Trigonelline nanoparticles: A novel tool to restore metabolic pathways in type 2 diabetic complications.

Jitesh A. Daunde[1*], Nitin D. Potphode[2] Pratiksha R. Pardeshi[3], Namrata A. Jadhav[4] , Madhuri V. Walvekar[5]

[1]Department of Zoology, Shivaji University, Kolhapur. 416004 (MS) India. E-Mail- jitesh.daunde@gmail.com

[2]Department of Zoology, Shivaji University, Kolhapur. 416004 (MS) India. E-Mail- potphoden@yahoo.com

[3]Department of Zoology, Shivaji University, Kolhapur. 416004 (MS) India E-Mail- pratiksha.daunde@gmail.com

[4]Department of Zoology, Shivaji University, Kolhapur. 416004 (MS) India, E-Mail- namrataj919@gmail.com

[5]Professor, Department of Zoology, Shivaji University, Kolhapur. 416004 (MS) India. E-Mail- mvw_zoo@unishivaji.ac.in

Abstract

Disturbed insulin signalling and glucose metabolism are the key features of type 2 diabetes mellitus (T2DM) Various phytomedicines have proved their anti hyperglycemic effects. This study has been done to evaluate the therapeutic potential of trigonelline nanoparticle in diabetic complication in mice model. 4 mice groups were used for this study, control, diabetic control, treated I and treated II. Treated I was treated with trigonelline and treated II treated with trigonelline nanoparticles (TNP) for 20 days and serum insulin concentration and blood glucose level were measured as well as glucokinase (GLK), glucose 6 phosphatase (G6Pase) and glucose 6 phosphate dehydrogenase (G6PD) activity were analysed by enzymatic assay. Treated II-showed improved glucose metabolic pathways and insulin sensitivity. Serum insulin concentration, blood glucose level and GLK activity was to be found significantly increased in this group and also G6PD, G6Pase activity were significantly decreased. Recovery of glucose metabolism and insulin sensitivity is due to nanoformulation of TNP indicating its increased bioavailability and therapeutic potential, these findings are useful for future studies related to diabetic complications such as insulin signalling and insulin resistance (IR).

Keyword- Trigonelline nanoparticles (TNP), T2DM, Glucokinase, G6Pase, G6PD, insulin.

Introduction

Now-a-days diabetes is spreading like cancer across the world and has become a reason of increased morbidity and mortality in the world population. Insulin promotes glycogen synthesis in the liver which is latter used through glycolysis. Conversely insulin restricts the pathways like glycogenolysis, gluconeogenesis and ketogenesis (Wu and Garvey, 2010). In carbohydrate metabolism GKL plays central role and act as a glucose sensor. GLK is believed to be key enzyme and plays crucial role in glucose metabolism. Pathogenesis of this enzyme can cause various forms of diabetes or hypoglycemia. Also, it is considered as rate limiting enzyme for glucose phosphorylation which

is also responsible for initiation of insulin signalling (Stefanovski D et al., 2012). Under certain conditions, it has been shown that disturbed activity of GLK may be responsible for development of diabetes. Insulin itself plays a regulatory role by its activity in liver. It has been demonstrated that G6PD is linked with metabolic disorders which are caused due to lipid like hyperlipidemia and lipotoxicity which are considered as players which induce obesity and diabetes (Park et al., 2005). Raised activity of G6PD increases the circulating free fatty acids (FFA). In obese subject, FFA invites various metabolic disorders such as hyperlipidemia, lipotoxicity and IR (Park et al., 2005; Arner, 2002; Boden, 1997; Boden and Shulman, 2002). Endogenous glucose production (EGP) in liver, intestine and kidney is mainly regulated by G6Pase which carries out rate limiting step of glycogenolysis and gluconeogenesis (Chou et al., 2010). Increased endogenous glucose production is considered as progressive step of T2DM (DeFronzo et al., 1992). Trigonelline (TRG) is a plant alkaloid which was first isolated from Trigonella foenumgraecum L. commonly known as fenugreek. It is commonly used as spice throughout India (Zia et al., 2001). TRG has been demonstrated that it has sedative, antibacterial, antiviral activity, inhibits platelet aggregation, shows anti-tumor effects, improves memory retention and also shows hypoglycemic, hypolipidemic, antimigraine effect (Tohda et al., 2005; Hirakawa et al., 2005; Hong et al., 2008,2009; Yoshinari et al., 2009; Yoshinari and Igarashi, 2010; Özçelik et al., 2010). In Mediterranean diet, coffee is considered as the important source of TRG, consumption of coffee helps in glucose homeostasis (Tresserra-Rimbau et al., 2014). In order to verify the effect of TRG v/s TNP we have studied the glucose level, insulin concentration and metabolic enzymes involved in glucose metabolism in T2DM mice model.

