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Hypoglycemic Activity of Methanolic Extract of *Talinum Triangulare* Leaves in Normal and Streptozotocin Induced Diabetic Rats

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ABSTRACT

Talinum Triangulare (Family: Portulacaceae) is an herbaceous perennial plant widely grown in tropical regions as a leaf vegetable with a blood-glucose-lowering effect, but its mechanism of action are still unknown. Here we report a hypoglycemic activity of Methanolic Extract of *Talinum Triangulare* Leaf (METTL) in healthy, glucose loaded and Streptozotocin (STZ)-induced diabetic rats. Animals either healthy or STZ-induced diabetic show significantly lowered blood glucose levels after 2 weeks of METTL treatment (250 mg/kg), it significantly reduced blood glucose levels compared with that in diabetic control rats. Additionally, the increase in blood glucose levels after administration of glucose (1.2 g/kg) in normal rats is significantly decreased and the oral glucose tolerance (OGTT) of STZ-induced diabetic rats is largely improved by METTL treatment. However, co-administration of METTL with glipizide, an oral hypoglycemic drug, it produces synergistic effect. We conclude that METTL has a significant anti hyperglycemic effect. Continuous glucose monitoring is needed in diabetic patients.

Keywords: Streptozotocin (STZ), Glipizide, *Talinum Triangulare*, Synergistic effect, Diabetes

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INTRODUCTION

Rapidly increasing prevalence of Type 2 Diabetes Mellitus (T2DM) as a major cause of morbidity and mortality worldwide, particularly in Asia, poses a severe challenge to the current management paradigm. The International Diabetes Federation (IDF) projects that without effective preventive action, the number of individuals with diabetes will increase from 285 million in 2010 to 439 million in 2030, of whom T2DM accounts for approximately 85–95% of cases in high-income countries and possibly a higher proportion in less affluent populations (International Diabetes Federation; 2009, Shaw *et al.*, 2010). The number of people with diagnosed diabetes could thus increase to 4 million by a few years. The burden of the disease is increasing both for the progressive aging of population and for the worsening of lifestyle (Zimmet *et al.*, 2001).

The largest increase is expected in low and middle income countries, which already bear some 70% of the total global burden of diabetes (International Diabetes Federation: 2009). In Asia, as elsewhere, healthy lifestyle modifications are the keystone of effective T2DM prevention and management. Nevertheless, additional anti hyperglycemic pharmacotherapy is the norm for most T2DM patients. Despite great progress in the pharmacological management of T2DM in past decades, the side effects of all current conventional therapies limit their uses and although they yield short-term improvements, none effectively arrest or reverse the inexorable worsening of the disease (Heine *et al.*, 2006). Herbal medicines are naturally occurring therapeutic compounds in biological organisms. The use of natural plant substances (botanicals) to treat and prevent illness has existed since prehistoric times and still flourishes today in many societies and cultures with many plants still in common use (Duke, 1998). Herbal medicine, which is also known as folk medicine, is known from every continent, essentially every tribe and, the world health organization estimates that up to 80% of the world's population relies mainly on herbal medicine for primary health care either in part or entirely (BGCI Fact Sheet, 2000). The importance of medicinal plants in pharmacology is very crucial because they contain active constituents (that are used in the management of various disease conditions) such as; quinine for malaria, opioid analgesics for cancer pain, NSAIDS for pyrexia, laxatives for constipation, etc which have side effects both on acute and chronic administration. Hence, their study is important to the development of new and safer drugs. *Talinum Triangulare* (TT) is a herbaceous perennial plant widely grown in tropical regions as a leaf vegetable. It is probably native to tropical America and the crop is grown in West Africa, Southeast Asia and warmer parts of North America and South America. Along with celosia species, it is one of the most important leaf vegetable of Nigeria. Common names include; water leaf, leaf ginseng, American ginseng, Surinam purslane, Surinam spinach, Phillipine spinach, Ceylon spinach, Florida spinach, potherb fameflower, Lagos bologi, sweetheart, poslen, biala, espinafre de ceilao galaghati grasse, krokot belanda, and kumu manus (Pain *et al.*, 2001). The aqueous root extract of TT does not possess any significant laxative effect but has anti diarrhoeal effect in doses of 500 to 2000 mg/kg (Olufunmilayo *et al.*, 2011). Antioxidant and hepatoprotective activities of polysaccharides from TT were reported (Liang *et al.*, 2011). TT leaves has hepatoprotective activity (Adefolaju *et al.*, 2009). TT leaves used as antidiabetic in Indian ayurvedic system (Agarwal, 1997). Till now no literature is available for this activity. Aim of our study was to evaluate hypoglycemic activity of METTL in healthy, glucose loaded and STZ induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

Streptozotocin was purchased from Sigma Chemicals Co., St. Louis, MO, USA. Glipizide was obtained from Digpati Roy,

Asst. Prof, KVSR Siddhartha College of pharmaceutical sciences, Vijayawada as gift sample. Johnson and Johnson one touch glucometer kit was purchased from local pharmacy, Narasaraopet. All other chemicals used were of analytical reagent grade.

