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ORIGINAL ARTICLE

Effect of *Psoralea corylifolia* on dexamethasone-induced insulin resistance in mice

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Abstract The whole plant of *Psoralea corylifolia* (PC) is traditionally used in the treatment of diabetes. Mice were treated with prestandardised dose of dexamethasone for 22 days and effect of PC at the doses of 100, 200 and 300 mg/kg, p.o. on plasma blood glucose level, serum triglyceride level, glucose uptake in skeletal muscle, levels of hepatic antioxidant enzymes (GSH, SOD, catalase and LPO), and body weight were observed. PC showed significant decrease in plasma glucose and serum triglyceride levels ($p < 0.01$) at the dose of 100 and 200 mg/kg, p.o. and also stimulated glucose uptake in skeletal muscle. The levels of antioxidant enzymes GSH, SOD, and catalase were significantly increased ($p < 0.01$) and there was significant decrease ($p < 0.01$) in level of LPO.

Hence it can be concluded that *Psoralea carylifolia* may prove to be effective in the treatment of Type-II Diabetes mellitus owing to its ability to decrease insulin resistance.

1. Introduction

Diabetes mellitus (DM) is the most common endocrine disorder. More than 150 million peoples are suffering from it worldwide and this is likely to be increase to 300 million by the year 2025. More than one-fifth of them are Indians. According to the International Diabetes Federation, India has been declared as "Diabetic Capital of the World" at the recent conference in Paris. Plants have been used as sources of drugs for the treatment of diabetes in developing countries, where the costs of conventional medicines are a burden to the population.

Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have found to be useful to successfully manage diabetes. One of the greatest advantages of traditional medicinal plants is that these are readily available and have no side effects. Even WHO has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas in which we lack safe modern drugs.

Glucocorticoids in excess inhibit insulin secretion from pancreatic beta-cells, decrease glucose utilization and stimulate glucagons secretion, lipolysis, proteolysis and hepatic glucose

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production. Glucocorticoids can modulate the insulin action at both binding sites and post binding sites and cause decreased glucose utilization in muscles. Glucocorticoids also cause insulin resistance by decreasing hepatic glucose utilization and decreasing glycogen synthesis. Free fatty acids may be elevated in insulin resistance because of impaired insulin-dependant down-regulation of lipolysis, hence leading to increase in triglyceride levels in muscles as well as in other tissues presumably because of excess of circulating free fatty acids, which are then deposited in these organs. The triglycerides are reported to be potent inhibitor of insulin signaling and result in acquired insulin resistance state (Andrews and Walker, 1999).

Traditionally, *Psoralea corylifolia* is used in the treatment of diabetes, lipid disorders, inflammation, ulcer and bronchitis (Kirtikar and Basu, 2005). *P. corylifolia* is reported to have antifungal (Xingyong et al., 2006), antidepressant (Chen et al., 2007), antioxidant, and antibacterial activity (Naznin and Khatunea, 2004). Taking into consideration the traditional claims and reported activities, PC has been studied for its anti-diabetic activity in diabetic animals. Hence the present study was planned to investigate the effect of *P. corylifolia* on dexamethasone-induced insulin resistance in mice.

2. Materials and methods

2.1. Plant material and preparation of extract

Fresh plant of *P. corylifolia* was collected from Aurangabad, Maharashtra, India. The specimen was authenticated at Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, with voucher specimen No. 08–12 and cataloged. The Plant was washed with distilled water and shade dried and latter powdered. This powder was then defatted with petroleum ether and then macerated with methanol for 72 h with occasional shaking. It was then filtered and the solvent was evaporated under vacuum. The yield of methanolic extract of *P. corylifolia* (PC) was 3.4% (w/w). PC when subjected for phytochemical study showed the presence of beta-sitosterol, terpenoids, phenolic compounds, saponins, glycosides and tannins.

2.2. Animals

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

2.3. Experimental design

2.3.1. Acute toxicity study (OECD, 425)

The acute toxicity study for methanolic extract of *P. corylifolia* was performed using albino mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. PC was found safe up to dose of 3000 mg/kg, orally.

