM-treated rats. Treatment with *W. coagulans* extract significantly (p ≤ 0.001) reduces the elevated levels of insulin (Groups III and IV) when compared to T2DM control rats. Treatment with 200 mg/kg of *W. coagulans* extract cut down insulin level by 25% (Group IV), whilst fall of 21% was observed with the dose of 150 mg/kg. On its own *W. coagulans* extract treatment (Group V) did not induce any significant change in the level of insulin. Hence, *W. coagulans* extract treatment was found to be effective in reducing insulin level of T2DM rats by preventing hyperinsulinemia.

An insulin resistance state is a key phase of metabolic syndrome, constituting the major risk factor for the development of glucose intolerance and diabetes mellitus (Groop, 2000). Thus, interventions to decrease insulin resistance may postpone the development of T2DM and its complications. When animals were subjected to OGGT, glucose disposal was found to significantly decreased and increased blood glucose was maintained up to 2 h in T2DM control rats (Group II). Treatment with *W. coagulans* extract for two different doses, rats (Groups III and IV) showed significant (p ≤ 0.001) decrease in blood glucose level at 1 and 2 h when compared with T2DM control rats. With 150 mg/kg *W. coagulans* extract treatment (Group III) blood glucose was found to diminish by 12 and 21% at 1 and 2 h, respectively, whilst 14 and 26% reduction was observed after 1 and 2 h, respectively with 200 mg/kg *W. coagulans* extract treatment (Group...
Figure 2. Effect of *Withania coagulans* extract on $K_{ITT}$ level in experimental rat groups. The data are expressed as mean ± S.E. ($n = 6$). *$p \leq 0.001$ as compared to citrate control rats (group I); **$p \leq 0.001$ as compared to T2DM-treated rats (group II).

Figure 3. Effect of *Withania coagulans* extract on HOMA-R level in experimental rat groups. The data are expressed as mean ± SE ($n = 6$). *$p \leq 0.001$ as compared to citrate control rats (group I); **$p \leq 0.001$ as compared to T2DM-treated rats (group II).
IV). Only *W. coagulans* extract treatment (Group V) did not register any significant change in the blood glucose level at 1 and 2 h during OGTT when compared with citrate control rats (Group I). Treatment with *W. coagulans* extract significantly improved glucose tolerance, as indicated by reduction in peak blood glucose level at 1 and 2 h in T2DM treated rats during OGTT. The extract of *W. coagulans* might enhance glucose utilization by peripheral tissues and increase the glycogen stores in the liver due to restoration of delayed insulin response, because it significantly decreased the blood glucose level in glucose loaded rats.

The results of this study showed that *W. coagulans* extract decreased blood glucose level, prevented hyperinsulinemia, and improved glucose tolerance in T2DM rat model.

Thus, $K_{ITT}$ and HOMA-R levels were determined to check insulin sensitivity. $K_{ITT}$ is used to assess peripheral insulin resistance (Bolzan and Bianchi, 2002), whereas HOMA-R is a useful clinical index of hepatic insulin resistance (Bonora et al., 2000). Treatment with *W. coagulans* extract significantly ($p \leq 0.001$) increased the level of $K_{ITT}$ and prevented increase in HOMA-R level in T2DM rats (Groups III and IV) when compared to T2DM control rats. With 150 mg/kg *W. coagulans* extract treatment (Group III) $K_{ITT}$ and HOMA-R level was found to be increased by 20% and diminished by 21%, respectively, whilst 60% increment and 74% reduction, respectively was observed later with 200 mg/kg *W. coagulans* extract treatment (Group IV). The results obtained clearly showed that $K_{ITT}$ was significantly improved by *W. coagulans* extract treatment to T2DM rats. Additionally, *W. coagulans* extract treatment significantly prevented the rise in HOMA-R in T2DM-treated rats. These findings suggest that *W. coagulans* extract is pharmacologically effective in improving insulin sensitivity.

If these results are extrapolated in human beings, then *W. coagulans* extract might prove useful in the treatment and/or prevention of hyperinsulinemia, impaired glucose tolerance and insulin resistance, and in addition, being an effective means of controlling glucose level.

**REFERENCES**