Collection and preparation of samples

The leaves of TT (water leaf) were collected from medicinal garden of MAM college of pharmacy, Narasaraopet, Guntur, Andhara Pradesh. A specimen was preserved in the laboratory for future reference.

Extraction

Leaves were air dried in shade, grinded mechanically and 100 g of coarse powder was extracted by using methanol in soxhlet apparatus. Extract was concentrated to a semi-solid alcohol free material and final extract yield was 8.74% (based on wet material).

Animals

Male wistar rats of weight between 150 to 200 gms obtained from National Institute of Nutrition (NIN), Hyderabad, India, were used in the study. They were housed in polypropylene cages at a temperature of $23 \pm 2^\circ\text{C}$ and relative humidity 60 ± 5 with 12 hour each of dark and light cycles. Water was allowed *ad libitum*. All the animal experiments were approved by Institutional Animal Ethics Committee and were done as per their guidelines.

Acute toxicity study

The rats were divided in to 3 groups of six rats each. Extract was suspended in to 2% gum acacia. Acute toxicity study on behavioral changes was carried for the dose of 500, 1000 and 2500 mg/kg body weight and observed for 24 hrs for mortality.

EXPERIMENTAL DESIGN

Effect of METTL on blood glucose of healthy rats

Rats were divided into 4 groups of six each after 18 hrs fasting. Group I served as control receives 2% gum acacia. Groups II, III and IV were treated with glipizide (5 mg/kg, p.o), (METTL (250 mg/kg, p.o) and both glipizide (5 mg/kg, p.o) and METTL (250 mg/kg, p.o) respectively. Blood glucose levels were estimated at 0, 30, 60 and 120 min.

Effect of METTL on glucose loaded rats (Etuk *et al.*, 2010)

This method is often referred to as physiological induction of Diabetes Mellitus (DM) because the blood glucose level of the animal is transiently increased with no damage to the pancreas. In the clinical setting, it is known as Glucose Tolerance Testing (GTT). GTT is a standard procedure often used for the diagnosis of border line diabetic patients. The method was found to produce a widely fluctuating level of hyperglycemia when compare to STZ induction method. Animals were fasted for 18 hr and blood was collected (at 0 hr) for glucose estimation. Rats were divided in to 4 groups of six rats each.

Group. I: (Control): Treated with 2% gum acacia suspension

Group. II: Treated with glipizide (5 mg/kg, p.o)

Group. III: Treated with METTL (250 mg/kg, p.o)

Group. IV: Treated with both glipizide (5 mg/kg, p.o) and METTL (250 mg/kg, p.o)

After half an hour glucose (1.2 g/kg, oral) solution was administered and blood samples were collected from tail vein for glucose estimation at 30, 60 and 120 min.

Induction of Diabetes Mellitus

Experimental diabetes was induced by single intraperitoneal injection of 60 mg/ kg of STZ, freshly dissolved in cold citrate buffer, pH 4.5 (Erejuwa et al., 2010; Pandit et al., 2010). Control animals received only citrate buffer. After 5 days of STZ injection, animals with fasting blood glucose above 250 mg/dl were considered as diabetic and included in the study. No adverse effect was observed at the tested concentration throughout the study.

Effect of METTL in STZ - induced diabetic rats

Animals were fasted for 18 hr and blood was collected (at 0 hr) for glucose estimation. Rats were divided in to 4 groups of six rats each.

Group. I: (Control): Treated with 2% gum acacia suspension.

Group. II: Treated with glipizide (5 mg/kg, p.o).

Group. III: Treated with METTL (250 mg/kg, p.o).

Group IV: Treated with both glipizide (5 mg/kg, p.o) and METTL (250 mg/kg, p.o). After 4, 7 and 15 days blood glucose levels were estimated by glucose kit. Results are expressed as the Mean ± S.D. for six rats in every group. Analyses were performed by one-way analysis of variance (ANOVA). Results were regarded significantly different if $p < 0.05$.

RESULTS

Acute toxicity study of METTL

In acute toxicity study, METTL treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weight and food consumption when compared to the vehicle treated group. LD₅₀ was considered as > 2500 mg/kg.

Effect of METTL on blood glucose of healthy rats

METTL decreases blood glucose levels in healthy rats but the results were not significant when compared with control. Co administration of METTL with glipizide significantly decreases blood glucose levels. Results were shown in table 1 and % blood glucose reduction was shown in fig 1.