2.3.2. Dexamethasone-induced insulin resistance in mice

All the mice were weighed before treatment, group I (normal control) received equivalent amount of 1% gum acacia (1 ml/kg, p.o.), and 30 mice were rendered hyperglycemic by daily administration of a prestandardised dose of dexamethasone (1 mg/kg, intramuscular) for consecutive 7 days and then divided into five groups of six animals each. Group II (DEXA-control) continued to receive only dexamethasone and 1%gumacacia (1 ml/kg, p.o.) for next 15 days, III received Pioglitazone (2 mg/kg, p.o.) along with dexamethasone respectively for 15 days. Groups IV–VI were treated with dexamethasone along with three different doses of PC100, 200, 300 mg/kg, p.o. respectively for 15 days. Simultaneously four other groups (groups VII–X), each with six normoglycemic animals, were administered equivalent amount of Pioglitazone and three different doses of PC100, 200, 300 mg/kg, p.o. respectively (Table 1). On the last day, after overnight fasting, all the animals were weighed and later sacrificed by cervical dislocation. Blood samples were collected and used for estimation of glucose and triglyceride (Gholap and Kar, 2005). Biochemical estimation of plasma glucose and serum triglyceride was done by GOD/POD and GPO/POD method respectively using standard diagnostic kits from Rajesh Chemical Ltd., India.

2.3.3. Hepatic antioxidant enzymes assay (estimation of MDA, GSH, SOD, and CAT)

Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Liver homogenates (5%, w/v) were prepared in cold 50 mM Tris buffer (pH 7.4) using Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 5000 rpm for 10 min using a Remi refrigerated centrifuge. The supernatant was used for the estimation of GSH (Ellaman, 1959), malondialdehyde (MDA) (Slater and Sawyer, 1971), superoxide dismutase (SOD) (Mishra and Fridovich, 1972) and catalase (Aebi and Bergmeyer, 1974; Colowick et al., 1984) levels (Table 2).

2.3.4. Effect on glucose uptake in isolated mice hemidiaphragm

Glucose uptake in mice hemidiaphragm was estimated by the method described by Chattopadhyay et al. (1992) with some modification. Twelve sets, containing graduated test tubes ($n = 6$) each, were used for study of non-insulin assisted and insulin assisted glucose uptake. The diaphragms were taken out quickly avoiding traumas and divided into two halves. The hemidiaphragms were rinsed in cold Tyrode solution (without glucose) to remove any blood clots. In non-insulin assisted glucose uptake study, one hemidiaphragm of each animal from groups I to VI was exposed to 2 ml Tyrode solution with glucose (2000 mg/l) in respective graduated test tubes. In insulin assisted glucose uptake study, the remaining hemidiaphragm of each animal from groups I to VI was exposed to 2 ml Tyrode solution with glucose (2000 mg/l) + insulin (0.25 IU/ml) in respective graduated test tubes. All the graduated test tubes were incubated for 30 min at 37 °C in an atmosphere of 95% O_2–5% CO_2 with shaking at 140 cycles per minute. Following incubation, the hemidiaphragm was taken out and weighed. The glucose content of the incubated medium was measured by GOD/POD, enzymatic method (Sabu and Subburaju, 2002; Ghosh et al., 2004).Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium (Table 3).

2.4. Statistical analysis

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

Table 1 Effect of *Psoralea corylifolia* on plasma glucose, serum triglyceride level and body weight in dexamethasone-induced insulin resistance.

Sr. No.	Groups	Plasma glucose (mg/dl)	Serum triglyceride (mg/dl)	Body weight change (g)
I	Normal control	63.37 ± 1.12	93.12 ± 1.21	+0.98 ± 0.06
II	DEXA-Control	85.32 ± 0.85[a**]	154.36 ± 2.34[a**]	−2.34 ± 0.16[a**]
III	DEXA + PIO	65.34 ± 0.87[b**]	95.84 ± 1.98[b**]	+0.97 ± 0.05[b**]
IV	DEXA + PC 100	82.56 ± 0.68	148.49 ± 1.59	−1.94 ± 0.13
V	DEXA + PC 200	76.45 ± 0.65[b**]	134.30 ± 2.00[b**]	−1.83 ± 0.18
VI	DEXA + PC 300	72.27 ± 1.23[b**]	121.23 ± 1.78[b**]	+0.95 ± 0.02[b**]
VII	PIO	62.24 ± 0.54	91.97 ± 1.93	+0.99 ± 0.06
VIII	PC 100	65.45 ± 0.87	96.78 ± 1.07	+0.98 ± 0.04
IX	PC 200	61.26 ± 0.76	96.23 ± 0.88	+1.24 ± 0.15
X	PC300	58.54 ± 0.51[a*]	94.54 ± 0.76	+1.15 ± 0.23

Values are expressed as mean ± SEM, $n = 6$, DEXA = dexamethasone 1 mg/kg, i.m., PIO = Pioglitazone 2 mg/kg, p.o., PC = *Psoralea corylifolia* 100, 200, 300 mg/kg, p.o.
(+) and (−) sign indicates increase and decrease in body weight.
[a*] $p < 0.05$ when compared with normal control.
[a**] $p < 0.01$ when compared with normal control.
[b**] $p < 0.01$ when compared with DEXA-control.