Subjects and methods

Animal model and groups

Animals were divided into 4 groups (Male mice of 3 months age) viz.

1. Control- Mice were given subcutaneous injection of citrate buffer pH 7.4 for 20 days.
2. Diabetic control- Mice were given single intraperitoneal injection of Streptozotocin 40mg /kg in citrate buffer pH 7.4 after high fat diet for 15 days (Srinivasan et al., 2005).
3. Treated I (Trigonelline (TRG) group)- Type 2 diabetic mice were given intraperitoneal injection of Trigonelline (TRG) at a dose of 100 mg /kg body weight daily for 20 days.
4. Treated II (Trigonelline nanoparticles (TNP) group)- Type 2 diabetic mice were given intraperitoneal injection of Trigonelline nanoparticles (TNPs) at a dose of 80 mg/ kg body weight daily for 20 days.

Experimental Procedure

1.1. Measurement of glucose level and serum insulin concentration.

Fasting blood glucose level was measured using glucometer (Accucheck, Roche diagnostics India Pvt. Ltd.). Serum insulin level was detected by enzyme-linked immunosorbent assay (ELISA) technique as per procedure provided in kit manual.

1.2. Enzymatic assay Glucokinase (GLK), glucose 6 Phosphatase (G6Pase) and glucose 6 phosphate dehydrogenase (G6PD)

The glucokinase activity was measured on UV-VIS spectroscopy (Davidson and Arion, 1987). A muscle and liver sample

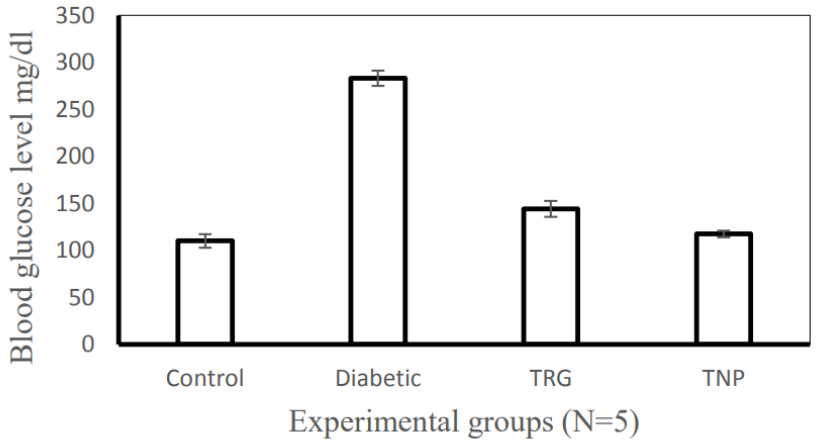
were homogenized in HEPES buffer (pH 7.5) and absorbance was measured from supernatant of homogenized sample and activity of GLK was calculated (Yoshinari et al., 2009). G6Pase activity was measured from microsomal fraction obtained from centrifugation of homogenate prepared in HEPES buffer (pH 6.5) absorbance was measured at 820 nm and G6Pase activity was calculated (Lange et al., 1986). Glucose 6 phosphate dehydrogenase activity was measured by the rate NADPH production (Park et al., 2005). Protein levels were determined for each sample by using a BCA assay kit (Pierce), and each enzyme activity was normalized by determining the protein concentration

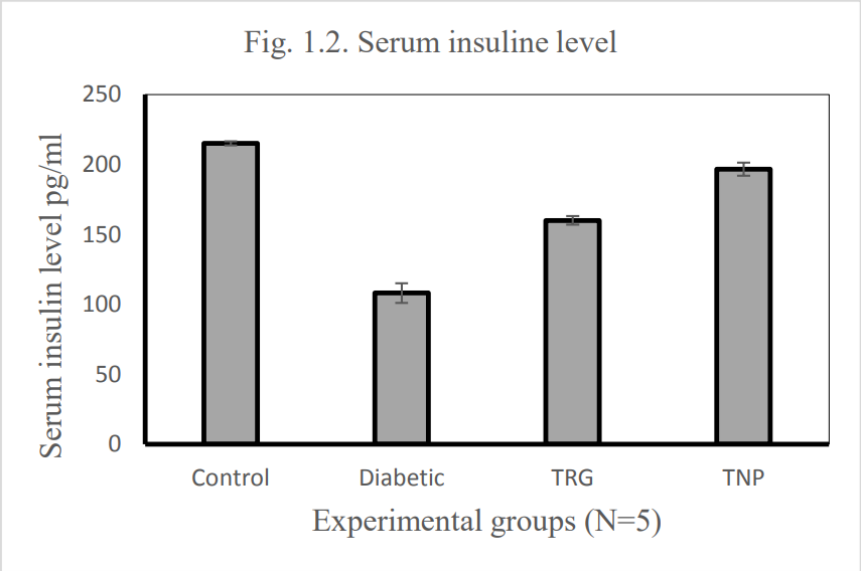
1.1. Therapeutic potential of TNPs on blood glucose level (mg/dl) (Fig 1.1.) and insulin concentration (pg/ml) (Fig 1.2.).

