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Antidiabetic, antihyperlipidemic and antioxidant potential of methanol extract of *Tectona grandis* flowers in streptozotocin induced diabetic ratsSubramaniam Ramachandran¹, Aiyalu Rajasekaran^{2*}, KT Manisenthil Kumar¹¹Department of Pharmacology, KMCH College of Pharmacy, Coimbatore – 641 048, Tamil Nadu, India²Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore – 641 048, Tamil Nadu, India

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ABSTRACT

Objective: To investigate antidiabetic, antihyperlipidemic and antioxidant activity of methanol extract of *Tectona grandis* (*T. grandis*) flowers (METGF) in streptozotocin (STZ) induced diabetic rats to supports its traditional use. **Methods:** Acute toxicity study of METGF was carried out in rat to determine its dose for the antidiabetic study. Oral glucose tolerance test (OGTT) was performed to evaluate METGF effect on elevated blood glucose level. Diabetes was induced in rats by administration of STZ (60 mg/kg, ip.) and it was confirmed 72 h after induction. METGF was orally given to the diabetic rats up to 28 days and blood glucose level were estimated each week. On 28 day of the experiment, diabetic rats were sacrificed after the blood collection for the biochemical parameters analysis and liver, kidney was collected to determine antioxidants levels. **Results:** In acute toxicity, METGF did not show toxicity and death up to a dose 2 000 mg/kg in rats. Administration of METGF 100 and 200 mg/kg significantly ($P < 0.001$) reduced blood glucose levels in OGTT and STZ-induced diabetic rats. Both doses of METGF treatment significantly ($P < 0.001$, $P < 0.01$ and $P < 0.05$) increased body weight, serum insulin, haemoglobin (Hb) and total protein levels in diabetic rats. Also, METGF treatment reduced elevated glycosylated haemoglobin (HbA1c) and other biochemical parameters levels significantly ($P < 0.001$) in diabetic rats. Altered lipid profiles and antioxidants levels were reversed to near normal in diabetic rats treated with METGF. **Conclusions:** These results concluded that METGF possesses antidiabetic, antihyperglycemic and antioxidant activity which supports its traditional use.

1. Introduction

Diabetes is a chronic metabolic disorder due to absolute or relative lack of insulin and characterized by hyperglycemia in the postprandial and or fasting state, associated with ketosis and protein wasting in severe condition[1]. Globally, it is the most common serious and largest endocrine disorder and considered to be one of the five leading causes of death in the world[2]. In both type 1 and 2 diabetics, cardiovascular diseases increases two to three fold morbidity and mortality rate compared to non-diabetic persons[3]. The free radicals derived from oxygen have been implicated in the pathophysiology of various disease conditions, including diabetes mellitus. The biomolecules

such as lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue are affected by free radicals. Also, the evidence showed diabetes induced changes in the activities of antioxidant enzymes in various tissues[4]. Moreover, oxidative stress is involved in the development and progression of diabetes associated complications. The activation of transcription factors, advanced glycated end products (AGEs) are believed to be partly responsible for the oxidative stress and diabetic complications[5].

Despite the availabilities of hypoglycaemic agents from natural and synthetic sources, diabetes and its complications continue to be a major healthcare problem. Also, there is a clinical unmet need is existing in the treatment of diabetes. In Ayurvedic and Siddha medicines, there are number of Indian medicinal plants which have found to be useful to successfully manage diabetes[6]. The advantage of traditional medicinal plants is no or lesser adverse effects with multiple therapeutic actions due to the presence of different bioactive compounds. World Health

*Corresponding author: Dr. A. Rajasekaran, Professor, KMCH College of Pharmacy, Kovai Estate, Kalapatti Road, Coimbatore – 641 048, Tamil Nadu, India.
Tel: +914222917282
Fax: +914222369302
E-mail: rsekaran2001in@yahoo.co.in

Organization (WHO) has recommended the evaluation of traditional plants for the diabetes treatment due to its non-toxic, with less or no side effects and efficacy are considered to be useful for diabetes management[7].