Table 2 Effect of *Psoralea corylifolia* on different antioxidant enzyme levels.

Sr. no.	Groups	GSH (_g of GSH/g of tissue)	SOD (units/mg of tissue)	Catalase (_Mof H_2O_2/g of tissue/min)	LPO (nM of MDA/g of tissue)
I	Normal control	26.42 ± 2.43	76.65 ± 2.95	7.83 ± 0.03	11.58 ± 0.94
II	DEXA-control	13.77 ± 1.67[a**]	25.98 ± 1.02[a**]	5.07 ± 0.02[a**]	23.94 ± 1.27[a**]
III	(III) DEXA + PIO	20.58 ± 1.76[b**]	75.55 ± 3.62[b**]	5.83 ± 0.03[b**]	13.29 ± 0.87[b**]
IV	DEXA + PC-100	15.60 ± 1.35	51.81 ± 2.12[b**]	4.53 ± 0.02	19.63 ± 1.20[b**]
V	DEXA + PC-200	18.21 ± 1.57[b**]	60.04 ± 2.79[b**]	5.14 ± 0.03	14.72 ± 0.64[b**]
VI	DEXA + PC-300	22.31 ± 2.08[b**]	71.55 ± 3.98[b**]	6.79 ± 0.03[b**]	14.76 ± 0.75[b**]
VII	PIO	25.73 ± 2.31	73.37 ± 3.21	7.81 ± 0.04	11.95 ± 1.10
VIII	PC-100	23.41 ± 1.87	72.66 ± 2.87	8.06 ± 0.05	11.83 ± 0.83
IX	PC-200	25.61 ± 2.10	71.80 ± 3.07	7.91 ± 0.07	11.80 ± 1.02
X	PC-300	24.80 ± 2.04	72.61 ± 2.11	7.51 ± 0.05	10.90 ± 0.79

Values are expressed as mean ± SEM, $n = 6$, DEXA = dexamethasone 1 mg/kg, i.m., PIO = Pioglitazone 2 mg/kg, p.o., PC = *Psoralea corylifolia* 100, 200, 300 mg/kg, p.o.
[a**] $p < 0.01$ when compared with normal control.
[b**] $p < 0.01$ when compared with DEXA-control.

3. Results

3.1. Effects of PC on plasma glucose, serum triglyceride and body weight

In DEXA-control group there was significant increase in plasma glucose level ($p < 0.01$) and serum triglyceride level ($p < 0.01$) when compared with normal control. All mice treated with DEXA and PC showed significant decrease ($p < 0.01$) in the levels of plasma glucose and serum triglyceride when compared with DEXA-control. The mice treated with DEXA and Pioglitazone showed significant decrease in plasma glucose level ($p < 0.01$) and serum triglyceride level ($p < 0.01$) when compared with DEXA-control. PC at the dose of 300 mg/kg, p.o. showed marginal hypoglycemia ($p < 0.05$) when compared with normal control. Whereas significant decrease in the plasma glucose was observed when compared with DEXA-control, PC at the dose of 100 & 200 mg/kg, p.o. showed significant reduction ($p < 0.01$) in plasma glucose and triglyceride level. The percent reduction in plasma glucose was found to be 65.45 for PC 100 mg/kg, p.o. and 61.26 for PC 200 mg/kg, p.o. Significant reduction in body weight was observed in DEXA-control group when compared with normal control. PC and Pioglitazone treatment significantly inhibited the dexamethasone induced decrease in body weight ($p < 0.01$) when compared DEXA-control. According to the result it is clear that the PC has the significant effect on DEXA induced insulin resistance.

3.2. Effects of PC on MDA, GSH, SOD and CAT levels

In DEXA-control group there was increase in the levels of MDA ($p < 0.01$) when compared with normal control, treatment with PC significantly prevented this rise ($p < 0.01$). DEXA-control group showed significant decrease ($p < 0.01$) in GSH, SOD and CAT when compared with normal control, where as significant increase ($p < 0.01$) in the levels of these enzymes was observed in the PC treated groups when compared with DEXA-control group. PC showed dose dependent increase in the level of enzyme. PC at 100 mg/kg,

Table 3 Effect of *Psoralea corylifolia* on glucose uptake in mice isolated hemidiaphragm.

