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Modulation of glucose transporter proteins by polyphenolic extract of *Ichnocarpus frutescens* (L.) W. T. Aiton in experimental type 2 diabetic rats

Kumarappan Chidambaram[1]*, Cini Mathew John[3], Sunil Christu Das[4], Kumar Venkatesan[2] & Rajinikanth Siddalingam[5]

[1]*Department of Pharmacology & Toxicology; [2]Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Abha - 62529, Saudi Arabia

[3]Department of Pharmacology, Faculty of Pharmacy, University Technologi Mara, Puncak Alam, Selangor - 42300, Malaysia

[4]Department of Ethno-Pharmacology, Entomology Research Institute, Loyola College, Chennai - 600 034, Tamil Nadu, India

[5]Department of Pharmaceutical Sciences, School of Biomedical and Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow - 226 025, Uttar Pradesh, India

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Traditionally, in India, the decoction of Black creeper, *Ichnocarpus frutescens* (L.) W. T. Aiton leaves is used to treatment diabetes mellitus. However, its molecular mechanisms of antihyperglycemic effects have not been completely studied. Due to the potential antidiabetic effect of *I. frutescens*, we hypothesized that the polyphonic extract might add to glucose uptake through improvement in the expression of genes of the glucose transporter (GLUT) family messenger RNA (mRNA) in the liver and adipose tissues. Experimentally, diabetes mellitus was induced in Wistar rats through i.p. injection of freshly prepared solution of streptozotocin (45 mg/kg). This was done 15 minutes after the administration of nicotinamide (120 mg/kg, ip). Serum level of insulin and C-peptide were analyzed using standard methods. Glucose metabolism by the hepatocytes and adipocytes were also analyzed by quantitative RT-PCR mRNA expression levels of phosphoenolpyruvate carboxykinase 1 (PCK1), GLUT2 in the hepatocytes, and GLUT4 in the adipocytes. The hemidiaphragm were also isolated and processed to study *in-vitro* peripheral glucose utilization. Results of the present investigation suggest that STZ-NA induced diabetes is associated with hyperglycemia, altered levels of PCK1 and glucose transporters gene expression as well as decreased levels of insulin and C-peptide. On the other hand, the outcome of the daily oral administration of PPE to STZ-NA induced diabetic rats at different doses (150 and 300 mg/kg bodywt.) for 30 days supports our hypothesis by showing significant improvement of insulin levels, C-peptide level, downregulation of PCK1 and upregulation of GLUT (2, 4) mRNA expression levels when compared to those of diabetic rats. The administration of PPE had also increased the uptake of glucose by rat hemidiaphragm significantly. Findings from this study demonstrate that PPE enhances peripheral glucose uptake through glycogenesis pathway, mediated by upregulation of GLUT2 and GLUT4, and downregulation of PCK1. Our study suggests that the leaf of *I. frutescens* is a rich source of polyphenolic compounds, including those with an insulin-sensitizing function that may have the potential for treating or managing diabetes or insulin resistance.

Keywords: Antidiabetic, Antihyperglycemic, Ayurveda, Black creeper, Glucose transporters, Glucose utilization, GLUT, Herbal, Streptozotocin-nicotinamide

Type 2 diabetes mellitus (T2DM) is a chronic and complicated metabolic disorder that has reached epidemic proportions with the estimated prevalence rates and trends escalating largely in developing countries. T2DM in people 20–79 years is further projected to increase to 629 million in 2045 compared to 425 million in 2017[1]. It is primarily associated with insulin secretory defects related to inflammation and metabolic stress among other contributors, including genetic factors. T2DM frequently goes undiagnosed for many years because hyperglycemia develops gradually and, at earlier stages, is often not severe enough for the patient to notice the classic diabetes symptoms. Nevertheless, even undiagnosed patients are at increased risk of developing macrovascular and microvascular complications[2].