Tectona grandis (*T. grandis*) Linn. f. (Verbenaceae), commonly known as teak, is distributed throughout India in deciduous forests and also cultivated. The whole tree of *T. grandis* is medicinally important and many reports on claims to cure several diseases in Indian traditional system of medicine particularly in Ayurveda and in folklore[8]. The flowers of *T. grandis* are acrid, bitter, refrigerant, depurative, diuretic and anti-inflammatory, and useful in vitiated conditions of pitta and kapha, burning sensation, dipsia, leprosy, skin diseases, strangury and diabetes[8,9]. The preliminary phytochemical analysis of METGF revealed presence of tannins and phenolic compounds[10]. It is well known that diverse phytoconstituents from this group were reported for many pharmacological actions including antidiabetic activity. Moreover, up-to-date literature research revealed that there is no scientific report on *T. grandis* flowers to supports its use in the treatment of diabetes. Hence, objective of the present study is to investigate antidiabetic, antihyperlipidemic and antioxidant potential of methanol extract of *T. grandis* flowers in STZ-induced diabetic rats to generate significant scientific data to supports its traditional use.

2. Materials and methods

2.1. Plant material and extraction

T. grandis Linn. f. (Verbenaceae) flowers were collected from Annaikatti, Coimbatore, Tamil Nadu, India. The specimen was authenticated at Botanical Survey of India (BSI), Coimbatore (BSI/SC/5/23/09–10/Tech.–486). The flowers (60 g) were shade dried, powdered and extracted with petroleum ether (60–80 °C) and methanol using soxhlet apparatus. The extracts was concentrated in rotary evaporator at 35–40 °C under reduced pressure. The METGF was stored at 2–8 °C until the completion of pharmacological studies and yield of the extract was 21.62% (w/w).

2.2. Experimental animals

Male Wistar albino rats (150–180 g) were used to assess the oral glucose tolerance test and anti-diabetic activity. In acute toxicity study, female Wistar rats (150–170 g) were used and all animals were kept and maintained under standard laboratory conditions. The animals were fed with standard laboratory diet and allowed to drink water *ad libitum*. Studies were carried out in accordance with institutional ethical guidelines for the care of laboratory animals of KMCH College of Pharmacy, Coimbatore, India (approval no. KMCRET/Ph.D/5/09).

2.3. Chemicals

Streptozotocin and all other chemicals used in this study were analytical grade and were procured from Himedia

Laboratories, Mumbai, India. For the estimation of biochemical parameters, kits were procured from Primal Healthcare Limited, Lab Diagnostic Division, Mumbai, India.

2.4. Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development guidelines 423[11]. After the oral administration of METGF, animals were observed individually at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 d observed for toxicity determination.

2.5. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed in overnight fasted normal rats as per reported method[12]. The blood glucose levels were determined by using glucose meter (Glucocard™ 01–mini, Arkray Factory, Inc., Japan) by glucose oxidase–peroxidase method using strips.

2.6. Induction of experimental diabetes

Diabetes was induced in overnight fasted rats by STZ (60 mg/kg, ip.) after dissolving in freshly prepared cold citrate buffer (0.1 mol/L, pH 4.5)[13]. STZ induce fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were provided with 5% dextrose solution after 6 h of STZ administration for next 24 h to prevent hypoglycemia[14]. Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels with the glucose meter. Diabetic rats were kept 14 d under standard laboratory condition for the stabilization of blood glucose level[15]. After 14 d induction of diabetes, blood glucose was again determined and only animals with a blood glucose level greater than 300 mg/dL were selected for the study.

