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Ripal P. Amin
St. John's University

Sanket N. Patel
St. John's University


Sunil Kumar
Molloy College, skumar@molloy.edu

S. William Zito

Sue Ford

See next page for additional authors

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Authors

Ripal P. Amin, Sanket N. Patel, Sunil Kumar, S. William Zito, Sue Ford, and Michael A. Barletta

Diuretic, Glucosuric and Natriuretic Effect of Pantoyltaurine in Diabetic Sprague-Dawley Rats

Ripal P Amin[#], Sanket N Patel[#], Sunil Kumar, S William Zito, Sue Ford and Michael A Barletta*

Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Jamaica, New York, United States

[#]Both are First Authors

***Corresponding Author:** Michael A Barletta, Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Jamaica, New York, United States.

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Abstract

Sodium-glucose co-transporter (SGLT) inhibitors offer a novel tool to control hyperglycemia and its complications. We present preliminary findings of pantoyltaurine, N-substituted analog of taurine, as diuretic, glucosuric and natriuretic agent in streptozotocin (60 mg/kg/mL, i.p.)-induced type 1 diabetic Sprague-Dawley rats and whether pantoyltaurine has an effect on regulation of SGLT isoforms that may further help in reducing hyperglycemia and improving renal function. After 14 days of persistent diabetes, phlorizin (0.4 g/kg/day, s.c.) or pantoyltaurine (2.4 mM/kg/day, p.o.) was administered for three weeks, days 15 - 35. As expected, diabetic rats showed persistent hyperglycemia, hyperphagia and weight loss. Pantoyltaurine and phlorizin-treated diabetic rats consumed less diet, showed significant weight loss, reduced persistent hyperglycemia as well as reduced glucose load after oral glucose tolerance test. Diuretic, glucosuric and natriuretic response of diabetic rats was enhanced by pantoyltaurine independent of renal and plasma oxidative stress, plasma insulin and renal expression of SGLT-2. Phlorizin and pantoyltaurine reduced renal expression of SGLT-1, which accounts for $\leq 10\%$ of glucose reabsorption. However, pantoyltaurine, but not phlorizin, normalized elevated fractional excretion of urea nitrogen and clearance of blood urea nitrogen in diabetic rats, suggesting lessening effect of pantoyltaurine on uremic toxicity associated with diabetes. Collectively, our preliminary findings show that chronic treatment with pantoyltaurine may help in an insulin-independent manner to lower diabetic hyperglycemia by producing diuresis, glucosuria and natriuresis that may have translated in improvement of renal function.

Keywords: *Pantoyltaurine; Glucosuric; Diuretic; Natriuretic; Sodium-Glucose Co-Transporter*

Introduction

Optimal control of hyperglycemia is always a challenge in diabetic renovascular complications. Current pharmacological therapies are focused on improving breakdown of carbohydrate (metformin), providing exogenous insulin, increasing insulin secretion (meglitinides, sulfonylureas) and action (thiazolidinediones), reducing insulin resistance, limiting glucagon secretion (dipeptidyl peptidase-4 inhibitors, DPP-4i) and lessening carbohydrate digestion (α -glucosidase inhibitors). Most anti-diabetic drugs clinically available suffers severe safety issues such as weight gain, renal or hepatic impairment (metformin, meglitinides, DPP-4i), hypoglycemia (sulfonylureas), gastric intolerance and discomfort (metformin, α -glucosidase inhibitors) and much more [1]. Moreover, the antidiabetic treatment is further confounded due to loss of efficacy over time, in part due to organ failure. Continuing research to identify novel targets in management of diabetes led to development of inhibitors of sodium-glucose co-transporter isoforms (SGLT).

Under physiological conditions, high capacity SGLT-2, located on luminal epithelium of early renal proximal tubules reabsorbs most filtered glucose load, and low capacity SGLT-1 of distal tubules reabsorbs the rest. Glucosuric response, i.e. increase in urinary excretion of glucose, is a sign of poor glycemic control and now recognized as a feasible insulin-independent target to reduce glucose load without causing hypoglycemia. Phlorizin is a naturally occurring inhibitor of SGLT-1/2 and reported to increase glucose excretion, control hyperglycemia and promote weight loss.

Phlorizin is not an ideal clinical candidate to lower glucose load because of its non-selectivity over SGLT-1/2 and GLUT-1, gastrointestinal side-effects, instability and poor absorption and bioavailability [2,3], while the use of recent SGLT-2 inhibitors (gliflozins) is associated with adverse reactions, including acute kidney injury, ketoacidosis, infection and edema [4]. Moreover, based on their IC_{50} values and plasma concentration, they are expected to inhibit ~90% of SGLT-2 function, however, they only result in ~20 - 50% SGLT-2 inhibition, suggesting their lower efficacy [5]. Hence, there is a pressing need of novel safer pharmacotherapies to lower hyperglycemic load. Recent study in our laboratory showed that single pre-treatment of pantoyltaurine, N-substituted taurine analog, controlled the rise in streptozotocin (STZ)-induced hyperglycemia and prevented diabetes-related acute oxidative changes in brain [6]. Whether pantoyltaurine is able to reverse or control the progression of STZ-induced hyperglycemia and its related renal complications is not known. The present study was undertaken to determine further its anti-hyperglycemic ability and whether pantoyltaurine has an effect on regulation of SGLT isoforms that may further help in improving renal function.