Over a period of time, this sustained chronic hyperglycaemia could end in microvascular and macrovascular injury, which significantly affects the quality of life and life expectancy[3]. The American Diabetes Association and the European Association for the Study of Diabetes consensus report "Management of Hyperglycemia in Type 2 Diabetes, 2018" recommends a patient-centered approach to choosing appropriate pharmacologic treatment of blood glucose[4]. However, in some instances, patients will require medication reduction or discontinuation. Common reasons for this include ineffectiveness,

intolerable side effects, expense, or a change in glycemic goals. Medicinal use of herbal medicine in the treatment and prevention of diseases including diabetes has a long history compared to conventional medicine[5]. Over the years, considerable progress has been made with traditional plant extracts and their active constituents in suppressing hyperglycemia, thus potentially alleviating the symptoms and complications of T2DM[6]. Traditionally, antidiabetic herbs and/or their active ingredients may accomplish this basic need. More than 200 species of medicinal herbs have antidiabetic properties which were assessed mostly by classical screening tests without excavating far for the exact molecular and cellular mechanisms[7]. Hanhineva *et al*.[8] have demonstrated that plants rich in polyphenols may reduce the risk of T2DM and hyperglycaemia. Hence, rigorous preclinical studies are still required to confirm the molecular mechanism in decreasing hyperglycemia. The observation of preclinical studies could provide more evidence in the treatment of T2DM, and may further encourage development of polyphenol based herbal medicines.

Enhancing or stimulating peripheral glucose utilization is one of the several mechanisms that could control hyperglycemia; therefore, an effort to treat T2DM should focus on this area. Fundamentally, numerous causes integrate to facilitate the glucose utilization process, comprising of the activation of GLUT-2 in the liver, GLUT-4 in adipocytes and skeletal muscles, as well as induction of peroxisome proliferator-activated receptor-gamma (PPARγ)[9]. However, earlier investigations of Udea *et al*.[10], have focused on insulin-sensitive GLUT4 and GLUT2 as novel targets of polyphenol compounds. Several investigators found that the increase in glucose uptake was associated with glucose transporters in the liver (GLUT2), adipose tissues and skeletal muscles (GLUT4) in experimental type 2 diabetic rats[11] As a result, the role of some GLUT transporters gene expressions and the importance of these expressions have been studied in this experiment. Similarly, phosphoenolpyruvate carboxykinase (PEPCK/PCK1) is another rate-limiting enzyme of gluconeogenesis and enhanced expression of the PEPCK gene in the liver is present in most models of diabetes and is thought to contribute to the increased hepatic glucose output seen in diabetes[12]. PCK1 expression levels and regulation have been shown to be a sensitive marker for blood glucose levels. Alternatively, insulin strongly represses PEPCK transcription through the activation of the phosphoinositide-3 kinase (PI3K) pathway[13]. The primarily metabolic abnormality in insulin-resistant type 2 diabetics is due to defective regulation of glucose transporter genes, understanding these genes' regulated (decreased/increased) expression may prove useful in the characterization of the major causes that lead to the development of diabetes mellitus. The compounds that are able to repress PEPCK expression and overcome insulin resistance could constitute a new class of glucose-lowering agents. Thus any medicine with the potential to alter hepatic gluconeogenesis or glycogenolysis or glucose utilization/uptake process might have a significant influence on glucose homeostasis in diabetes mellitus.

The leaves of Black creeper, *Ichnocarpus frutescens* (Apocynaceae) have been used extensively in the form of decoction by the tribes in Karnataka and Utter Pradesh states for the treatment of diabetes and jaundice[14]. Due to the presence of active polyphenolic and flavonoid compounds in Apocynaceae family species, *I. frutescens* has been considered as a motivating choice to be studied further on its antihyperglycemic mode of action. Phenolic acids and terpenoids and flavonoids were three major classes of the chemical constituents of *Ichnocarpus frutescens* leaves. Babita *et al*.[15], *I. frutescens* has been shown to contain several flavonoids and polyphenolic compounds[15]. Interestingly, various parts of *I. frutescens* have been shown to have hepatoprotective, antioxidant, antihyperlipidemic and antidiabetic activities[16-18]. Despite its reported use, no systematic study related to its molecular mechanism of action has been carried out in diabetic animals. In this study, to ascertain the molecular mechanism of actions of this plant in the management of diabetes, we investigated the insulin-mimetic effect of polyphenolic extract of *Ichnocarpus frutescens* on the molecular mechanisms of glucose utilization on STZ-induced diabetic rats and normal rat hemidiaphragm tissues.