2.7. Experimental design for antidiabetic activity

The rats were divided into five groups each consists of a minimum of six animals. The group 1: control rats received propylene glycol (5 mL/kg); group 2: STZ-induced diabetic rats received propylene glycol (5 mL/kg); group 3: diabetic rats received METGF 100 mg/kg; group 4: diabetic rats received METGF 200 mg/kg and group 5: diabetic rats received glibenclamide 5 mg/kg[16]. The vehicle, METGF and glibenclamide were administered orally to the respective group animals for 28 d. The fasting body weight, blood glucose level were estimated on 0, 7, 14, 21 and 28 d periodically. Urine glucose and ketone levels determined using commercially available strips. At the end of experimental period, overnight fasted animals were received respective treatment and after 1 h treatments all animals were anaesthetized with ketamine (100 mg/kg, i.p.); blood sample was collected through retro-orbital plexus puncture and stored in with or without disodium ethylene diamine tetra-acetate for the biochemical parameters estimation.

2.8. Measurement of biochemical parameters

Blood glucose, Hb, HbA1c was estimated using whole blood. The high density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG), serum glutamate–pyruvate transferase (SGPT) and glutamate–oxaloacetate transaminase (SGOT), total protein, creatinine, uric acid and urea were estimated in serum by kits specific for the test using semi–autoanalyzer (Photometer 5010V5+, Germany). The serum insulin was determined by radioimmunoassay method^[17]. The LDL and VLDL levels was calculated by the following equation^[18]:

$$\text{VLDL} = \text{Triglycerides}/5$$

$$\text{LDL} = \text{TC} - \text{HDL} - \text{VLDL}$$

2.9. Determination of antioxidant levels

Liver and kidney samples were dissected out and washed immediately with ice cold saline to remove blood. The lipid peroxidation determined by determined measuring thiobarbituric acid reactive substances (TBARS). The antioxidant such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were estimated in liver and kidney^[19].

2.10. Histopathological investigation

The animals were sacrificed and pancreas was dissected out, washed in normal saline, for histopathological studies. Pancreatic tissues were fixed in 10% formalin, dehydrated with 50%–100% ethanol solution, and embedded in paraffin. The sections of 5 μ m thick were cut and stained with hematoxylin–eosin then examined under light microscope.

2.11. Statistical analysis

All the data expressed as mean \pm SEM were evaluated by one–way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons using Prism GraphPad version 5.0 and values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Acute toxicity study

The oral administration of METGF in rats up to the dose 2 000 mg/kg did not exhibit any signs of toxicity for 14 d and no animals were died. It indicates that METGF was nontoxic in rats up to an oral dose of 2 000 mg/kg of body weight. Therefore, the biological evaluation was carried out using 1/5 and 1/10 dose of METGF *ie.* 100 and 200 mg/kg dose levels.

3.2. Effect of METGF on OGTT

Glucose challenge to normal rats increased blood glucose levels with maximum level at 60 min and returned to normal level at 240 min. The METGF administration improved glucose tolerance significantly ($P < 0.001$ and $P < 0.01$) at 30 min to 120

min compared to diabetic control animals (Figure 1).

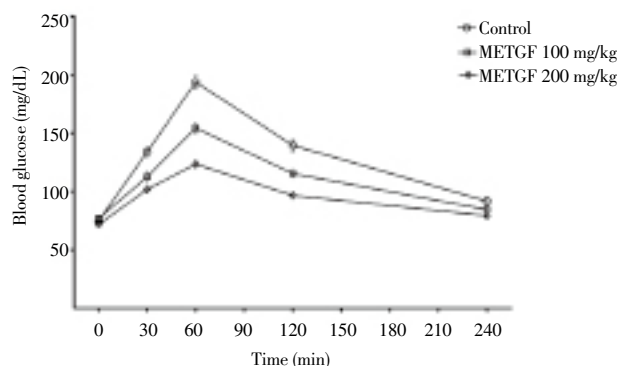


Figure 1. Effect of METGF on oral glucose tolerance test ($n=6$).

^a $P < 0.001$ METGF 100 and 200 mg/kg compared with diabetic control group; ^b $P < 0.01$ METGF 100 mg/kg compared with diabetic control group.

