Short Communication

Effect of *Thespesia populnea* Linn. on dexamethasone induced insulin resistance in mice

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In traditional medicine, plants have been used to treat diabetes mellitus as valuable alternative therapy since synthetic oral hypoglycemic agents are known to exhibit adverse side effects. The present study was planned to investigate the effect of ethanolic extract of bark of *Thespesia populnea* Linn (TP) on plasma glucose, serum triglyceride and body weight in dexamethasone-induced insulin resistance in mice. The animals were divided into diabetic and non-diabetic groups consisting of six animals in each group. TP was administered at doses of 100, 200, and 400 mg/kg per oral (p.o.) in mice which were concomitantly treated with pre-standardized dose of dexamethasone (1 mg/kg intramuscular (i.m.)) for 22 days and effect on plasma glucose, serum triglyceride level and change in body weight were recorded. TP showed significant decrease in plasma glucose (p<0.01), serum triglyceride (p<0.01) levels and significant increase in body weight (p<0.01) as compared to dexamethasone control group. This study revealed that the ethanolic extract of bark of *T. populnea* may prove to be effective in the treatment of insulin resistance owing to its ability to decrease peripheral insulin resistance and can be used in the treatment of type-II diabetes mellitus.

**Key words:** *Thespesia populnea*, dexamethasone, insulin resistance.

INTRODUCTION

Diabetes mellitus has been a common problem of the world from centuries. It is a disease related to the sweetness, characterized by the presence of excessive sugar in blood and urine due to deficiency in the production of insulin by ‘β’ cell of pancreas or presence of ineffective insulin. The growth of disease is rapid due to the heredity, endocrine imbalance, dietary imprudence, severe and continued mental stress, and reduction in physical labour and differences in social structure, etc., which is providing a productive atmosphere to diabetes (Gupta et al., 2006).

Insulin resistance has emerged as a far reaching endocrine phenomenon which signifies an impaired biological response and insulin resistance is known to underlie the
deterioration of glucose homeostasis that lead to typical forms of type-II diabetes mellitus. The development of insulin resistance met initially with compensatory increase in insulin secretion (Bailey, 1999). Worldwide, over 1200 species of plants have been recorded as traditional medicine for diabetes (Bailey, 1999). Some of these plants have been evaluated in laboratories and in a number of cases their efficacy has been confirmed, for instance, *Panax ginseng* (ginseng), *Opuntia cactus* (cactus), *Tecoma stans* (trompeta), *Syzygium cumini* (jambolao) (Boden et al., 2001). Specific chemical constituents of these plants, such as polysaccharides, alkaloids, triterpenoids and xanthones, are believed to be responsible for the hypoglycemic effects and they can be related to actions including increased insulin release and increased glucose metabolism in the body periphery, among others (Wang and Ng, 1999).

The therapeutic potential of *Thespesia populnea* Linn is known from many years, and it has key place in Ayurvedic medicine (Anonymous, 2006). The alcoholic extract of unripe fruits reported for the antibacterial activity (Gaidn and Bapna, 1967), anti-hyperglycemic (Satyanarayana et al., 2004), and wound healing potential (Nagappa and Binu, 2001). The bark has been reported for anti-oxidant activity (Ilavarasan et al., 2003), antinociceptive and anti-inflammatory activity (Vasudevan and Parle, 2006), alzheimers disease (Vasudevan et al., 2007), and antidiarrheal activity (Viswanatha et al., 2007). The bark contains thespesone, which is having 2-hydroxy-2, 4, pyranonaphthaquinone as a parent nucleus. Various plants like *Lawsonia alba* having this nucleus are reported for their anti-diabetic potential (Neeli et al., 2007). Hence, considering these correlation and traditional claims related to this plant, the ethanolic extract was evaluated for dexamethasone induced insulin resistance in mice.

**EXPERIMENTAL**

**Plant material and preparation of extract**

The bark of *T. populnea* Linn. (Malvaceae) was collected from fields near Dehu Road in Pune, Maharashtra. The specimen was authenticated at Agharkar Research Institute, Pune with voucher specimen No. Auth08-006 and was documented. The bark was dried in shed and then powdered. The ethanol extract was prepared using soxhlet apparatus and concentrated under vacuum. The yield of ethanol extract of whole plant of *T. populnea* (TP) was 13.45% w/w. TP when subjected for phytochemical study showed the presence of flavonoids, beta-sitosterol, terpenoids and tannins (Viswanatha et al., 2007).

**Animals**

Albino mice weighing 25 to 30 g were used for the study and were kept in animal house at 26 ± 2°C with relative humidity 44 to 56% along with light and dark cycles of 12 h, respectively. Animals were provided with standard diet and *water ad libitum*. The food was withdrawn 18 to 24 h before the start of the experiment.

**Design**

**Acute toxicity study**

The acute toxicity study for ethanol extract of *T. populnea* was performed using albino mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. TP was administrated orally in increasing doses and found safe up to the dose of 2,000 mg/kg (OECD, 2001).