Blood glucose level (mg/dl) measured after the treatment period of 20 days, in TRG and TNP group was found decreased compared to diabetic control group. The percentage deference of 49% in TRG group and 59% in TNP group was observed as compared to diabetic control group. On its contrary insulin concentration in both the groups was seen to be elevated significantly i.e. about 33% and 45% in TRG and TNP group respectively as compared to diabetic control group (TRG and TNP v/s diabetic control, p= <0.01).

Results

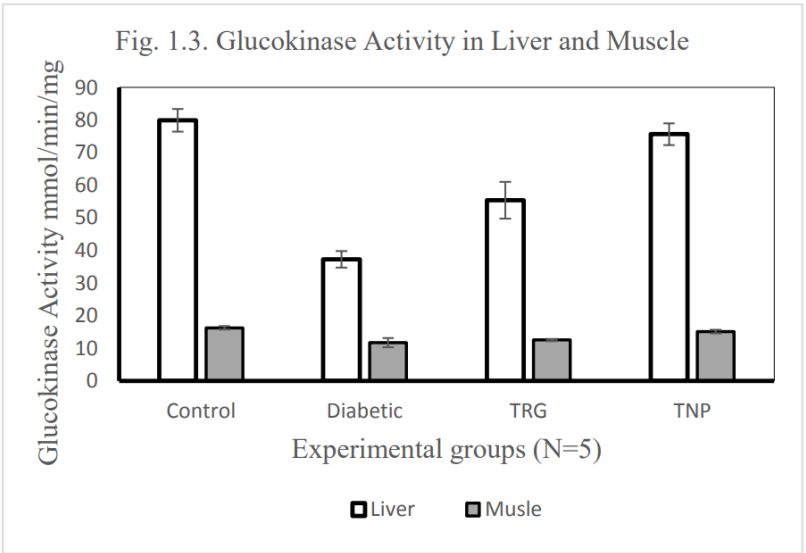
Fig. 1.1. Blood glucose level





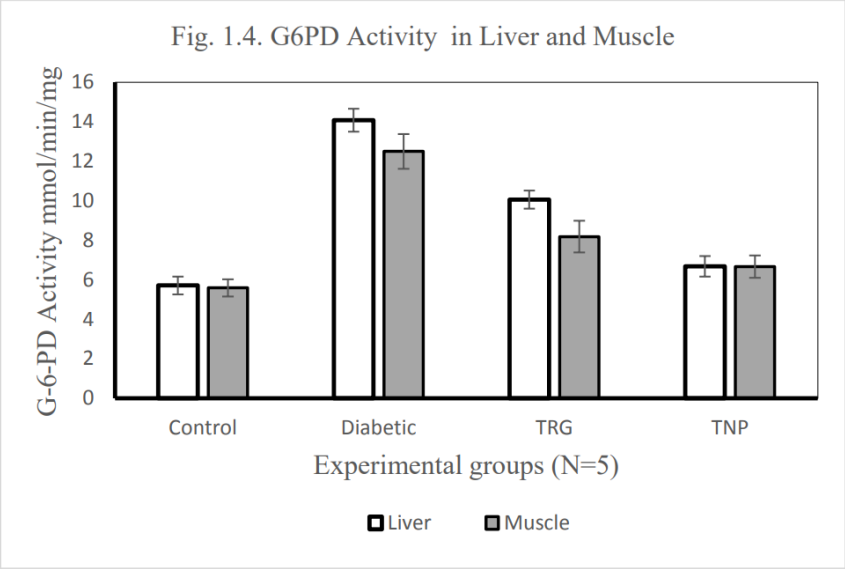
1.2. Therapeutic potential of TNPs on enzymatic activity of GLK (mmol/mg/min) in liver and muscle (Fig 1.3.)