3.3. Effect of METGF on blood glucose and body weight

The administration of STZ increased blood glucose levels significantly compared to the control rats. The oral treatment of METGF (100 and 200 mg/kg) and glibenclamide (5 mg/kg)

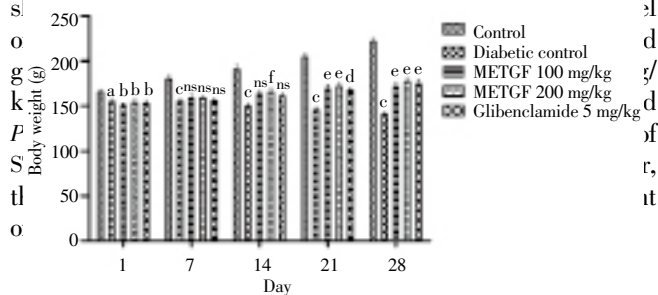


Figure 2. Effect of METGF on body weight in STZ–induced diabetic rats ($n=6$).

^a $P < 0.05$ and ^b $P < 0.01$ diabetic control compared with control group; ^c $P < 0.001$ diabetic control compared with control group; ^d $P < 0.01$ glibenclamide 5 mg/kg compared with diabetic control group; ^e $P < 0.001$ METGF 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control group; ^f $P < 0.05$ METGF 200 mg/kg compared with diabetic control group; ns: No significance.

3.4. Effect of METGF on serum insulin, HbA1c, Hb, urine sugar and ketone levels

The elevated levels of HbA1c, decreased level of Hb and serum insulin were observed in STZ–induced diabetic rats were significantly ($P < 0.001$) reverted towards normal levels after the treatment of METGF and glibenclamide. The METGF at a dose 200 mg/kg showed higher ($P < 0.001$) efficacy than 100 mg/kg dose in diabetic rats. The urine sugar and ketone was drastically controlled in diabetic rats treated with METGF and glibenclamide compared to diabetic control rats (Table 2).

3.5. Effect of METGF on creatinine, total protein, SGOT, SGPT, urea and uric acid levels

The effects of METGF on the levels of serum creatinine,

total protein, SGOT, SGPT, uric acid and urea in control and experimental diabetic rats are given in Table 3. In STZ-induced diabetic rats, the above biochemical parameters are significantly ($P < 0.001$) altered compared to control rats. The increased levels of creatinine, SGOT, SGPT, uric acid and urea were significantly ($P < 0.001$) reduced after the administration of both doses of METGF and glibenclamide in diabetic rats. The METGF 200 mg/kg treatment showed higher reduction of SGOT, urea and uric acid significantly ($P < 0.05$ and $P < 0.001$) compared to 100 mg/kg dose. The total protein levels in STZ-induced diabetic rats showed significant ($P < 0.001$) reduction than control rats and administration of METGF and glibenclamide increases total protein levels significantly ($P < 0.01$ and $P < 0.001$).

3.6. Effect of METGF on lipid profiles

In diabetic rats, significant ($P < 0.001$) elevated level of cholesterol, triglycerides, LDL, and VLDL as well as decreased level of HDL was observed compared to control animals. The administration of both the doses of METGF significantly ($P < 0.001$) reduced elevated TC, TG, LDL and VLDL levels in diabetic rats. At a dose METGF 200 mg/kg showed significant ($P < 0.01$ and $P < 0.001$) higher reduction of TC, TG, LDL and VLDL levels in diabetic rats than METGF 100 mg/kg. Also, diabetic rats treated with METGF 200 mg/kg showed significant ($P < 0.001$ and $P < 0.05$) greater reduction in TC, VLDL and TG levels compared to glibenclamide. Moreover, HDL level was significantly ($P < 0.001$) increased in diabetic rats treated with METGF and glibenclamide than diabetic control rats. The administration of METGF 200 mg/kg increases HDL levels significantly ($P < 0.001$) than METGF 100 mg/kg (Table 4).