Table. 1: Effect of METTL on blood glucose of healthy rats (n = 6).

Time (Min)	Group I	Group II	Group III	Group IV
0	75 ± 1.47	78 ± 4.61	76 ± 2.64	78 ± 2.06*
30	73 ± 2.1	71 ± 3.57	72 ± 4.75	70 ± 3.21*
60	73 ± 3.5	68 ± 3.26*	70 ± 4.50	63 ± 3.42*
120	71 ± 2.08	62 ± 2.97*	69 ± 3.2	58 ± 5.76*

* (p < 0.05) when compared to control

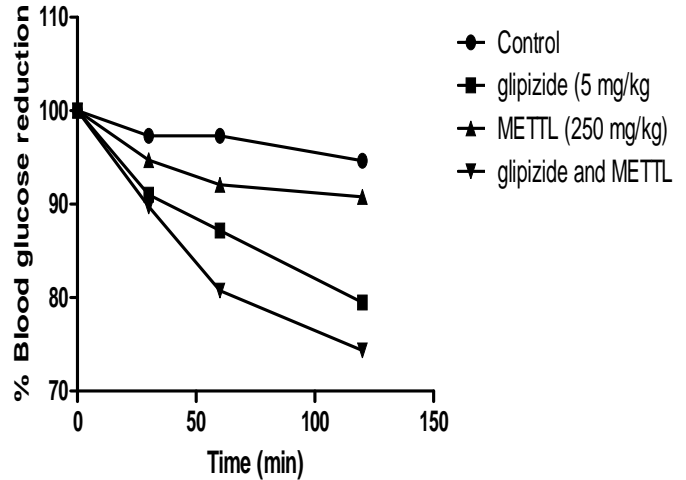


Fig. 1: % blood glucose reduction with control, glipizide, METTL and combination of glipizide and METTL in healthy rats.

Effect of METTL on glucose loaded rats

Fasting blood glucose levels were estimated in 18 hrs fasted rats in all groups. After 30 min of treatment glucose (1.2 g/kg) was given. Blood glucose levels in all groups increased rapidly and gradually decreased there after. METTL extract at 250 mg/kg caused a significant reduction of the rise of blood glucose levels after 60 min, but it is not significant when compared to standard drug glipizide. METTL extract produces synergistic effect with glipizide. Results were shown in table 2. % blood glucose reduction with METTL, glipizide and combination in glucose loaded rats shown in fig 2.

Table. 2: Effect of METTL on glucose levels in glucose loaded rats.

Time (Min)	Group I	Group II	Group III	Group IV
0	96 ± 2.31	90 ± 3.54	92 ± 2.60	90 ± 5.12
30	164 ± 4.28	141 ± 3.52*	156 ± 6.48*	137 ± 5.43*
60	158 ± 6.02	125 ± 5.41*	137 ± 3.57*	111 ± 4.65*
120	143 ± 8.45	103 ± 7.05*	114 ± 8.15*	97 ± 6.50*

* (p < 0.05) when compared to control

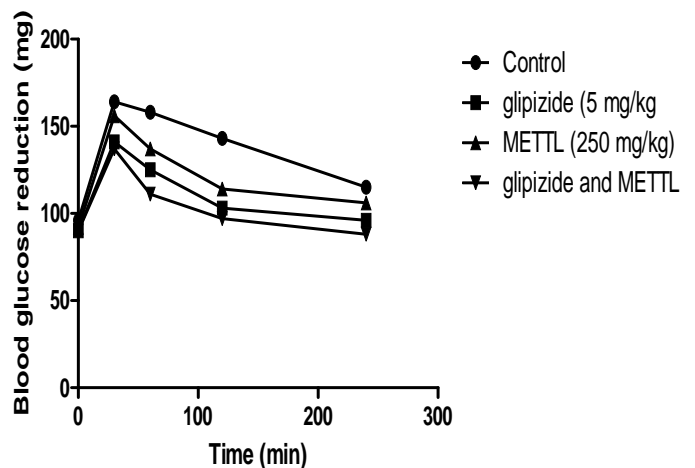


Fig. 2: Blood glucose reduction with METTL, glipizide and combination in glucose loaded rats.

Effect of METTL in STZ - induced diabetic rats

The effects of METTL on STZ-induced diabetic rats were evaluated. Diabetic rats received METTL (250 mg/kg/day) for 2 weeks. Fig. 3 shows the hypoglycemic effect of METTL on the % blood glucose level of rats with STZ-induced diabetes during the experimental period. The blood glucose level of STZ rats treated with METTL was significantly lower than that of untreated rats at 2nd week. Results were shown in table 3. In addition, for the OGTT, a significant reduction of oral glucose tolerance was observed in the METTL-treated group (Fig. 1). This suggests that METTL-treated rats showed a better effect of improved oral glucose tolerance and blood glucose regulation than rats with STZ-induced diabetes. Blood glucose reduction in STZ- induced rats on 15th day were shown in fig 4.