GLK is considered as rate limiting enzyme of glycolytic pathway. After the treatment, its activity (mmol/min/mg) in the liver significantly increased in TRG and TNP groups by about 32% and 50% respectively (TRG and TNP v/s diabetic control). Similarly in muscle, TNP group its activity was significantly increased as compared to diabetic control group i.e. about 17% (TNP v/s diabetic control, P= <0.01).



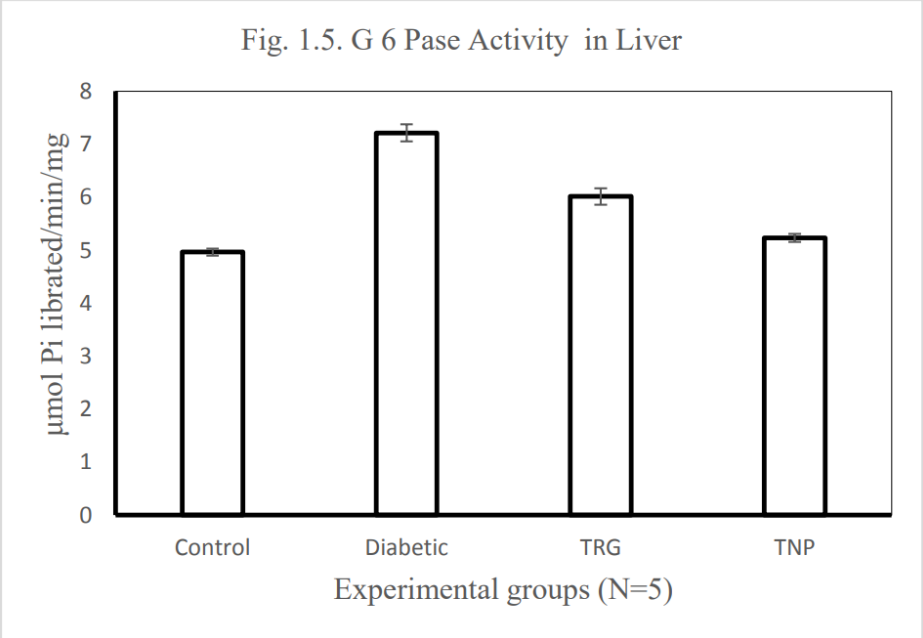
1.3. Therapeutic potential of TNPs on enzymatic activity of G6Pase (µmol Pi liberated/min/mg) in liver and muscle (Fig 1.4.)

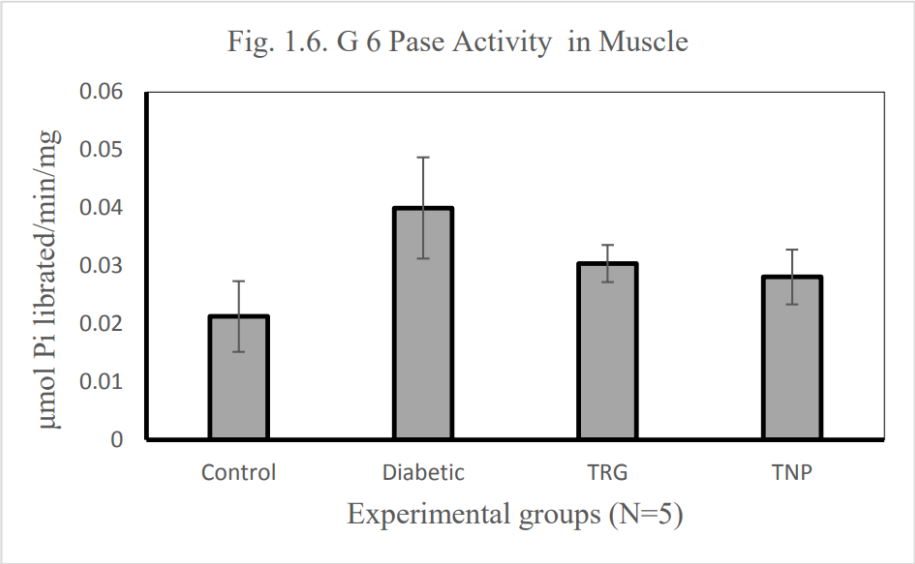
G6Pase is an enzyme involved in gluconeogenetic pathway, its activity (µmol Pi liberated/min/mg) in the liver and muscle significantly decreased after the treatment. It was 16% and 24% in liver and muscle of TRG group and 27.5% and 29.75% in liver and muscle of TNP group (TRG and TNP v/s diabetic control). When both the treated groups compared to its activity, significant difference was observed i.e. about 13% in liver and 7.13% in muscle (TRG v/s TNP, P=<0.01).