3.7. Effect of METGF on liver and kidney antioxidant levels

There was a significant ($P < 0.001$) elevation in TBARS levels and reduction in SOD, CAT, GPx and reduced GSH levels in liver and kidney of diabetic rats compared to control rats. The administration of METGF 100 and 200 mg/kg and glibenclamide significantly ($P < 0.001$) reversed these changes to near normal level (Table 5). A significant ($P < 0.01$ and $P < 0.05$) greater efficacy was observed in METGF treated diabetic rats on liver GPx than glibenclamide.

3.8. Histopathological investigation

In control animals, histopathological examination showed normal structure of β -cells in the islet of Langerhans on the endocrine portion and normal structure of acini in the exocrine portion (Figure 3A). In diabetic control rats, nuclear changes, karyolysis and residue of destroyed cells were visible. Relative reduction of size and number of islets and drastic reduction of β -cells were clearly seen (Figure 3B). Administration of METGF 100 mg/kg to the diabetic rats showed minimal necrosis of β -cells and lesser damage of acini when compared to diabetic control rats (Figure 3C). At a dose 200 mg/kg of METGF treated diabetic rats prevented β -cells necrosis, increases number of β -cells and size of the islets of Langerhans (elongated). Also, METGF treatment

prevented destruction of exocrine part or reversed the damaged acini towards normal when compared to diabetic control and METGF 100 mg/kg treated diabetic rats (Figure 3D). The reference drug, glibenclamide, also prevented the destruction of β -cells and increases its number as well as size than diabetic control rats (Figure 3E).

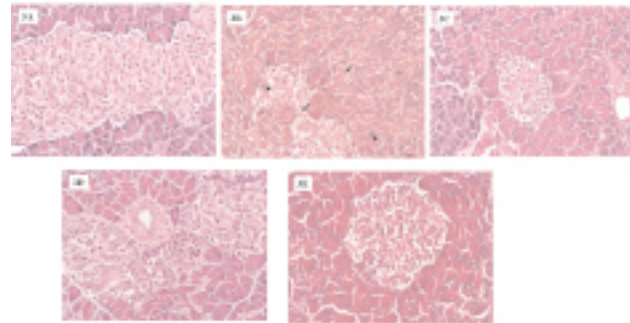


Figure 3. Histopathology of pancreas

3A: Control; 3B: Diabetic control (Black arrow indicates necrosis of β -cells, karyolysis, nuclear changes); 3C: Diabetic rat treated with METGF (100 mg/kg); 3D: Diabetic rat treated with METGF (200 mg/kg); 3E: Diabetic rat treated with glibenclamide (5 mg/kg). All photos were taken at $40\times$ magnification (H & E).

4. Discussion

In diabetic patients, objective of the treatment is to lower blood glucose to near-normal levels[20]. In present investigation, the oral glucose tolerance test revealed that METGF has potential to lower the elevated blood glucose levels. The STZ-induced diabetic rat is one of the animal models that mimic the human diabetes mellitus. The necrosis of pancreatic β -cells by STZ causing degranulation and reduction of insulin secretion leads to diabetes[21]. The alkylation nature of STZ causes β -cells DNA strands breaks that induce the activation of poly ADP-ribose polymerase followed by depletion of lethal nicotinamide adenine dinucleotide (NAD). Also, generation of potential free radicals such as nitric oxide (NO) by intracellular metabolism of STZ aggravates the situation and precipitates the further β -cells DNA strands breaks[22]. The decrease in body weight, increase in food and water intake was commonly observed in diabetes and it may be due to metabolic changes caused by lack or deficiency of insulin due to destruction of β -cells[23]. In diabetic rats, drastic reduction in body weight changes observed might be the result of degradation or catabolism of structural proteins due to unavailability of carbohydrate for the energy metabolism[24]. A significant increase in body weight of diabetic rats treated with METGF showed the blood glucose stabilization effect which in turn prevents the loss of body weight. Administration of METGF lowers blood glucose in STZ-induced diabetic rats significantly and its effect was almost equal to that of glibenclamide. Further, this antidiabetic activity was associated with an increase in the serum insulin level indicates that METGF may stimulates insulin secretion from the remaining β -cells or regenerated β -cells. The histopathological investigation

Table 1

Effect of METGF on blood glucose in STZ-induced diabetic rats (n=6).