Materials and Methods

Plant Materials

Fresh leaves of *Ichnocarpus frutescens (*L.) R.Br. were collected from the delta region of Cauvery River, Thiruchirappalli, Tamil Nadu, India, and was authenticated at the Botanical Survey of India (BSI), Central National Herbarium (CNH), Howrah, India. An authentic voucher specimen was deposited in the Herbarium Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

Preparation of Polyphenolic Extract (PPE)

Dried leaves of *I. frutescens* (500 g) were finely powdered, mixed with 70% methanol and kept at room temperature (~25°C for 5 days. After 5 days it was filtered and the solvent was evaporated. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 mL/L). Extraction of polyphenols was carried out for 36 h at room temperature and the combined ethyl acetate layer was concentrated. The residue was lyophilized and stored at −70°C. The total polyphenolic content and flavonoid of the extract were assayed using the standard methods[19].

Animals and ethical approval

Healthy male Wistar albino rats, weighing about 200-250 g/body wt. were used in this experiment. Animals were collected from the standard breeding colony from our university's animal house and acclimatized to the laboratory condition for two weeks. They were housed in macrolon cages under standard laboratory conditions (light period 7.00 am. to 7.00 pm, 21±2ºC, and relative humidity 55-70 %). The animals were fed with standard commercial diet and free access to water (*ad libitum*) during the experiment. Experiments performed complied with the rulings of the Institutional Animal Care and the study was permitted by the Institutional Animal Ethical Committee (IAEC).

Induction of experimental diabetes mellitus

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1M citrate buffer, pH 4.5, was injected i.p. in a volume of 0.1 mL/kg, 15 min after the administration of 120 mg/kg nicotinamide according to the methods previously reported[20]. After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycaemia (i.e., with a blood glucose of 200-300 mg/dL) were taken for the experiment. The blood elevated glucose levels were determined at 72 h and then 7 days after injection, to simulate hyperglycemia. Rats that were found with constant values of elevated glucose levels were used for the study.

Experimental design

In the experiment, a total of 30 rats (24 diabetic surviving rats, 6 normal rats) were used. The rats were divided into five groups of six rats each. Two different doses of PPE (150 and 300 mg/kg/day) were administered orally for 30 days. All the doses were initiated 48 h after the elevation of blood glucose followed by STZ-NA injection. At the end of the 30 days, all the rats were killed by decapitation (pentobarbitone sodium) under anaesthesia (60 mg/kg). All animals received human care, according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland, and the Institutional Animal Ethical Committee.

Measurement of C-peptide and insulin levels

Blood samples (approximately 0.5 mL) collected from the retro-orbital venous plexus using hematocrit-capillary tubes containing heparin were centrifuged at 10000 rpm at 4ºC for 10 min. Plasma samples were then stored at –20ºC. Plasma insulin level was assayed by Mercodia Rat Insulin ELISA kit (Sweden) from the plasma collected on the day of sacrifice[21]. The optical density for the reading of the sample was set to 450 nm. The concentration of insulin level was obtained by computerized data reduction of the absorbance for the calibrators, except for Calibrator 0, versus the concentration using cubic spline regression. The insulin level of the samples was calculated using the formula generated. Plasma C-peptide level was assayed according to the standard method by Mercodia Rat C-peptide ELISA kit (Sweden) from the plasma obtained from retro-orbital puncture[22]. The optical density for the reading was also set to 450 nm. The concentration of C-peptide was obtained using cubic spline regression the same way as the Insulin ELISA kit mentioned above.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from liver and adipose tissues by TRI reagent solution (Ambion, Austin, Texas, USA) according to the manufacturer's instructions. The total RNA concentration was determined by measuring the absorbance at 260 nm. The purity of the extracted RNA was determined by measuring the ratio of the optical density at 260 and 280 nm using a spectrophotometer (BioRad, USA), ranging between 1.8 and 2.0 according to earlier described method[23]. Briefly, a reaction volume of 25 µL contained 12.5 µL master mix, 1 µL of 5 M each forward and reverse primers, and 1 µL of the template RNA at a concentration of 100 ng under the following conditions: 42°C for 30 min (this step was included to synthesize cDNA), 95°C for 10 min, 40 cycles at 95°C for 15 s, 53°C for 30 min and 72°C