Methods

Animal treatment: Male Sprague-Dawley rats were obtained from Taconic Farms, Germantown, NY and were kept in a room maintained at a temperature of $23 \pm 1^\circ\text{C}$ and in a 12 hr light/dark cycle. All experimental groups were maintained on laboratory rodent diet (LabDiet® 5001, PMI Nutrition International, Brentwood, MO) and water ad libitum. Animals were randomly divided into four groups ($n = 8/\text{group}$): normal rats treated with 10 mM citrate buffer (control, i.p.), pH 4.5; untreated diabetic rats (STZ); diabetic rats treated with phlorizin (0.4 g/kg/day, s.c., days 15-35) (STZ+phlorizin); diabetic rats treated with pantoyltaurine (2.4 mM/kg/day, p.o., days 15 - 35) (STZ+DL-pantoyltaurine). The dose of pantoyltaurine was selected based on our previous work [6]. Experimental diabetes was induced by a single administration of STZ (60 mg/kg, i.p.) dissolved in citrate buffer (10 mM, pH 4.5) on day 0. Control rats received the same volume of citrate buffer (10 mM, pH 4.5). After 72 hr of STZ administration, the blood glucose concentration was determined in all animals on day 0, 4, 7, 10, 14, 21, 28 and 35, via tail snip method (< 2 mm) using commercially available glucometer (TRUEtrack, Nipro Diagnostics, Fort Lauderdale, FL), and animals having blood glucose over 300 mg/dL were considered diabetic and included for further studies. All animal procedures were performed in accordance with guidelines established by the United States Department of Agriculture (USDA) and were approved by the Institutional Animal Care and Use Committee (IACUC) of St. John's University, NY. The changes in body weight was monitored every week throughout the study. To evaluate the effect of phlorizin on renal excretion of glucose and electrolytes, urine samples were collected at 4, 8 and 24 hour interval following drug administration on day 21, 28, 35 while the animals were kept in metabolic cages. On Day 35, oral glucose tolerance test (OGTT) was performed on all animals to evaluate the effect of UA on glucose intolerance. All overnight fasted animals were received oral challenge with glucose solution (2 g/kg). Blood glucose for OGTT was measured via tail pricking method (~1 mm) using a commercially available glucometer, before (0 min) and after (15, 30, 45, 60, 90, 120, 240 and 480 minutes) glucose challenge. Urine was also collected in parallel while animals were kept in metabolic cages following oral glucose challenge [7]. After OGTT, the blood sample was collected by cardiac puncture under isoflurane anesthesia and used for plasma preparation. The kidneys were excised by the freeze clamp technique and stored at -80°C . The kidney homogenate (10% w/v) was prepared in phosphate buffered saline (PBS), pH 7.4, containing protease-phosphatase inhibitors. The suspension was centrifuged at 3,000 rpm at 4°C for 30 minutes and the resulting supernatant was stored for further analysis.

Biochemical analysis: Plasma insulin content was measured by solid-phase, two-side, direct sandwich ELISA (Calbiotech Inc., Spring Valley, CA). Plasma glucose and glucosuria were measured using a commercial colorimetric assay kit (Procedure No. 510, Sigma Chemical Co., St. Louis, MO) representing a minor modification of the method [8]. Expression of SGLT-1 and -2 was quantified via direct sandwich ELISA (Mybiosource Inc., San Diego, CA). Urea nitrogen (UN) and creatinine (Cr) in plasma and urine and urinary sodium (Na^+) were measured using commercially available colorimetric kit (Stanbio Laboratory, Boerne, TX). The UN and Cr values were used to derive indices of renal function such as fractional excretion of urea nitrogen ($FE_{\text{UN}} (\%) = 100 \times (\text{urine urea}/\text{plasma urea})/(\text{urine creatinine}/\text{plasma creatinine})$) and blood urea nitrogen clearance.

Statistical analysis

The experimental results are expressed as mean ± S.E.M. (standard error of the mean) for n = 8 per group. The data were subjected to ANOVA with Bonferroni’s multiple comparison post hoc test using GraphPad Prism 5 and were considered significant at p ≤ 0.05.

Results

Effects on body weight, food intake, plasma insulin and oral glucose tolerance

In the present study, control rats showed normal weight gain (Figure 1A) and food intake (Figure 1B) while type 1 diabetic rats showed typical hyperphagia but weight loss (control: 77 ± 6 vs. STZ: -8 ± 8 g, STZ+phlorizin: -26 ± 1 g, STZ+pantoyltaurine: -80 ± 10 g). Plasma insulin levels were significantly decreased in untreated diabetic rats compared to control rats (STZ: 7.5 ± 0.1 vs. control: 15.4 ± 3.4 mU/L), which were not improved by phlorizin or pantoyltaurine (Figure 1C). After oral glucose load (OGTT), diabetic rats showed significantly higher blood glucose levels as compared to respective controls (STZ: 430 - 550 vs. control: 90-174 mg/dL) (Figure 1D). Phlorizin was able to prevent rise in blood glucose after oral challenge immediately (STZ+phlorizin: at 15 minutes 259 ± 19 mg/dL), but not at later time point (STZ+phlorizin: at 480 minutes 400 ± 60 mg/dL) while that effect of pantoyltaurine was delayed, modest but consistent decrease in blood glucose load (STZ+pantoyltaurine: at 240 minutes, 373 ± 29 and at 480 minutes 346 ± 30 mg/dL) as compared to untreated diabetic rats (STZ: at 240 minutes 485 ± 33 and at 480 minutes 518 ± 22 mg/dL).