Treatment	Dose (mg/kg)	Blood glucose (mg/dL)				
		Day 1	Day 7	Day 14	Day 21	Day 28
Control	Vehicle	59.50 ± 1.83	64.33 ± 1.92	70.66 ± 0.88	68.00 ± 1.52	65.83 ± 1.42
Diabetic control	Vehicle	557.67 ± 12.18	451.17 ± 10.39*	440.01 ± 11.02*	445.50 ± 12.23*	430.67 ± 9.96*
METGF	100	482.17 ± 13.73	352.17 ± 7.40 [†]	264.67 ± 9.33 [†]	176.83 ± 8.40 [†]	145.50 ± 11.69 [†]
METGF	200	527.33 ± 17.65	305.33 ± 9.49 ^{†, ‡}	212.17 ± 11.68 ^{†, ‡}	148.33 ± 6.85 [†]	119.17 ± 10.20 [†]
Glibenclamide	5	514.50 ± 18.25	285.50 ± 13.48 [†]	172.67 ± 8.86 [†]	125.17 ± 9.88 ^{†, ‡}	96.50 ± 4.61 ^{†, ‡}

*P<0.001 Diabetic control compared with control group; [†]P<0.001 METGF 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control group; [‡]P<0.01 METGF 200 mg/kg or glibenclamide 5 mg/kg compared with METGF 100 mg/kg group.

Table 2

Effect of METGF on HbA1c, haemoglobin, serum insulin, urine sugar and ketone in STZ-induced diabetic rats (n=6).

Treatment	Dose (mg/kg)	HbA1c (%)	Hb (g/dL)	Serum insulin (μ IU/mL)	Urine sugar	Urine ketone
Control	Vehicle	6.73 ± 0.20	13.93 ± 0.22	8.54 ± 0.12	Nil	Nil
Diabetic control	Vehicle	14.16 ± 0.36*	6.63 ± 0.18*	3.55 ± 0.15*	++++	Trace
METGF	100	10.76 ± 0.27 [†]	8.76 ± 0.16 [†]	6.37 ± 0.14 [†]	Nil	Nil
METGF	200	6.81 ± 0.18 ^{†, ‡}	14.88 ± 0.25 ^{†, ‡}	8.26 ± 0.12 ^{†, ‡}	Nil	Nil
Glibenclamide	5	6.72 ± 0.11 [†]	11.45 ± 0.36 [†]	8.40 ± 0.21 [†]	Nil	Nil

*P<0.001 diabetic control compared with control group; [†]P<0.001 METGF 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control group; [‡]P<0.001 METGF 200 mg/kg compared with METGF 100 mg/kg group. ++++ - indicates 110 mmol/L of glucose; Trace - indicates 0.5 mmol/L of ketone in urine.

Table 3

Effect of METGF on serum creatinine, SGOT, SGPT, urea, uric acid, total protein in STZ-induced diabetic rats (n=6).

