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Short Communication

Polyphenol extract of *ichnocarpus frutescens leaves* modifies hyperglycemia in dexamethasone (dex) treated rats

Chidambaram Kumarappan[1]* and Subhash Chandra Mandal[2]

Department of Pharmacology[1],
School of Pharmacy, Taylor's University,
Subang Jaya 47500, Malaysia

Phytotherapy Research Laboratory (PRL),
Department of Pharmaceutical Technology[2],
Jadavpur University, Kolkata – 700 032, India

Abstract

Introduction – The leaves of *Ichnocarpus frutescens* are used extensively as a decoction for the treatment of diabetes mellitus by the tribals of Karnataka and Uttar Pradesh states.

Methods – Anti-diabetic activity of polyphenol extract of *Ichnocarpus frutescens* was investigated using dexamethasone (DEX) induced hyperglycemia in Wistar rats. Experimental animals were injected subcutaneously with dexamethasone 3 mg/kg/day. After one week, hyperglycemic rats were orally treated with polyphenol extract (PPE) extracts at the dose of 300 mg/kg/day and 150 mg/kg/day for a period of 14 days.

Results – Administration of DEX to fasted rats for 21 days resulted in insulin resistance evidenced by the significant increase in mean fasting blood glucose level (162.33±4.72 mg/dl). Both 300 mg/kg and 150 mg/kg of PPE markedly reversed DEX induced mean fasting blood glucose level to 104.00±3.30 mg/dl and 145.5±1.99 mg/dl, respectively when compared with the positive control ($p<0.01$).

Conclusion – The possible mechanism by which polyphenol extract of *I. frutescens* brings about its antihyperglycemic action might be through potentiation of insulin sensitivity enhanced transport of blood glucose to the peripheral tissues. However, these findings suggest that polyphenol extract of *I. frutescens* therapy may reduce the risk of dexamethasone induced hyperglycemia in Wistar rats. These observations suggest that *I. frutescens* is a potential glucose lowering agent to ameliorate glucocorticoids induced hyperglycemia.

***Corresponding author :**

Chidambaram Kumarappan, Senior Lecturer, Department of Pharmacology, School of Pharmacy, Taylor's University, Taylor's Lakeside Campus, No. 1, Jalan Taylor's – 47500, Subang Jaya, Selangor Darul Ehsan, (+603-5629 5000 (Extn: 5825), (+6016-22-42696 (Mobile), Email: ctkumarrx@gmail.com, Kumarappan.chidambaram@taylors.edu.my

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Introduction

The metabolic syndrome is characterized by a group of metabolic risk factors. The dominant underlying risk factor for metabolic syndrome is insulin resistance. Currently glucocorticoids are among the most commonly prescribed drugs for various

disorders. Dexamethasone is a potent synthetic member of glucocorticoids class of steroid hormones. Unfortunately, chronic glucocorticoid therapy has most serious side effects (1). Glucocorticoids may be involved in subsets of patients with diabetes mellitus and insulin resistance. The mechanism responsible for steroid induced diabetes and insulin resistance is unclear. Down regulation of insulin receptor substrate-1 by dexamethasone may account at least partly for the insulin resistance (2). Whether these mechanisms are primarily for the actions or are secondary for the improved efficiency of whole pathways of insulin resistance remain unclear. Glucocorticoids increase hepatic glucose production by the liver and decrease peripheral glucose uptake (3). Dexamethasone induced insulin resistance can lead to hyperglycemia resulting from impaired insulin-stimulated glucose disposal in peripheral tissues. Apart from currently available therapeutics options, many herbal medicines have been recommended for the treatment of diabetes. Numerous medicinal plantshave been used to treat diabetes mellitus and other formsof glucose intolerance or insulin resistance (4).

The leaves of *Ichnocarpusfrutescens* (L). Br (Family: Apocynaceae) are used extensively as a decoction for its anti-diabetic activity by the tribal of Karnataka and Uttar Pradeshstates for treating diabetes (5). Previous studies have been shown that different extract of *Ichnocarpus frutescens* have antioxidant, anti-diabetic activity, antihyperlipidemic and α-glucosidase inhibitory activities (6-8). Active constituents in *I.frutescens* include flavonoids, simple phenolic acids, coumarins, and pentacyclic triterpenoids (9). Ethno medical documentation revealed that consumption of the leaves of *I.frutescens* decoction helps lower the blood glucose levels. The objective of this investigation was taken to ascertain the scientific basis for the use of this plant in the management of diabetes mellitus caused by insulin resistance. Therefore, the present study was designed to evaluate the glucose lowering effect of polyphenol extract of *I.frutescens* on dexamethasone induced hyperglycemia model.