Table 3: Effect of METTL on glucose levels of STZ- induced diabetic rats.

Time (day)	Group I	Group II	Group III	Group IV
0	284 ± 6.07	296 ± 8.62	267 ± 10.5	305 ± 9.71
4	274 ± 6.27	263 ± 9.30*	240 ± 11.27*	256 ± 11.64*
7	269 ± 8.53	218 ± 7.61*	224 ± 8.42*	193 ± 10.57*
15	261 ± 11.25	186 ± 10.2*	203 ± 12.38*	148 ± 10.54*

* (p < 0.05) when compared to control

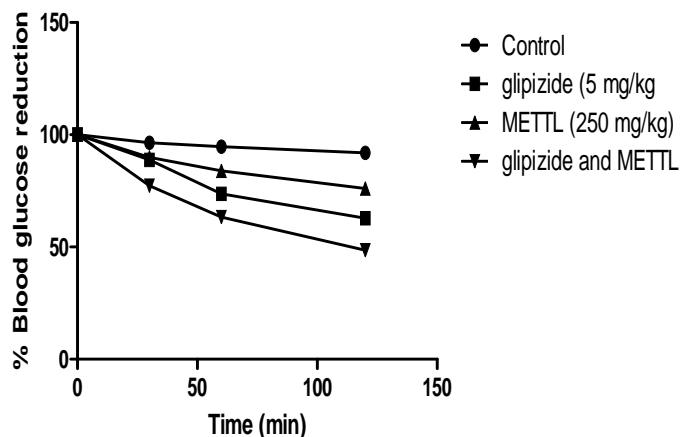


Fig. 3: % blood glucose reduction with control, glipizide, METTL and combination of glipizide and METTL in STZ - induced rats.

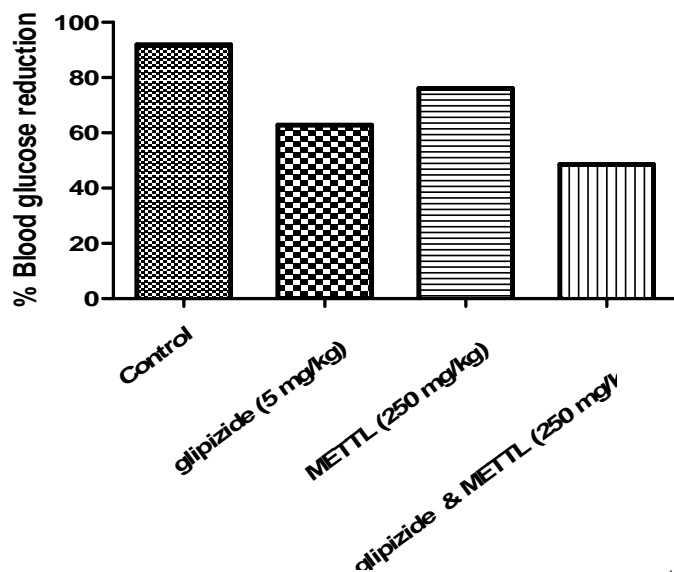


Fig. 4: Effect of METTL on glucose levels of STZ- induced diabetic rats on 15th day.

DISCUSSION

DM is an endocrine dysfunction resulting from insulin deficiency or incapability of peripheral tissues to respond to insulin (Singh et al., 2008). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Tiwari et al., 2002). DM is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes (Kesari 2005), but only a few have been scientifically evaluated. Therefore, we have investigated the effect of METTL on glycemic control in healthy, glucose loaded and STZ-induced diabetic rats.

METTL decreases blood glucose level in healthy rats, but not significant when compared with control. METTL showed significant effect in glucose loaded and diabetic rats at dose of 250 mg/kg in diabetic rats. It produces synergistic effect with glipizide. The capacity of METTL to decrease the elevated blood glucose to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The possible mechanism by which METTL exerts its hypoglycemic action in diabetic rats may be due to potentiating the insulin release, since the percentage fall in blood glucose levels was very significant (p < 0.05) at 250 mg/kg.

CONCLUSION

The leaves of TT are a good candidate as alternative and/or complementary medicine in the management of DM. The results of the present study indicate that the METTL is capable of exhibiting significant anti-hyperglycemic activity in STZ - induced diabetic rats. TT used as leafy vegetable in India. Continuous blood glucose monitoring is needed in diabetes patients because it produces synergistic effect with oral hypoglycemic agents. TT may be altering the pharmacokinetic of glipizide; further investigations are needed to identify the lead molecule and to elucidate exact mechanism of action for antidiabetic effect.

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