Treatment	Dose(mg/kg)	Creatinine(mg/dL)	SGOT(U/L)	SGPT(U/L)	Urea(mg/dL)	Uric acid(mg/dL)	Total protein(g/dL)
Control	Vehicle	0.43 ± 0.05	42.50 ± 1.05	26.67 ± 0.76	14.67 ± 0.55	1.55 ± 0.06	7.85 ± 0.25
Diabetic control	Vehicle	0.85 ± 0.04*	115.20 ± 0.79*	50.83 ± 1.07*	94.25 ± 1.51*	4.05 ± 0.13*	5.38 ± 0.12*
METGF	100	0.47 ± 0.03 [†]	75.33 ± 1.28 [†]	41.00 ± 0.85 [†]	75.83 ± 2.23 [†]	2.88 ± 0.11 [†]	6.18 ± 0.11 ^{††}
METGF	200	0.42 ± 0.04 [†]	69.83 ± 0.94 ^{†, ‡}	38.67 ± 0.88 [†]	34.18 ± 1.08 ^{†, §}	1.92 ± 0.04 ^{†, §}	7.08 ± 0.10 ^{†, §}
Glibenclamide	5	0.37 ± 0.02 [†]	46.83 ± 1.81 ^{†, §}	27.33 ± 1.05 [†]	29.10 ± 0.97 [†]	1.43 ± 0.03 ^{†, §}	7.67 ± 0.09 [†]

*P<0.001 diabetic control compared with control group; [†]P < 0.001 METGF 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control group; [‡]P < 0.05 METGF 200 mg/kg compared with METGF 100 mg/kg group; [§]P < 0.001 METGF 200 mg/kg and glibenclamide 5 mg/kg compared with METGF 100 mg/kg group; ^{††}P < 0.01 METGF 100 mg/kg compared with diabetic control group.

Table 4

Effect of METGF on lipid profile in STZ-induced diabetic rats (n=6).

Treatment	Dose (mg/kg)	Serum lipid profile (mg/dL)				
		Total cholesterol	Triglycerides	HDL	LDL	VLDL
Control	Vehicle	119.05 ± 0.98	131.27 ± 1.05	60.15 ± 0.74	32.64 ± 1.20	26.25 ± 0.21
Diabetic control	Vehicle	145.72 ± 1.15*	161.67 ± 1.09*	44.46 ± 1.18*	63.93 ± 1.87*	32.33 ± 0.22*
METGF	100	112.07 ± 1.39 [†]	140.70 ± 1.14 [†]	51.08 ± 1.01 [†]	32.85 ± 1.19 [†]	28.14 ± 0.17 [†]
METGF	200	104.77 ± 1.31 ^{†, ‡, §}	126.92 ± 0.98 ^{†, ‡, ††}	57.53 ± 0.79 ^{†, ¶}	21.86 ± 0.83 ^{†, ‡}	25.38 ± 0.20 ^{†, §, ¶}
Glibenclamide	5	116.55 ± 1.71 [†]	135.45 ± 0.70 [†]	56.13 ± 0.93 ^{†, ‡}	33.32 ± 1.01 ^{†, §}	27.09 ± 0.14 [†]

*P<0.001 diabetic control compared with control group; [†]P<0.001 METGF 100 and 200 mg/kg, glibenclamide 5 mg/kg compared with diabetic control group; [‡]P<0.01 METGF 200 mg/kg or glibenclamide 5 mg/kg compared with METGF 100 mg/kg group; [§]P<0.001 METGF 200 mg/kg compared with glibenclamide 5 mg/kg group; ^{††}P<0.05 METGF 200 mg/kg compared with glibenclamide 5 mg/kg group; [¶]P<0.001 METGF 200 mg/kg compared with METGF 100 mg/kg group.

Table 5Effect of METGF on liver and kidney antioxidant enzymes in STZ-induced diabetic rats ($n=6$).