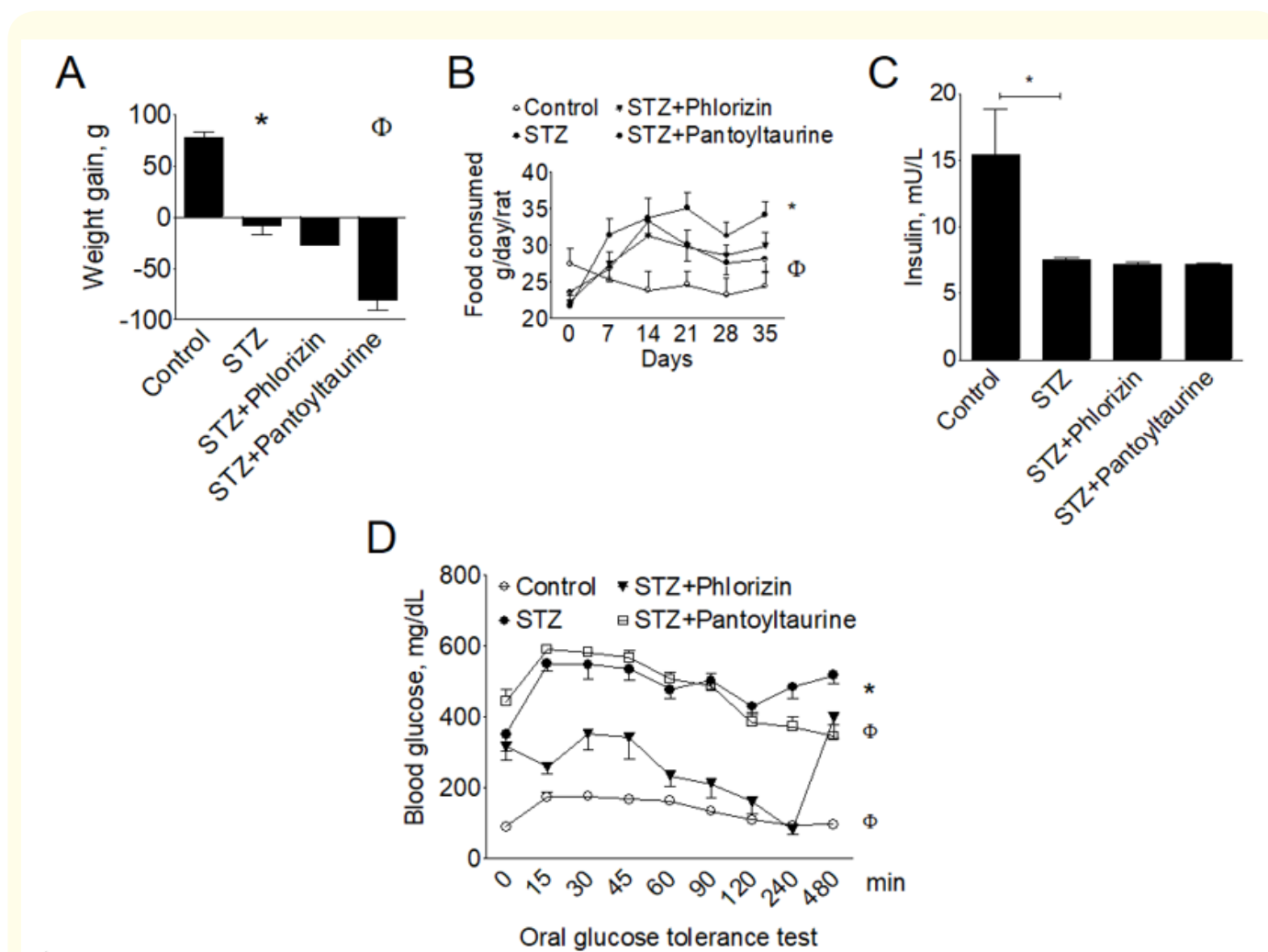


Figure 1: Effect of chronic treatment of phlorizin and pantoyltaurine on weight gain (A), food consumption (B), plasma insulin content (C) and oral glucose tolerance test (D) in streptozotocin-induced type 1 diabetic rat. Results were presented as mean ± SEM, analyzed by one-way ANOVA followed by Bonferroni’s multiple comparison test and were found significantly different at *p ≤ 0.05 vs. Control and at ϕp ≤ 0.05 vs. STZ; n = 8.