Sr. No.	Group	Non-insulin assisted glucose uptake mg/g/30 min	Insulin assisted glucose uptake mg/g/30 min
I	Normal	9.79 ± 0.31	15.47 ± 0.87
II	DEXA-control	6.15 ± 0.35[a**]	9.74 ± 0.54[a**]
III	DEXA + PIO	8.54 ± 0.45	25.46 ± 1.34[c**]
IV	DEXA + PC-100	8.20 ± 0.48	16.50 ± 0.45[c**]
V	DEXA + PC-200	13.44 ± 0.47	19.15 ± 0.89[c**]
VI	DEXA + PC-300	19.56 ± 0.59[b**]	25.41 ± 1.12[c**]

Values are expressed as mean ± SEM, $n = 6$, DEXA = dexamethasone 1 mg/kg, i.m.
PIO = Pioglitazone 2mg/kg, p.o., PC = 100, 200, 300 mg/kg, p.o.
[a**] $p < 0.01$ when compared with normal control or normal + insulin.
[b**] $p < 0.01$ when compared with DEXA-control.
[c**] $p < 0.01$ when compared with DEXA + insulin group.

p.o. showed slight variation in the enzyme level, whereas PC 300 mg/kg, p.o. showed significant increase in the enzyme level.

3.3. Effect of PC on glucose uptake in mice isolated hemidiaphragm

Hemidiaphragm of the mice treated with dexamethasone showed significant decrease ($p < 0.01$) in glucose uptake when compared with normal control. Pioglitazone did not show any significant increase in glucose uptake by mice isolated hemidiaphragm. PC at higher doses i.e. 200, 300 mg/kg, p.o. showed significant increase ($p < 0.01$) in glucose uptake when compared with DEXA-control. In insulin assisted glucose uptake, Pioglitazone showed significant increase ($p < 0.01$) in glucose uptake when compared with DEXA + insulin group. At higher doses PC 200, 300 mg/kg, p.o. along with insulin showed significant increase ($p < 0.01$) in glucose uptake when compared with DEXA + insulin group.

4. Discussion

In the present study, the effect of PC has been studied for its anti-diabetic activity in diabetic animals. Dexamethasone administration at a dose of 1 mg/kg intramuscular resulted in significant increase in blood glucose (63.37 ± 1.12) and triglyceride level (93.12 ± 1.21). Animals which show blood glucose level more than 150 mg/dl were selected for the further studies. PC showed dose dependent decrease in elevated plasma glucose and triglyceride levels caused by dexamethasone. The effect of PC on the plasma glucose level and triglyceride level may be due to the glucocorticoid antagonism and the presence of other chemical constituents like terpenoids and tannins which are reported to have antihyperglycemic action (Matsudha et al., 2002). PC showed significant increase in insulin assisted glucose uptake, which indicates that there was increase in the insulin sensitivity.

Glucocorticoid treatment is known to induce insulin resistance and catabolic states in rats (Harber and Weinstein, 1992). Pharmacological doses of glucocorticoids induce *ob* gene expression in rat adipocyte tissues within 24 h which is followed by complex metabolic changes like hyperleptinemia, resulting in decrease in food consumption, reduction in body weight with enhanced blood glucose and triglyceride levels (Kim et al., 2002; Shalam et al., 2006). As expected, in the present study, dexamethasone group showed reduction in body weight, while PC and Pioglitazone treatment inhibited dexamethasone-induced reduction in body weight and showed marginal increase in body weight. The effect of PC on the body weight may be attributed to the increase in the sensitivity to insulin and the subsequent increase in the glucose uptake (Schmidt et al., 1984).

Oxidative stress can be generated by hyperglycemia and for a long time it has been accused to cause insulin resistance. Insulin resistance induces release of cytokines like TNF-alpha, IL-8 which leads to development of oxidative stress in liver by reducing the mitochondrial levels of Cu/Zn SOD, glutathione and producingH_2O_2 radicals and leads to increase in lipid peroxidation. Mitochondria are significant source for generation of superoxide radicals (Marfella et al., 2001). Insulin resistance is associated with increase in fat accumulation and mitochondrial oxidative stress. In present study, *P. corylifolia* showed significant increase in the concentration of various antioxidant enzymes, which could be beneficial in countering, the hyperglycemia induced oxidative stress. Methanolic extract of PC contains tannins, terpenoids and saponins (Kirtikar and Basu, 2005). Tannins and saponins play a major role in reducing oxidative stress associated with diabetes (Bruneton, 1999), probably by scavenging the free radicals and preventing the depletion of endogenous antioxidant.