1.4. Therapeutic potential of TNPs on enzymatic activity of G6PD (mmol/min/mg) in liver and muscle (Fig 1.4)

G6PD activity (mmol/min/mg) in the liver and muscle significantly decreased after the treatment. It was 29% and 35% in liver and muscle of TRG group and 53% and 19% in liver and muscle of TNP group (TRG and TNP v/s diabetic control). When both the treated groups were compared to its activity, significant difference was observed i.e. about 34% in liver and 19% in muscle (TRG v/s TNP, P= <0.01).





Discussion

Blood Glucose level and Serum Insulin level

In T2DM, increased activity of G-6-Pase and decreased activity of GLK in liver were responsible for endogenous glucose production (EGP) by liver (Barzilai and Rossetti, 1993; Haber et al., 1995). Lack of insulin activity, increased activity of glycogenolytic and gluconeogenic enzymes are while decreased activity of glycolytic enzymes responsible for imbalanced glucose metabolism (Sundaram et al., 2012; Latha and Daisy, 2013). Our study suggests parallel observations, in diabetic mice increased blood glucose level could be due to above mentioned reasons. In TRG and TNP group, blood glucose level was significantly lowered than the normal level; these findings suggest that trigonelline has antihyperglycemic effect which lowers the blood glucose level (Al-Habori M, Raman, 1998; Zhou et al., 2012). In T2DM, insulin signalling was severely disrupted and glucose uptake and glycogen storage was depleted because of insulin resistance (Bogardus et al., 1984). Trigonelline improves insulin sensitivity and reduces insulin resistance. As well as trigonelline reduces lipid accumulation in epididymal adipose tissue and reduces chances of insulin resistance (Yoshinari and Igarashi. 2010). In another study it has been demonstrated that fenugreek seed extract causes increase in number of ß-cells of islets of Langerhans, which promote the number of insulin secretary granules in pancreatic ß-cells of the treated diabetic mice (Walvekar et al., 2014).

Activity of Glucokinase

In present investigation, we found that in diabetic mice the activity of glucokinase was diminished. Earlier work had indicated that GLK activity is chiefly regulated by insulin in the liver and muscle (Barzilai and Rossetti, 1993; Magnuson, 1990). This suggests that lowered activity of GLK could be due to decreased insulin level in diabetic mice. In diabetic rat model it has been demonstrated that the activity of enzyme GLK is significantly decreased (Pilkis, 1970). These suggestions may have contributed to our findings. In T2DM mice model trigonelline increases GLK activity and glycolysis (Yoshinari and Igarashi. 2010).

Activity of Glucose 6 phosphatase

The observed activity of G-6-Pase in the liver of diabetic mice was decreased (Durruty et al., 2019). It was demonstrated that insulin resistance is liable to increase activity of G-6-Pase in the liver which once dephosphorylates glucose-6-phosphate cannot be metabolized in glycolysis. This dephosphorylated glucose gets into circulation supporting to hyperglycemia. However, in vivo studies proved that increased insulin level reduces the G-6-Pase activity (Speth and Schulze, 1981; Suzuku et al., 1984). Our observation agrees to this report because decreased G-6-Pase activity in TRG and TNP groups could be due to increased level of insulin. It has been also reported that HGP suppressed by decreased flux of G-6-Pase in liver, despites upholding of average glucose level (Newgard et al., 1984).

Glucose 6 phosphate dehydrogenase

G-6-PD at higher activity brings on insulin resistance as well as reduces insulin signalling (Park et al., 2005). Consistence with this observation our data suggest that in diabetic mice increased level of G-6- PD could be due to HFD-STZ induced diabetes. In support of these observations, it has been shown that in obesity, increased FFA accumulated within skeletal muscle cells results in insulin resistance (Boden, 2002). It has been supposed that G-6-PD is a key regulatory enzyme responsible for FFA biosynthesis that results in diabetic consequences. Consistent with previous reports, in T2DM trigonelline decreases G-6-PD activity which aids the decrease in fatty acid synthesis (Yoshinari and Igarashi, 2010). In our observation, TRG and TNP group that showed reduced activity of G-6-PD in liver and muscle is due to hypolipidimic activity of TRG.

Conclusion

Present investigation was carried out compare the efficacy of TNP on diabetological complications with TRG and this study was found that TNP showed promising results than trigonelline alone. Future applications of TNP in diabetological studies may prove it as good antidiabetic drug.

Declaration of competing interest

All authors declare that there is no duality of interest associated with their contribution to this manuscript.

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