Materials and Methods

The fresh leaves of *Ichnocarpusfrutescens* (L.) R.Br. were collected from the Delta region of Cauvery River, Thiruchirappalli, India and was authenticated at Botanical Survey of India (BSI), Central National Herbarium (CNH), Howrah, India (REF NO: CNH/I-I/87/2005-TECH/1326). An authentic voucher specimen was deposited in the Herbarium of Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

Dried leaves of *Ichnocarpus frutescens* (500 g) were finely powdered, mixed with 70% methanol and kept at room temperature for 5 days. After 5 days it was filtered using Whatman filter paper no. 2 and the solvent was evaporated using rotary vacuum evaporator (Superfit, India) at 60ºC under reduced pressure. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleumether was obtained. The lower layer was then treated with ethylacetate containing glacial acetic acid (10 ml/l). Extractionof polyphenols was carried out for 36 h at room temperatureand the combined ethyl acetate layer was concentrated (12%). The residue was lyophilized and stored at –70°C. Freshly collected and dried leaves of *I. frutescens* were used for the extraction of polyphenol for each study. The total polyphenol content and flavonoid of the extract were assayed using the standard methods.

Male Wistar rats weighing about 200-250 g/body wt were used in the present study (M/s Ghosh Enterprises, Kolkata, India). Animals were collected from breeding colony and acclimatized to the laboratory condition for two wk. They were housed in macrolon cages under standard laboratory conditions (12 hr light and 12 hr dark cycle, 21±2ºC, and relative humidity 55-70%). The animals were fed with commercial diet from Hindustan Lever Ltd (Bangalore, India) and free access to water (*ad libitum*) during the experiments. Experiments were performed complied with the rulings of the Committee for the

Purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi, India (Registration No: 0367/01/C/CPCSEA) and the study was permitted by the institutional animal ethical committee (IAEC) of the Jadavpur University, Kolkata, India.

Animals were grouped into four groups consisting of eight rats each. Group I was used as control. Groups II–IV were injected (sc) with dexamethasone 3 mg/kg/day body wt for 21 days (10). After one week, groups III and IV were orally treated with PPE extracts at the dose of 150 and 300 mg/kg/day, respectively for a period of 14 days. Group II considered as a dexamethazone insulin resistance control which received distilled water for 14 days. At the end of the experimental period from the tail vein mean fasting blood glucose levels were estimated at the morning by glucose oxidase peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). The experimental data were expressed as mean±SEM. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison test using GraphadInstat Software (San Diego, USA). $p<0.05$ was considered as statistically significant.

Results

As shown in Table I, PPE at the doses of 150 mg/kg and 300 mg/kg reduced the mean fasting blood glucose levels in dexamethazone induced diabetic rats. PPE significantly is also able to reduce against the impairment of glucose tolerance induced by dexamethazone. Results showed that administration of dexamethasone (3 mg/kg) resulted in hyperglycemia as evidenced by the significant in increase in mean glucose (162.33±4.72 mg/dl) of the positive control (Dexamethasone)when compared with the baseline (negativecontrol) that had mean glucoseof 86.83±1.60 mg/dl.Group C which received 150 and 300 mg/kg of the polyphenol extract of *I.frutescens* after dexamethatone administration had mean glucose (GLU)) levels of 145.5±1.99 and 104.00±3.30 mg/dl respectively, which were significantly different from the positive control (Dexamethazone), indicating an antagonism to the glucose and elevating property ofdexamethasone. In dexamethasone induced insulin resistance model, the polyphenol extract of *I.frutescens* treatment showed statistically significant change inreducing hyperglycemia (Table I; $p<0.01$).

TABLE I : Effect of polyphenolic extracts of *I. frutescens* leaves on dexamethazone induced hyperglycemia in Wistar rats.