for 4 s. Data were acquired on SYBR channel at the end of each extension step. Melt curves were analyzed to check for the absence of mispriming. The possibility of a genomic DNA influence on the results was eliminated by the use of primers. Primers specific for GLUT2, GLUT4, PCK1 and β-actin genes were designed from the gene sequence of rat (*Rattus norvegicus*) adopted from NCBI (National Center for Biotechnology Information) GenBank Database (www.ncbi.nlm.nih.gov) and supplied by NextGene. The run was performed using Sensi Mix one-step RT-PCR kit with SYBR Green (Quantace, London, UK) according to manufacturer's instructions. Each experiment was performed three times. Expression levels for each gene relative to beta-actin were calculated for all samples using the Rotor-Gene software (Version 1.7, Corbett Research) and Microsoft Excel. Analysis of gene expression data was carried out by the CT method of relative quantification according to the methods previously reported[24]. Forward and reverse primer sequences were: Forward primer: 5′ ATGGTGGGTATGGG TCAG 3′ and reverse primer: 5′ CAATGCCGTGT TCAATGG 3′ for ACTB; forward primer: 5′ TCTGTGCTGCTTGTGGAG 3′ and reverse: 5′ CTGACGAAGAGGAAGATGG 3′, for GLUT2; forward primer: 5′ AACGTTGGCTGGCTCTC 3′ and reverse: 5′GAACCTGGCGTTGAATGC3′ for PCK1; forward primer: 5′ GCCTTCTTTGAGA TTGGTCC6] 3′ and reverse: 5′ CTGCTGTTTCCT TCATCCTG 3′, for GLUT4.

Effect on glucose utilization by rat hemidiaphragm

Glucose uptake by rat hemidiaphragm was estimated using the previously reported methods with minor modifications[25]. Four sets containing six graduated test tubes (n=6), were taken as follows: Group I, 2mL of Tyrode solution with 2% glucose; Group II, 2 mL of Tyrode solution with 2% glucose and regular insulin (Nova Nordisk) 0.62 mL of 0.4 units per mL solution; Group III, 2mL of Tyrode solution and 1.38 mL of PPE (0.1%); and Group IV, 2 mL of Tyrode solution with 2% glucose and regular insulin 0.62 mL of 0.4 units per mL solution and 1.38 mL of PPE (0.1%). The volumes of all the test tubes were filled with 4 mL of distilled water to match the volume of the test tubes of Gr. IV. Twelve albino rats were fasted overnight and killed by decapitation. The diaphragms were dissected out quickly with minimal trauma and divided into two halves. Two diaphragms from the same animal were not used for the same set of experiment. Six diaphragms were used for each group. The hemidiaphragm were placed in test tubes and incubated for 30 min at 37°C in an atmosphere of 100% oxygen with shaking at 140 cycles/min. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

Statistical analysis

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 and two-way ANOVA followed by Dunnett's multiple comparisons and Bonferroni post-hoc test, respectively using GraphPad Prism Software (San Diego, USA). P <0.05 was considered as statistically significant.

Results

Diabetic rats treated with PPE for 30 days displayed a notable improvement in plasma insulin and C-peptide levels as compared to untreated STZ-diabetic rats. Plasma insulin and C-peptide were analyzed to check the appropriate endogenous insulin release from β-cells after PPE treatment. Fig. 1 A and B illustrate the effect