Effects on diuresis, natriuresis, urinary glucose excretion and blood glucose levels

Diabetic rats showed marked diuresis (STZ: 67 ± 5 vs. control: 14 ± 1 mL/day) (Figure 2A) and increase in fractional excretion of sodium ($U_{Na}V$) (STZ: 0.55 ± 0.04 vs. control: 0.08 ± 0.02 mmol/hr) (Figure 2B) which was enhanced by pantoyltaurine (STZ+pantoyltaurine: 81 ± 2 mL/day and 0.66 ± 0.01 mmol/hr, respectively), but not by phlorizin. However, the glucosuric response in diabetic rats was not improved by phlorizin or pantoyltaurine till day 21 (Figure 3A). On day 28, phlorizin and pantoyltaurine showed further increase in urinary glucose excretion as compared to untreated diabetic rats (STZ+phlorizin: 40 ± 2 at 4 hr and 55 ± 7 at 8 hr; STZ+pantoyltaurine: 38 ± 4 at 4 hr and 44 ± 6 at 8 hr; STZ: 19 ± 3 at 4 hr and 27 ± 7 at 8 hr) (all in mg/hr). On day 35, glucosuric effects of phlorizin and pantoyltaurine remained higher than untreated diabetic rats till 24 hr (STZ+phlorizin: 46 ± 4 at 4 hr, 50 ± 4 at 8 hr and 33 ± 1 at 24 hr; STZ+pantoyltaurine: 38 ± 5 at 4 hr, 45 ± 5 at 8 hr and 34 ± 1 at 24 hr; STZ: 20 ± 2 at 4 hr, 29 ± 2 at 8 hr and 21 ± 3 at 24 hr) (all in mg/hr).

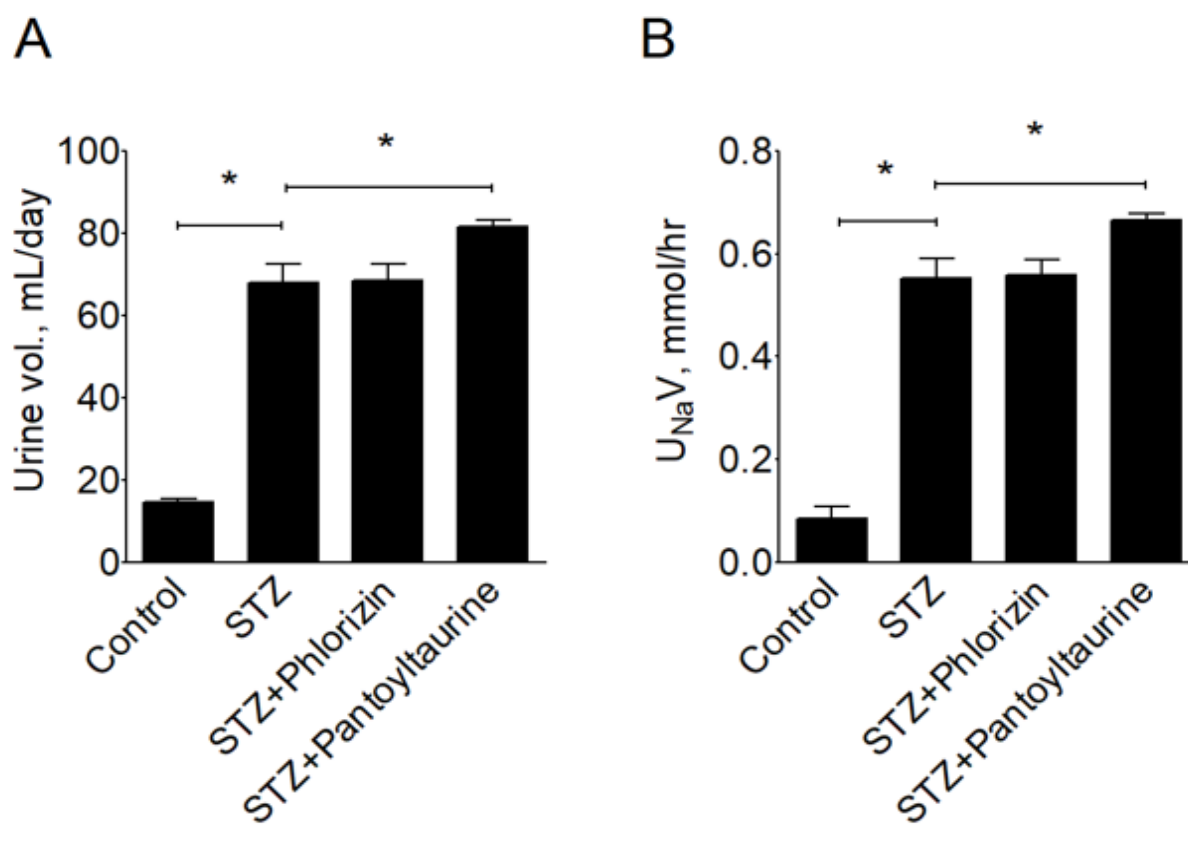


Figure 2: Effects of chronic treatment of phlorizin and pantoyltaurine on urine volume (A) and fractional excretion of sodium $U_{Na}V$ (B) in streptozotocin-induced type 1 diabetic rats. Results were presented as mean \pm SEM, analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test and were found significantly different at $*p \leq 0.05$; $n = 8$.