Treatment groups	*Mean fasting blood glucose (mg/dl)*	
	Initial	*Final*
Normal control	80.10±1.66	86.83±1.60
Dexamethazone control (3 mg/kg)	84.15±1.98	162.33±4.72
PPE (150 mg/kg)	84.10±1.64	145.5±1.99**
PPE (300 mg/kg)	85.03±2.23	104.00±3.30**

Dexamethazone control-3 mg/kg for 21 days PPE (150 and 300 mg/kg) for 14 days; Values are expressed as mean±S.E.M. n=6; **p<0.01 vs dexamethzone hyperglycemic control.

Discussion

Glucocorticoids in excess inhibit insulin secretion from pancreatic beta-cells, decrease glucose utilization and stimulate glucagons secretion, lipolysis, proteolysis and hepatic glucose 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 (11). Dexamethasone causes insulin resistance as measured by several markers, including a reduction in insulin-stimulated glucose uptake and a decrease in glucose oxidation. Excess exogenous glucocorticoids have been shown to increase gluconeogenesis and decrease tissue glucose uptake thus resulting in hyperglycemia, potentially inducing diabetes (12, 13). Glucocorticoids can worsen glycemic control in patients with overt diabetes and can precipitate the onset of hyperglycemia in patients who are otherwise predisposed. Hyperglycemia caused by dexamethasone is often associated with a group of risk factors such as obesity, dyslipidemia, hypertension and impaired glucose tolerance (14).

Plant products have been used in folk medicine and traditional healing systems and are being evaluated for their hypoglycemic effects. The study was planned

to evaluate the glucose lowering effect of polyphenolic extract of *I.frutescens* in dexamethasone-induced hyperglycemia in Wistar rats. A larger number of medicinal plants have been reported to have significant anti-hyperglycemic and anti-diabetic in diabetes mellitus with insulin resistance as well as improved glucose tolerance (15). It was demonstrated that oral administration of polyphenol extract of *I.frutescens* could reverse the above mentioned diabetic effects. The possible mechanism by which polyphenol extract of *I.frutescens* brings about its antihyperglycemic action may be through potentiation of insulin sensitivity enhanced transport of blood glucose to the peripheral tissues, as was evident from our previous studies. This was clearly demonstrated by the decreased levels of mean fasting blood glucose in diabetic rat treated with polyphenol extract of *I.frutescens* (Table I). Similar antihyperglycemic and insulin release stimulatory effects have been reported *I.frutescens* (16). We early confirmed that polyphenol extract of *I.frutescens* could ameliorate peripheral glucose uptake (17). Phytochemical analyses of the leaves of I.frutescens have shown presence of flavonoid and polyphenol compounds (18).

Flavonoids and polyphenolic are a large group of phytochemicals found in a variety of plants, vegetables and fruits. It was reported flavonoids and polyphenolic can suppress insulin resistance, possibly mediated via activation of PPARγ, reduced oxidative stress enhanced glucose uptake and insulin sensitivity (19, 20). The unique properties of polyphenol and flavonoid as insulin sensitizers suggested that the mechanism underlying increases insulin sensitivity with better therapeutic profiles. Administration of dexamethasone alone at 3 mg/kg for continuous 21 days results in increase in serum glucose. The present study clearly demonstrates the efficacy of using polyphenol extract of *I.frutescens* extract to prevent effect of dexamethasone induced hyperglycemia in Wistar rats. *I.frutescens* leaves and flower mainly contain simple phenolic acid, flavones, flavonoids and glycoflavones (21, 22). Furthermore, high polyphenol and flavonoid content of *I. frutescens* may be responsible for the alleviation of dexamethazone induced derangement in glucose homeostasis. Taken together, these data show that polyphenol extract of *I.frutescens* hold promising therapeutic potential in treatment of diabetes mellitus and reduces dexamethasone induced adverse effects in diabetes mellitus. Because dexamethazone causes derangements in glucose homeostasis, it is striking to speculate homeostasis in individuals or those receiving exogenous glucocorticoids. The hypoglycemic action can be due to release of insulin, insulin-sensitizing action or a combination of both. Hence further chronic studies need to be undertaken to determine the mechanism of action to reverse the insulin resistance by measurement of insulin or 'C' peptide level, body weight or oral glucose tolerance studies.