**Dexamethasone-induced insulin resistance in mice**

All the mice were weighed before treatment, group I (Normal control) received 1% gum acacia (1 ml/kg per oral (p.o.)), and 36 mice were rendered hyperglycemic by daily administration of a prestandardised dose of dexamethasone (1 mg/kg intramuscular (i.m.)) for consecutive 7 days and then divided into six groups of six animals in each. Group II (Dexa-control) continued to receive only dexamethasone and 1% gum acacia (1 ml/kg, p.o.), groups III and IV received ketoconazole (24 mg/kg, p.o.) and pioglitazone (2 mg/kg, p.o.) along with dexamethasone, respectively. Groups V, VI, and VII were treated with dexamethasone along with three different doses of TP 100, 200, and 400 mg/kg, p.o., respectively. Simultaneously, five other groups (Groups VIII, IX, X, XI, and XII), each with six normoglycemic animals, were administered equivalent amount of ketoconazole and pioglitazone and three different doses of TP 100, 200, and 400 mg/kg, p.o., respectively (Gholap and Kar, 2005; Shalam et al., 2006).

**Biochemical analysis**

On the last day, all the animals were weighed. Blood samples were collected and plasma and serum separated for estimation of glucose and triglyceride, respectively. Biochemical estimation of plasma glucose and serum triglyceride was done by glucose oxidase (GOD)/POD and glycerol-3-phosphate oxidase (GPO)/PAD methods, respectively using standard diagnostic kits (Crest Biosystems, Goa, India).

**Statistical analysis**

The results were expressed as mean ± standard error of mean (SEM) and statistically analyzed by ANOVA followed by Dunnett test, with level of significance set at p<0.05 and p<0.01.

**RESULTS AND DISCUSSION**

**Effect on plasma glucose level**

In dexamethasone induced insulin resistance, it was found that the Dexa group showed significant increase (p<0.01) in plasma glucose level when compared with normal control group. Dexa + Keto and Dexa + Pio groups showed significant decrease (p<0.01) in plasma glucose level when compared with Dexa control group. Dexa + TP-400 group showed significant decrease in plasma glucose (p<0.01) when compared with Dexa control group. Dexa + TP-200 group showed significant decrease in plasma glucose (p<0.05) when compared with Dexa control group. In non-diabetic animals, Keto
Table 1. Effect of *T. populnea* on plasma glucose, serum triglyceride and body weight in dexamethasone induced insulin resistance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose (mg/dl)</th>
<th>Serum triglyceride (mg/dl)</th>
<th>Body weight change (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>54.68 ± 0.51</td>
<td>83.68 ± 1.17</td>
<td>0.88 ± 0.05</td>
</tr>
<tr>
<td>Dexa-control</td>
<td>80.29 ± 0.30**</td>
<td>144.38 ± 2.42**</td>
<td>-2.17 ± 0.13**</td>
</tr>
<tr>
<td>Dexa + Keto</td>
<td>65.02 ± 0.65**</td>
<td>92.65 ± 1.96**</td>
<td>0.98 ± 0.05**</td>
</tr>
<tr>
<td>Dexa + Pio</td>
<td>55.29 ± 0.94**</td>
<td>85.48 ± 1.91**</td>
<td>0.98 ± 0.07**</td>
</tr>
<tr>
<td>Dexa + TP-100</td>
<td>76.98 ± 0.50</td>
<td>139.76 ± 1.16</td>
<td>-1.88 ± 0.11</td>
</tr>
<tr>
<td>Dexa + TP-200</td>
<td>76.46 ± 0.21*</td>
<td>138.13 ± 1.22</td>
<td>-1.86 ± 0.08</td>
</tr>
<tr>
<td>Dexa + TP-400</td>
<td>71.77 ± 1.31**</td>
<td>121.02 ± 1.92**</td>
<td>0.54 ± 0.10**</td>
</tr>
<tr>
<td>Keto</td>
<td>46.73 ± 0.63**</td>
<td>85.75 ± 1.92</td>
<td>0.99 ± 0.12</td>
</tr>
<tr>
<td>Pio</td>
<td>53.51 ± 0.51</td>
<td>81.87 ± 1.83</td>
<td>0.98 ± 0.07</td>
</tr>
<tr>
<td>TP-100</td>
<td>56.20 ± 0.68</td>
<td>88.48 ± 1.08</td>
<td>0.96 ± 0.13</td>
</tr>
<tr>
<td>TP-200</td>
<td>54.15 ± 0.34</td>
<td>88.76 ± 1.34</td>
<td>1.18 ± 0.13</td>
</tr>
<tr>
<td>TP-400</td>
<td>52.35 ± 0.48</td>
<td>89.59 ± 1.04</td>
<td>1.08 ± 0.16</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6), ANOVA followed by Dunnett test. **p<0.01 when compared with normal control, *p<0.05, *p<0.01 when compared with Dexa-control. Normal control: Recieved 1% Gum Acacia (1 ml/kg, p.o.); Dexa: Dexamethasone (1 mg/kg, i.m.); Keto: Recieved Ketoconazole (24 mg/kg, p.o); Pio: Recieved Pioglitazone (2 mg/kg, p.o); TP-100, TP-200 and TP-400: Received ethanol *T. populnea* extract 100, 200, and 400 mg/kg, respectively. SEM: Standard error of mean.