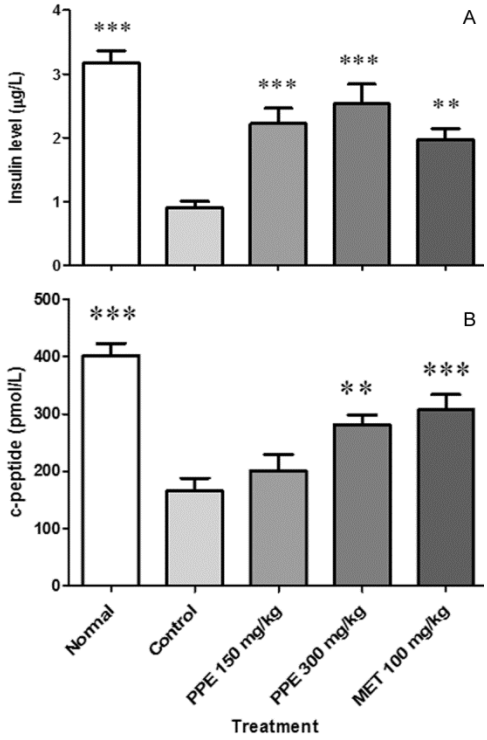

Fig. 1 — Effect of polyphenolic extract of *I.frutescens* on insulin (A); and C-peptide (B) levels in streptozotocin-nicotinamide induced diabetes after 30 days of drug treatment. [Values are expressed as mean ± S.E.M of six rats (n=6). Statistically different from control, *** P <0.001, ** P <0.01]

of the polyphenolic extract on plasma insulin and C-peptide levels, respectively in normal and experimental animals.

The *in vivo* effect of PPE on gene expression of GLUT4, GLUT2 and PCK1 was performed by RT-PCR using respective specific primers. In the present study, hepatic GLUT2 expression was lower in control of diabetic rats, but the expression was significantly (P <0.001 and P <0.01)) higher in the treated groups. This suggests that the expression of hepatic GLUT2 may also be triggered by insulin secretion. Significant improvement was observed in the expression of GLUT2 from the liver and the GLUT4 from the adipocytes in response to glucose uptake (Fig. 2 A and B). The treatment with PPE significantly reduced the expression of the genes encoding the regulatory enzyme of gluconeogenesis and phosphorylation (PCK1) in the liver of diabetic rats. The current study thus reveals that PPE significantly (P <0.001) represses hepatic PCK1 gene expression in diabetic rats compared to diabetic control rats (Fig. 3).

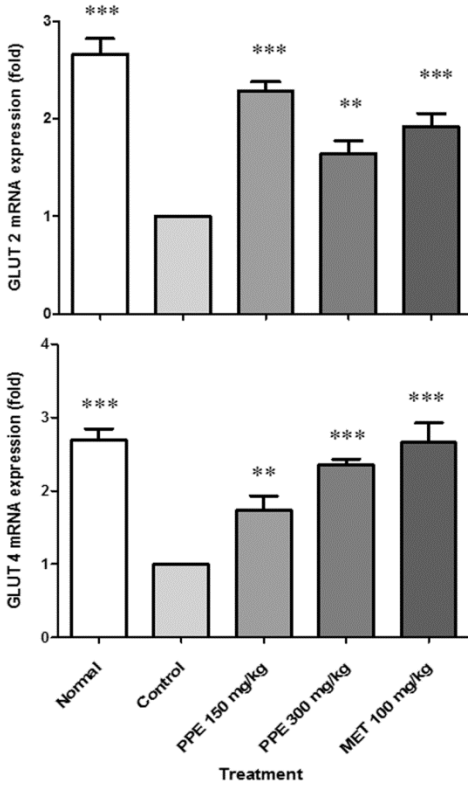

Fig. 2 — Effect of polyphenolic extract on the mRNA level of GLUT2 (A); and GLUT4 (B) genes in diabetic rat liver and adipose tissues, respectively by quantitative RT-PCR analysis. [The number of products for each sample was measured according to the quantity of β-actin while the number of fold expression was calculated by $2^{-\Delta\Delta Ct}$. Values are expressed as mean ± S.E.M of six rats. Statistically different from control, *** P <0.001, **P <0.01]

Fig. 4 illustrates glucose uptake (mg/g tissue wt./ 30 min) by isolated rat hemidiaphragm muscle in the absence and presence of insulin and PPE. Glucose uptake by isolated rat hemidiaphragm muscle of control groups in the presence of insulin (0.4 U/mL)