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References

1. Moghadam-Kia S, Werth VP. Prevention and treatment of systemic glucocorticoid side effects. *Int J Dermatol* 2010; 49(3): 239–248.
2. Turnbow MA, Smith LK, Garner CW. The oxazolidinedione CP-92, 768-2 partially protects insulin receptor substrate from dexamethasone down-regulation in 3T3-L1 adipocytes. *Endocrinology* 1995; 136(4): 1450–1458.
3. Novelli M, De Tata V, Bombara M, Lorenzini A, Masini M et al. Insufficient adaptive capability of pancreatic endocrine function in dexamethasone-treated aging rats. *J Endocrinol* 1999; 162(3): 425–432.
4. Bajaj S, Srinivassan BP. Investigation into the antidiabetic activity of *Azadirachta indica*. *Indian J Pharmacol* 1999; 31(2): 138–141.
5. Parinitha M, Harish GU, Vivek NC et al. Ethno-botanical wealth of Bhadra wild life sanctuary in Karnataka. *Indian J Trad Knowledge* 2004; 31(1): 37–50.
6. Kumarappan CT, Mandal SC. Polyphenolic extract of *Ichnocarpus frutescens* attenuates diabetic complications in streptozotocin-treated diabetic rats. *Ren Fail* 2008; 30: 307–322.
7. Kumarappan CT, Nageswara RT, Subhash CM. Polyphenolic extract of *Ichnocarpus frutescens* modifies hyperlipidemia status in diabetic rats. *J Cell Mol Biol* 2007; 6(2): 175–187.

8. Barik R, Jain S, Qwatra D, Joshi A, Tripathi GS, Goyal R. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. *Indian J Pharmacol* 2008; 40(1): 19–22.
9. Singh RP, Singh RP. Flavanoids of the flowers of *Ichnocarpus frutescens*. *J Indian Chem Soc* 1987; 64: 715–716.
10. Severino C, Brizzi P, Solinas A et al. Low dose dexamethazone in the rat: a model to study insulin resistance. *Am J Physiol Endocrinol Metab* 2002; 283(3): E367–E373.
11. Harber RS, Weinstein SP. Role of glucose transporter in glucocorticoid induced insulin resistance GLUT4 isoform in rat skeletal muscle is not decreased by dexamethasone. *Diabetes* 1992; 41: 728–735.
12. Gholap S, Kar A. Gymnemic acids from *Gymnema sylvestre* potentially regulates dexamethasone induced hyperglycemia in mice. *Pharm Biol* 2005; 43(2): 192–195.
13. Wang M. The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. *Nutr Metab* 2005; 2(1): 3.
14. Bruder ED, Lee PC, Raff H. Metabolic Consequences of Hypoxia from birth and dexamethasone treatment in the neonatal rat: Comprehensive hepatic lipid and fatty acid profling. *Endocrinology* 2004; 145(11): 5364–5372.
15. Patel DK, SK Prasad, R Kumar, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacc J Trop Biomed* 2012; 2(4): 320–330.
16. Rakesh B, Sanjay J, Deep Q et al. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. *Indian J Pharmacol* 2008; 40(1): 19–22.
17. Kumarappan CT, Thilagam E, Vijayakumar M, Mandal SC. Modulatory effect of polyphenolic extracts of *Ichnocarpus frutescens* on oxidative stress in rats with experimentally induced diabetes. *Indian J Med Res* 2012; 136: 815–821.
18. Daniel M, Sabnis SD. Chemotaxonomical studies on apocynaceae. *Indian J Exp Biol* 1978; 16: 512–513.
19. Nijveldt RJ, Van Nood E, Van Hoorn DE et al. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 2001; 74(4): 418–425.
20. Xiao-ke Z, Li Z, Wei-wei W et al. Anti-diabetic activity and potential mechanism of total flavonoids of *Selaginella tamariscina* (Beauv.) Spring in rats induced by high fat diet and low dose STZ. *J Ethnopharmacol* 2011; 137(1): 662–668.
21. Khan MSY, Javed K, Khan MH. Chemical constituents of the leaves of *Ichnocarpus frutescens*. *Br J Chem Soc* 1995; 72: 65–66.
22. Singh RP. Flavanoids of the flowers of *Ichnocarpus frutescens. J Indian Chem Soc* 1987; 64(11): 715–716.