The blood glucose was found decreased upon treatment with phlorizin or pantoyltaurine as urinary excretion of glucose was found increased (Figure 3B). All diabetic rats showed persistent hyperglycemia during induction period, days 0 - 14 (> 374 vs. control: < 120 mg/dL). During treatment, phlorizin consistently lowered blood glucose (STZ+phlorizin: on day 21 113 ± 19 at 4 hr, 143 ± 26 at 8 hr and 279 ± 23 at 24 hr; day 28 91 ± 6 at 4 hr 250 ± 29 at 8 hr and 250 ± 30 at 24 hr; day 35 135 ± 21 at 4 hr, 206 ± 36 at 8 hr and 257 ± 24 at 24 hr). However, pantoyltaurine was modestly effective in lowering blood glucose (STZ+pantoyltaurine: on day 21 406 ± 28 at 4 hr, 354 ± 21 at 8 hr and 349 ± 42 at 24 hr; day 28 460 ± 25 at 4 hr, 496 ± 26 at 8 hr and 376 ± 26 at 24 hr; day 35 328 ± 24 at 4 hr, 355 ± 17 at 8 hr and 375 ± 34 at 24 hr) as compared to untreated diabetic rats.

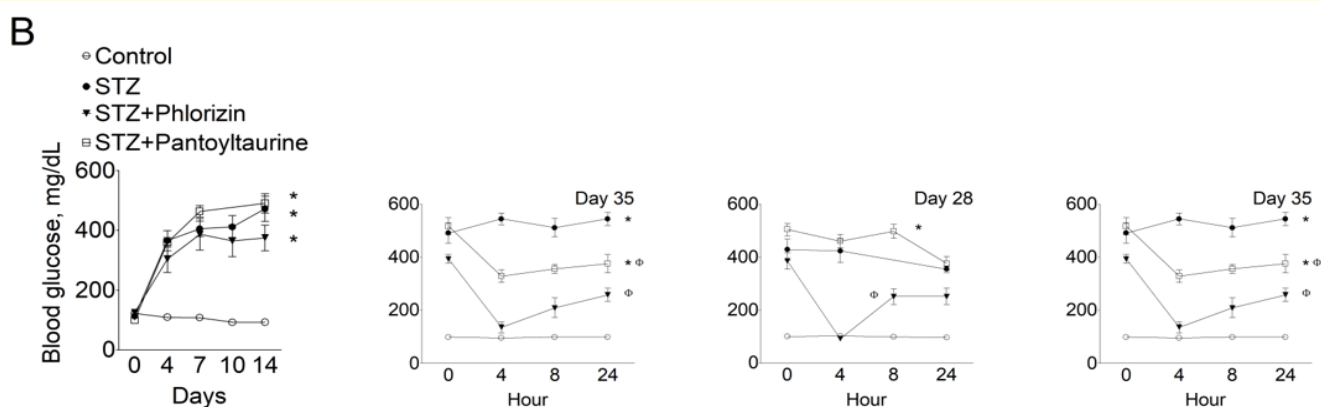
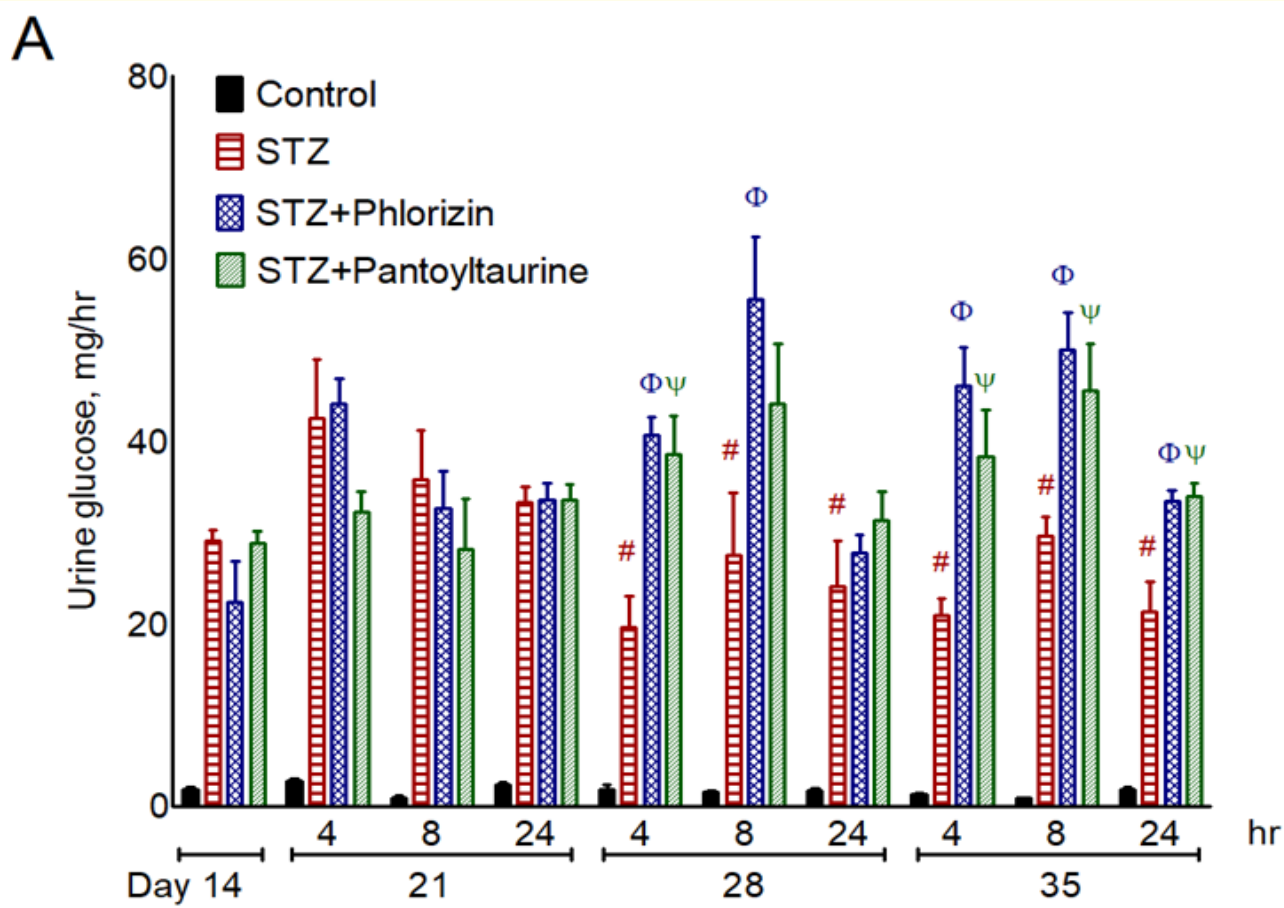
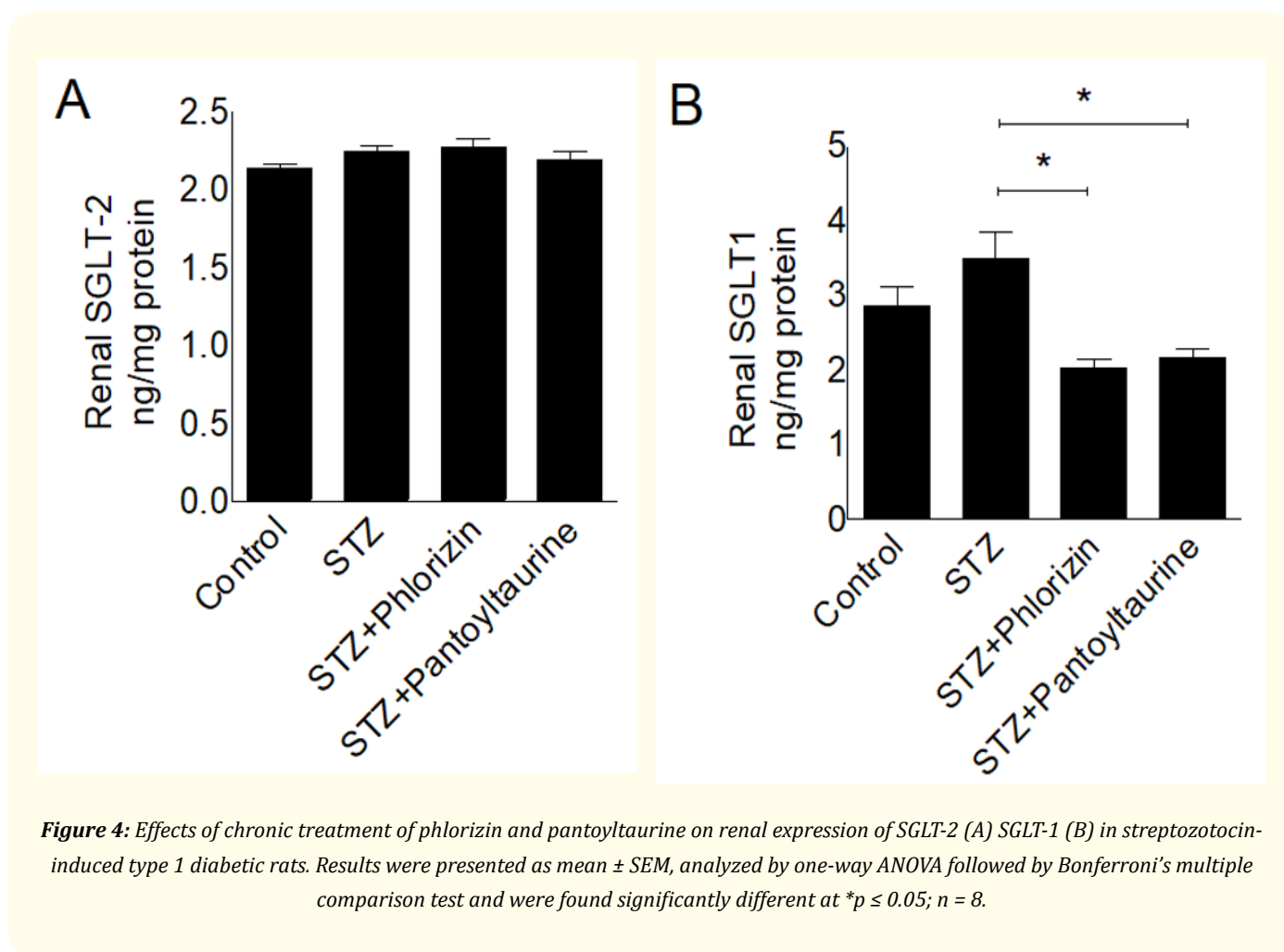


Figure 3: Effects of chronic treatment of phlorizin and pantoyltaurine on urinary excretion of glucose (glucosuria) (A) and blood glucose levels at days 0 - 14, day 21, day 28 and day 35 (B) in streptozotocin-induced type 1 diabetic rats. Results were presented as mean \pm SEM, analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test and were found significantly different at $*p \leq 0.05$, $\#p \leq 0.05$ vs. control, $\phi, \psi p \leq 0.05$ vs. STZ; $n = 8$.