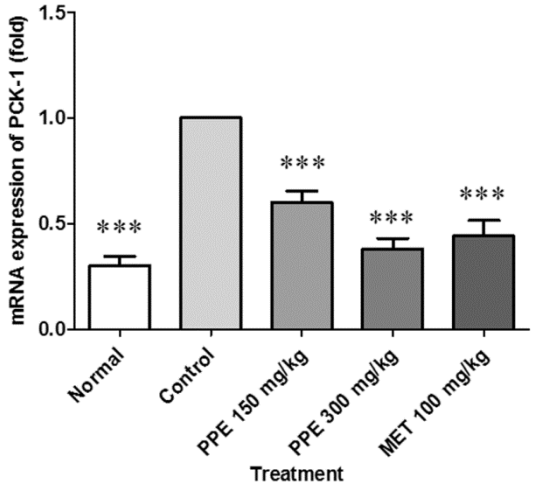

Fig. 3 — Effect of polyphenolic extract on the mRNA level of PCK-1 gene expression in diabetic rats by quantitative RT-PCR analysis. [The number of products for each sample was measured according to the quantity of β-actin while the number of fold expression was calculated by $2^{-\Delta\Delta Ct}$. Values are expressed as mean ± S.E.M of six rats. Statistically different from control, *** P <0.001, **P <0.01]

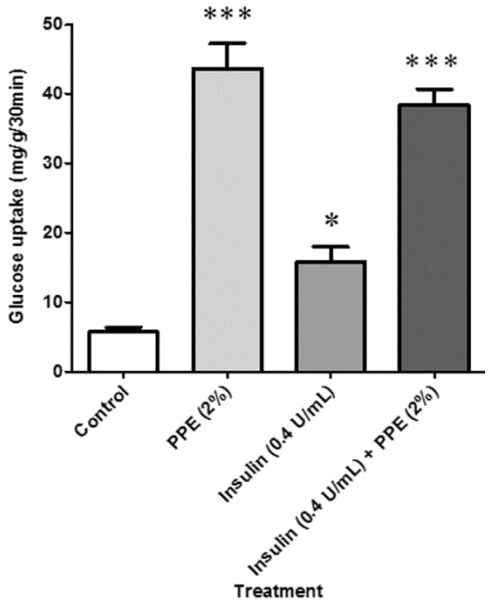

Fig. 4 — Effect of polyphenolic extract of *I. frutescens* on glucose uptake in rat hemidiaphragm. [*P < 0.01 significant when compared to control glucose in the incubation medium. Values are expressed as mean ± S.E.M of three different hemidiaphragm tissues (n=3). Statistically different from control, *** P <0.001, *P <0.05]

was significantly higher (15.75 ± 2.25 mg/g tissue wt./30 min) than in the absence of insulin (5.70 ± 0.695 mg/g tissue weight/30 min). PPE (0.1%) treated rat hemidiaphragm muscles showed significant (P <0.001) glucose uptake (43.65 ± 3.60 mg/g tissue wt./30 min) when compared to control and insulin treatment alone (P <0.05). The *ex-vivo* study on glucose utilization/uptake by isolated rat hemidiaphragm revealed that glucose uptake by the PPE is significantly higher than that of insulin. Thus, overall glucose utilizing process and glycogen synthesis (glycogenesis) stimulated by PPE is significantly more effective than insulin. The combination of PPE and insulin showed a marked increase in glucose uptake by rat hemidiaphragm.

Discussion

Non-insulin dependent diabetes mellitus is characterized by abnormal β-cell function caused by chronic hyperglycemia[26]. The fundamental mechanism underlying chronic hyperglycemia in a diabetic patient involves excessive production and decreased uptake of glucose by the tissues. Controlling the postprandial blood glucose excursions can prevent chronic hyperglycaemia and improve insulin resistance[27]. Glucose uptake in peripheral tissues is largely mediated by a group of intrinsic membrane proteins known as facilitative glucose transporters[28]. Maintenance of glucose homeostasis depends on insulin by inhibiting expression of PCK1 enzyme and stimulating expression of GLUT2 and GLUT4 transporters. The GLUT4 expression is downregulated when there is a relative insulin deficiency, such as in STZ-induced diabetes[29]. Consequently, decreased utilization of glucose by the peripheral tissues results in the elevation of fasting blood glucose. Since the amelioration of abnormal glucose metabolism in diabetes mellitus is shown to be largely due to an increase in the glucose uptake in muscle and adipose tissues, the alteration in GLUT transporters expression or their translocation to the plasma membrane is regarded as a possible target in the treatment of diabetes mellitus[30]. For this reason, we investigated whether PPE improves GLUT2 in the liver or GLUT4 expression in insulin-sensitive adipose tissues. In addition, we have also investigated the effects of PPE on glucose uptake by isolated rat hemidiaphragm tissues. Therefore, we hypothesized that PPE may improve hyperglycemia and insulin resistance by ameliorating impaired GLUT2 expression in liver and GLUT4 expression in adipose tissues from STZ-NA induced diabetic rats.