Thus the results obtained in the present investigation indicate that *P. corylifolia* may prove to be useful in insulin resistance owing to its antioxidant activity and ability to increase the glucose uptake.

References

Aebi, H., Bergmeyer, Hu., 1974. Methods of Enzymatic Analysis. Chemic Academic Press Inc. 3, 673–685.

Andrews, R.C., Walker, B.R., 1999. Glucocorticoids and insulin resistance: old hormone, new targets. Clinical Science 96, 513–523.

Bruneton, J., 1999. Pharmacognosy. In: Phytochemistry of Medicinal Plants, vol. 2. Lavoisier Publishers, London, pp. 386–387.

Chattopadhyay, R.R., Sarkar, S.K., Ganguli, S., Banerjee, R.N., Basu, T.K., 1992. Effect of extract of leaves of *Vinca rosea* Linn. on glucose utilization and glycogen. Indian Journal of Physiology and Pharmacology 36, 137–138.

Chen, Y., Wangb, H., Xiaa, X., 2007. Behavioral and biochemical studies of total furocoumarins from seeds of *Psoralea corylifolia* in the chronic mild stress model of depression in mice. Phytomedicine 14, 523–529.

Colowick, S.P., Kaplan, N.O., Packer, L., 1984. Methods in Enzymology, Vol. 105. Academic Press, London, pp. 121–125.

Ellaman, G.L., 1959. Tissue sulfhydryl group. Archives Biochemistry and Biophysiology 6, 70–77.

Gholap, S., Kar, A., 2005. Gymnemic acids from *Gymnema sylvestre* potentially regulate dexamethasone-induced hyperglycemia in mice. Pharmaceutical Biology 43, 192–195.

Ghosh, R., Sharatchandra, K., Rita, S., Thokchom, I.S., 2004. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. Indian Journal of Pharmacology 36, 222–225.

Harber, R.S., Weinstein, S.P., 1992. Role of glucose transporter in glucocorticoid induced insulin resistance GLUT4 isoform in rat skeletal muscle is not decreased by dexamethasone. Diabetes 41, 728–735.

Kim, D.S., Kim, T.W., Park, I.K., Kang, J.S., Om, A.S., 2002. Effect of chromium picolinate supplementation on insulin sensitivity, serum lipid, and body weight in dexamethasone-treated rats. Metabolism 51, 589–594.

Kirtikar, K.R., Basu, B.D., 2005. Indian medicinal plants, Vol. 2. International book publisher, Dehradun, pp. 718–721.

Marfella, R., Quagliaro, L., Nappo, F., Ceriello, A., Giugliano, D., 2001. Acute hyperglycemia induces an oxidative stress in healthy subjects. Journal of Clinical Investigation 108, 635–636.

Matsudha, H., Morikawa, T., Yoshikawa, M., 2002. Antidiabetogenic constituents from halogenoalkanes or peroxidative reactions in rat liver fraction in vitro. Biochemical Journal 123, 805–814.

Mishra, H.P., Fridovich, I., 1972. Role of superoxide anion in auto-oxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247, 3170–3175.

Naznin, A., Khatunea, M., 2004. Ekramul Islam Antibacterial compounds from the seeds of *Psoralea corylifolia*. Fitoterapia 75, 228–230.

Sabu, M.C., Subburaju, T., 2002. Effect of *Cassia auriculata* Linn. on serum glucose level, glucose utilization by isolated rat hemidiaphragm. Journal of Ethnopharmacology 80, 203–206.

Schmidt, T.J., Miller-Diener, A., Litwack, G., 1984. Lapachone, a specific competitive inhibitor of ligand binding to the glucocorticoid receptor. The Journal of Biological Chemistry 259, 9536–9543.

Shalam, M.D., Harish, M.S., Farhana, S.A., 2006. Prevention of dexamethasone and fructose-induced insulin resistance in rats by SH-01D, a herbal preparation. Indian Journal of Pharmacology 38, 419–422.

Slater, T.F., Sawyer, B.C., 1971. The stimulatory effect of carbon tetrachloride and other several natural medicines. Pure and Applied Chemistry 74, 1301–1308.

Xingyong, Y.A., Jun, L.A., Xiaowen, W., 2006. Psc-AFP, an antifungal protein with trypsin inhibitor activity from *Psoralea corylifolia* seeds. Peptides 27, 1726–1731.