All the non-diabetic groups treated with TP and Pio did not show any significant decrease in plasma glucose when compared with normal control.

Effect on serum triglyceride level

In this study, it was found that Dexa group showed significant increase (p<0.01) in serum triglyceride level when compared with normal control group. Dexa + Keto and Dexa + Pio groups showed significant decrease (p<0.01) in serum triglyceride level when compared with Dexa control group. Dexa + TP-400 group showed significant decrease in serum triglyceride (p<0.01) when compared with Dexa control group. All the non-diabetic groups treated with Pio, Keto and TP did not show any significant decrease in serum triglyceride when compared with normal control.

Effect on body weight

It was found that Dexa group showed significant decrease (p<0.01) in body weight when compared with normal control group. Dexa + Keto and Dexa + Pio groups showed significant increase (p<0.01) in body weight when compared with Dexa control group. Dexa + TP-400 group showed significant increase (p<0.01) in body weight when compared with Dexa control group.

Insulin resistance is defined where a normal or elevated insulin levels produces an attenuated biological response and it refers to acute regulation of carbohydrate metabolism, which leads to impaired insulin sensitivity to insulin mediated glucose disposal (Reaven, 2004). It generally occurs systemically for e.g. in liver or it may occur locally in e.g. adipose tissue or in skeletal muscle (Nandi et al., 2004), and evidence is now accumulating that ectopic lipid accumulation is the central feature of insulin resistance (Phipelx and Mensink, 2008). Insulin normally lowers the level of blood glucose through the suppression of hepatic glucose production and stimulation of peripheral glucose uptake, but the dysfunction in any step of this process can result in insulin resistance. In healthy individuals, an increased gluconeogenesis is compensated by a decreased glycogenolysis, due to concomitant hyperinsulinemia, thereby maintaining hepatic glucose output at the same levels, called hepatic auto regulation (Clore et al., 1991). But in type-II diabetes mellitus, a breakdown of hepatic auto regulation is suggested to underlie the increased glucose output (Boden et al., 2001).

The influence of glucocorticoids on insulin sensitivity is the most important in syndromes of cortisol excess (Cushing’s syndrome) or deficiency (Addison’s disease). In Cushing syndrome, the patient develops glucose intolerance and central obesity, while Addison’s disease is associated with increased tissue in insulin sensitivity. There are numerous potential sites of action of glucocorticoids to affect insulin sensitivity. The principal effects of glucocorticoids are to oppose the actions of insulin in the regulation of carbohydrate, lipid and protein metabolism by effects of three main target tissues of liver, skeletal muscle, and fat (Andrews and Walker, 1999). Glucocorticoids increase blood glucose by mobilizing substrates for hepatic gluconeogenesis and stimulate the release of amino acids from skeletal muscle, fatty acids and glycerol from adipose tissue and increase the expression of gluconeogenic enzymes, such as phosphoenolpyruvate.
carboxykinase (PEP-CK) (Hanson and Reshef, 1997); hence, enhancing gluconeogenesis in liver. Glucocorticoids stimulate glyceroneogenesis by activating glycogen synthase and inactivating glycogen mobilizing enzyme glycogen phosphorylase. Glucocorticoids inhibit peripheral glucose uptake and utilization partly as a result of decreased translocation of glucose transporters (GLUT 4) to cell surface (Dimitriadis et al., 1997). Acute effects of glucocorticoids provide gluconeogenic substrates for fat metabolism which result in stimulation of lipolysis. Glucocorticoids increase triglyceride levels causing imbalance in lipid metabolism leading to heperlipidemia with increase in glucose levels leading to hyperglycemia (Shalam et al., 2006). Various pharmacological doses of glucocorticoids induce ob gene expression within 24 h which is followed by reduction in body weight, and development of insulin resistance (Shalam et al., 2006).

Intracellular triglycerides and products of fatty acids result in acquired insulin resistance state which results in the lipotoxic effect due to decrease in activity of lipoprotein lipase activity which results in decrease in insulin signalling pathway. There occurs an inhibitory interaction between two major fluids, glucose and free fatty acids which leads to insulin resistance. These interactions constitute a mechanism beyond hormonal regulation for controlling the circulating hormones like insulin, corticosteroid, and adrenalin can modify the control.

In this study, dexamethasone administration resulted in increased blood glucose and triglyceride level and decrease in body weight. TP prevented the rise in blood glucose and triglyceride level and decreases in body weight might be because of significant increase in glucose uptake which might be due to increase in the insulin sensitivity.

In conclusion, it was observed that ethanolic extract of the bark of T. populnea may prove to be useful in treatment of conditions like type II diabetes mellitus (NIDDM) probably by overcoming the insulin resistance.

**Competing Interests**

The authors hereby declare that there are no competing interests.

**REFERENCES**