STZ induced hyperglycemia in rodents is considered to be a good preliminary screening method and is most widely used. STZ enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA. STZ induced hyperglycemia may be attributed to the enhancement of gluconeogenesis as a result of the absence of insulin[31]. Repeated (30 days) administration of the PPE improved plasma insulin and C-peptide levels in the STZ-NA induced diabetic rats compared with control diabetic rats. The anti-hyperglycemic effects revealed by the PPE against STZ-NA induced hyperglycemia were relatively similar to that of standard reference drug, metformin.

Glucose uptake in peripheral tissues is dependent on the translocation of GLUT4 glucose transporters to the plasma membrane. Numerous peripheral tissues play an important part in maintaining blood glucose homeostasis. Amongst these, the liver, pancreatic β-cells, skeletal muscle and adipose tissue are of greatest importance since they can sense and respond to varying blood glucose levels. Glucose is taken up into the cell through GLUT2 and GLUT4 in the plasma membrane of the cells[32]. Changes in these glucose transporters gene expressions are an important component of the pathogenesis of diabetes**.** In recent years, numerous plant extracts and plant formulations have been shown to regulate the expression of genes in metabolic pathways of different diabetic animal models[33]**.** In diabetic subjects, GLUT-2 and GLUT-4 expression are decreased before the loss of glucose-stimulated insulin secretion[34]. First, we showed that there are marked increases in fasting blood glucose level in STZ-NA diabetic rats and impaired glucose tolerance in the normal vehicle control rats. Consistent with earlier reports, we also showed that the GLUT4 expression in adipose tissue is drastically diminished in diabetic rats, as compared with vehicle control**.** Previous reports suggest that GLUT4 gene expression is down-regulated in STZ-induced diabetes, a state of insulin deficiency. A decrease in the GLUT4 mRNA was observed in STZ-NA induced diabetic animals which account for the impaired glucose disposal[35]. The principal glucose transporter protein that mediates this uptake is GLUT4, which plays a key role in regulating whole-body glucose homeostasis[36]. Our reports support the present study, which demonstrated a decrease in GLUT4 expression in adipose tissue of STZ-induced diabetic rats. Our previous studies have reported the antidiabetic effect polyphenolic extract of *I. frutescens* in STZ-NA

induced diabetes in rats[37], while the mode of action of this plant is not yet reported.

The current results from our current investigation confirm that hyperglycemia and impaired glucose tolerance are at least in part due to a decreased expression of these GLUT proteins. Since, both GLUT2 and GLUT4 are expressed in liver and insulin-sensitive tissue such as adipose, and they participate in the basal and insulin-stimulated glucose uptake. Interestingly, PPE treatment improves hyperglycemia and impaired glucose tolerance observed in STZ-NA diabetic rats. Our previous evidence has also suggested that PPE significantly inhibited postprandial excursion in experimental rats**.** Moreover, the circulating C-peptide and insulin levels were restored back to normal in PPE treated rats. An important finding in the current present investigation is that the mRNA expression of GLUT4 in adipose tissue and GLUT2 expression in liver from diabetic rats is dramatically increased by PPE. Therefore, the beneficial effect of PPE on STZ-NA induced hyperglycemia may be due to a remarkable improvement of GLUT4 and GLUT2 expression in adipose tissue and liver, respectively. Thus, increasing the expression of hepatic GLUT2 might add another efficient mechanism of PPE through response to insulin action. Taken together, the results from this study suggest that the modulation of GLUT2 and GLUT4 mRNA expression could thus be one of the mechanisms of the anti-hyperglycemic potential of PPE of *I. frutescens* leaves.