Effects on expression of renal SGLT-1 and SGLT-2

Among all treatment groups, renal expression of SGLT-2 remained statistically unchanged (Figure 4A). The renal expression of SGLT-1 was found increased in untreated diabetic rats (STZ: 3.49 ± 0.34 vs. control: 2.85 ± 0.26) that was significantly decreased upon chronic treatment with phlorizin (STZ+Phlorizin: 2.02 ± 0.12) and pantoyltaurine (STZ+pantoyltaurine: 2.15 ± 0.13) (all in ng/mg protein) (Figure 4B).



Effects on indices of renal function

Diabetic rats showed marked increase in fractional excretion of urea nitrogen (FE_{UN}) (STZ: 239 ± 36 vs. control: 104 ± 6) (Figure 5A) and BUN clearance (STZ: 93.5 ± 14.8 vs. control: $9.8 \pm 0.4 \mu\text{L}/\text{min}$) (Figure 5B). Phlorizin treatment did not reduce FE_{UN} and BUN clearance as compared to untreated diabetic rats. Pantoyltaurine treated diabetic rats showed decrease in FE_{UN} (77 ± 8) and BUN clearance ($29.6 \pm 1.97 \mu\text{L}/\text{min}$).

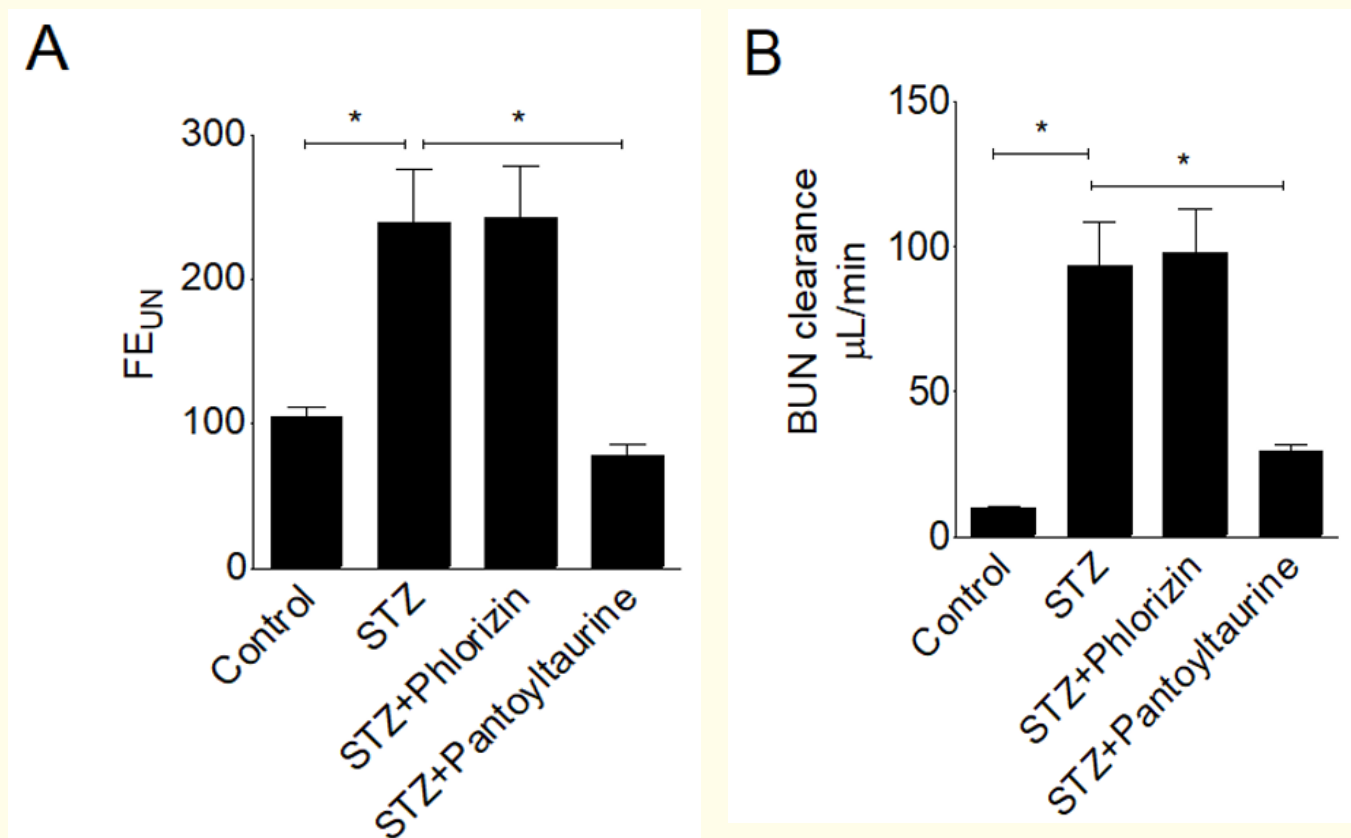


Figure 5: Effects of chronic treatment of phlorizin and pantoyltaurine on fractional excretion of urea nitrogen (FEUN) (A) and BUN clearance (B) in streptozotocin-induced type 1 diabetic rats. Results were presented as mean \pm SEM, analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test and were found significantly different at $*p \leq 0.05$; $n = 8$.