In this line, our results suggest that PPE could more efficiently contribute towards promoting glucose uptake from the blood when glucose levels are postprandially elevated in comparison with diabetic rats, as described for metformin. The investigation also revealed a significant increase in serum insulin and C-peptide level in rats treated with PPE and metformin. Thus, the antidiabetic effect of PPE could be attributed to upregulation of these protein expressions resulting in potentiation of pancreatic secretion of insulin from existing β-cells of islets. Overall, PPE has been demonstrated to have an insulin-like effect on glucose uptake[38]. PEPCK is one of the major enzymes responsible for the regulation of gluconeogenesis. In addition, phosphoenolpyruvate gluconeogenesis and hepatic glucose production have been found to be increased in diabetic[39]. It has also been shown that PPE significantly inhibited the expression of PCK-1 in diabetic rats. In agreement with our results, the phenolic compounds in PPE reduced the levels of this gluconeogenic enzyme (PEPCK) mRNA expression in the liver during STZ-NA induced hyperglycemia situation and, consequently, reduced blood glucose level. PEPCK overexpression restoration by PPE reflects increased insulin sensitivity in STZ diabetic rats**.** Over the years, scientific evidence has strongly supported that the polyphenolic compounds improve hyperglycaemic status through enhanced the expression of AMPK and GLUT4[40]. Additionally, it has been observed that flavonoids restore 5'AMP-activated protein kinase (AMPK) and GLUT4 expression in skeletal muscle and adipose tissues in high-fat diet-induced obese mice[41]. However, additional molecular mechanistic studies are needed to define the detailed role of PPE in the regulation of the insulin pathways, other glucose transporters and glucose utilization in other peripheral tissues.

In addition, the possibility of enhanced hemidiaphragm tissues uptake of glucose by PPE treatment cannot be ruled out. The estimation of glucose content in the rat hemidiaphragm is a commonly employed and reliable method for in vitro study of peripheral uptake of glucose. Furthermore, the glucose reducing effect of PPE was more significant when compared to normal rats, suggested that it could be caused by an increase in peripheral glucose utilization; this reinforces the hypothesis that the hypoglycemic mechanism involves insulin-like effect through peripheral glucose uptake by PPE. A decrease in blood glucose may be attributed to the stimulation of glucose uptake by peripheral tissue and a decrease in the gluconeogenesis. It appears that PPE has direct peripheral action by promoting glucose utilization of skeletal muscle. Several herbal preparations increase the beta cell regeneration and peripheral tissue glucose utilization in streptozotocin-induced diabetic animal supports the above hypothesis[42]. Our *in vitro* studies also showed that PPE significantly increased glucose uptake in rat hemidiaphragm tissues. These results indicate that the inhibition in liver gluconeogenesis process and the enhancement in adipocyte tissue glucose uptake are involved in the PPE induced antihyperglycemic action.

These observations suggest a possible role for polyphenolic compounds of *I. frutescens* in regulation hyperglycemia, although the effects appear to be the result of direct action on peripheral tissue. However, since unpurified extracts were used,

it is not possible to assign the effects to any particular compounds(s). To conclude, the data presented in this investigation support our primary hypothesis that the antihyperglycemic action of the polyphenolic extract of *I. frutescens* comprises an array of actions and provides direct evidence for the involvement of GLUT4 in controlling glucose homeostasis. Lastly, with a better understanding of insulin signalling pathways, it would be possible to assess, exactly and molecularly, the importance of PPE on glucose uptake and homeostasis. Furthermore, it would be possible to assess the action of PPE that might optimize glucose uptake and consequently be an important step in controlling the blood glucose levels.

Conclusion

In vivo antidiabetic effects of the polyphenol extract from the *Ichnocarpus frutescens* leaves were studied in nicotinamide-streptozotocin induced diabetic animal model. Our experimental data has shown that administration of the polyphenol extract to diabetic rats enhances peripheral glucose utilization by activating glucose GLUT2 and GLUT4 gene expression while downregulating PCK1 gene expression in liver. According to the present study, PPE of *I. frutescens* possesses a potent antidiabetic activity and could be used as a safe remedy for the treatment of diabetes mellitus. While the effects of *I. frutescens* polyphenol extracts are important as it is derived from an established herbal medicine used for human consumption, the side effects from prolonged polyphenol extract administration as described herein need to be completely assessed.

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Conflict of Interest

Authors declare no conflict of interests.

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