Discussion and Conclusion

The significance of current preliminary study rests on the glucosuric response (i.e. urinary excretion of glucose) of pantoyltaurine in lowering persistent hyperglycemia and oral glucose load in diabetic rats. These beneficial effects were partially attributed to osmotic diuresis, decrease in food intake and reduced weight gain which may have improved metabolic capacity. In pantoyltaurine- or phlorizin-treated diabetic rats, loss of $\sim 25 - 40$ mg/hr urinary glucose excretion equates a significant deficit in daily energy usage. Although the glucosuric response of pantoyltaurine was comparable to that of positive control phlorizin, the blood glucose lowering effect of pantoyltaurine was delayed and less potent as compared to phlorizin. At moment we do not know the mechanism behind such delayed effects. Moreover, as expected, glucosuric response with pantoyltaurine or phlorizin treatment was independent of changes in plasma insulin and renal expression of sodium-glucose co-transporter (SGLT-2), a major pump responsible for glucose reabsorption. Thus, it is highly likely that phlorizin and pantoyltaurine may have reduced the activity of SGLT isoforms and/or glucose transporters (GLUT-1/-2), primarily in renal proximal tubule. Collectively, based on delayed observed effect of chronic pantoyltaurine treatment, less hypoglycemic incidences can be presumed with pantoyltaurine that have been reported with commercially available potent SGLT-2 inhibitors. However, further detailed investigation is needed to clarify the mechanism(s) involved in reduction of food intake, weight gain and glucosuric effects upon chronic treatment with pantoyltaurine.

Moreover, upon chronic treatment with pantoyltaurine, glucosuria (after 14 days of treatment) appeared before the noticeable lowering of blood glucose (after 21 days of treatment). Therefore, we speculate that pantoyltaurine may have reduced blood glucose by inhibiting SGLT isoforms. At present pharmacokinetic and pharmacodynamics of pantoyltaurine in normal or diabetic rat model is not available to further discuss its efficacy on glucose excretion. Also, during mild to moderate renal impairment in diabetes, renal blood flow has been reported to be decreased as a consequence of decline in glomerular filtration rate. This limits the amount of glucose delivered to kidney for filtration, thus less glucose is available to be re-absorbed limiting glucosuric efficacy. Although, we did not measure direct renal blood flow. But, this may have been a reason that glucosuric effect of phlorizin and pantoyltaurine was not seen till day 21. In contrast to effects of pantoyltaurine, effects of phlorizin remained dramatic and unexpected, i.e. reductions in blood glucose by phlorizin were observed (after 14 days of treatment) before appearance of glucosuric effect (after 21 days of treatment), which indicates that phlorizin may have improved glucose metabolism in addition to inhibition of SGLT. In depth elucidation of such notions require another mechanistic study.

FE_{UN} and BUN clearance possess sensitive, specific and positive predictive value in diagnosis of renal injury. Classical cases of renal injury are highly associated with elevated FE_{UN} and BUN clearance. Typically, the fractional excretion of urea nitrogen (FE_{UN}) is inversely but non-linearly associated with renal perfusion and proximal tubular water reabsorptive capacity [9,10]. In the present study, diabetic rats showed marked diuresis, elevated FE_{UN} and BUN clearance which allows us to speculate impaired urinary concentrating ability, proximal tubular defects in urea reabsorption with or without increased formation of urea. Results suggest that chronic treatment of diabetic rats with pantoyltaurine have reduced urea clearance and thus may have improved renal function. Untreated diabetic rats also showed diuresis-dependent elevation in fractional excretion of sodium ($U_{Na}V$). Pantoyltaurine treatment of diabetic rats showed further increase in $U_{Na}V$ which suggests that pantoyltaurine also have protected against inflammatory actions of sodium. Unfortunately, the effects of pantoyltaurine on blood pressure, natriuretic targets such as sodium pump, renal or hepatic gluconeogenesis, enteric absorption of sodium or glucose, tubuloglomerular feedback or connecting tubule glomerular feedback is not available. However, in the present preliminary study we did not observe an imbalance of plasma electrolytes (not shown) or abnormalities such as dehydration or hypoglycemia. It cannot be ruled out that diuretic-natriuretic effect of pantoyltaurine may also have contributed to glucosuric effect resulting in improvement of renal function changes.

The preliminary study provided glimpse of beneficial effects pantoyltaurine on glucoregulation and renal function. Based on pantoyltaurine's diuretic, glucosuric and natriuretic response, we suggest that pantoyltaurine should further be exploited as an insulin-independent approach to gain additive efficacy in alleviating hyperglycemia and renal complications. It should be noted that, in comparison to phlorizin, pantoyltaurine showed significant renoprotection against uremia associated with diabetes. It is too early to predict the beneficial effects of pantoyltaurine in polygenic pathologies such as type 2 diabetes and obesity.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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