Treatment	Dose (mg/kg)	Organ	SOD (U/mg protein)	GPx (mg of GSH utilized/ min/mg protein)	Catalase (μ mol/L of H ₂ O ₂ / min/mg protein)	GSH (mg of GSH/mg protein)	TBARS (μ M of MDA/ min/mg protein)
Control	Vehicle	Liver	97.17 \pm 1.22	0.045 7 \pm 0.001 3	18.94 \pm 0.89	0.565 \pm 0.014	112.71 \pm 3.27
		Kidney	98.58 \pm 1.85	0.043 7 \pm 0.001 0	15.56 \pm 0.60	0.568 \pm 0.012	140.57 \pm 5.21
Diabetic control	Vehicle	Liver	75.64 \pm 1.58*	0.029 6 \pm 0.001 0*	10.63 \pm 0.51*	0.379 \pm 0.016*	272.33 \pm 6.48*
		Kidney	72.51 \pm 1.36*	0.035 8 \pm 0.001 1*	10.85 \pm 0.32*	0.466 \pm 0.014*	311.27 \pm 6.03*
METGF	100	Liver	92.53 \pm 1.59 [†]	0.045 3 \pm 0.001 5 ^{†, ‡}	15.83 \pm 0.58 [†]	0.573 \pm 0.021 [†]	203.17 \pm 5.46 [†]
		Kidney	90.42 \pm 0.82 [†]	0.041 3 \pm 0.001 3 ^{†¶}	13.26 \pm 0.26 ^{§ §}	0.582 \pm 0.013 [†]	264.32 \pm 6.67 [†]
METGF	200	Liver	99.26 \pm 1.21 ^{†, ¶}	0.046 8 \pm 0.001 7 ^{†, §}	17.05 \pm 0.45 [†]	0.633 \pm 0.018 [†]	168.06 \pm 4.49 [†]
		Kidney	97.66 \pm 1.64 ^{†, ††}	0.042 6 \pm 0.001 5 ^{§ §}	14.02 \pm 0.43 ^{††}	0.535 \pm 0.016 ^{§ §}	211.30 \pm 6.99 [†]
Glibenclamide	5	Liver	94.68 \pm 1.29 [†]	0.038 9 \pm 0.001 2 [†]	16.26 \pm 0.61 [†]	0.584 \pm 0.020 [†]	149.09 \pm 3.86 [†]
		Kidney	92.64 \pm 1.93 [†]	0.0423 \pm 0.001 2 ^{§ §}	12.66 \pm 0.40 ^{¶¶}	0.561 \pm 0.015 [†]	191.32 \pm 5.60 [†]

* $P<0.001$ diabetic control compared to control group; [†] $P<0.001$ METGF 100 and 200 mg/kg, glibenclamide 5 mg/kg compared to diabetic control group; ^{††} $P<0.001$ METGF 200 mg/kg compared to diabetic control group; [‡] $P<0.05$ METGF 100 mg/kg compared to glibenclamide 5 mg/kg group; ^{‡‡} $P<0.05$ METGF 200 mg/kg compared to METGF 100 mg/kg group; [§] $P<0.01$ METGF 200 mg/kg compared to glibenclamide 5 mg/kg group; ^{§ §} $P<0.01$ METGF 100, 200 mg/kg and glibenclamide 5 mg/kg compared to diabetic control group; [¶] $P<0.05$ METGF 200 mg/kg compared to METGF 100 mg/kg group; ^{¶¶} $P<0.05$ METGF 100 mg/kg and glibenclamide 5 mg/kg compared to diabetic control group.

clearly supported that METGF has an ability to prevent pancreatic β -cells damage, increases islets of Langerhans size and β -cells number and it may leads to increase in insulin secretion. Also, increase in insulin level may prevent catabolism of structural protein and increase the peripheral utilization glucose leading to improvement in body weight and reduction in blood glucose levels in the METGF treated diabetic rats.

In diabetic rats, observed increase in the levels of HbA1c is due to the persistent hyperglycemia and results in glycation of haemoglobin. The concentration of HbA1c correlated with diabetic retinopathy, nephropathy and neuropathy and it is considered as a tool for the diagnosis and prognosis of diabetes associated complications[25]. The increased level of HbA1c up to 16% was reported in diabetic patients[26]. In diabetes, relative deficiency of insulin leads to decrease in protein synthesis in all tissues and thus the synthesis of haemoglobin is also reduced[27]. In our study, administration of METGF significantly decreased the HbA1c and increased Hb levels in diabetic rats. The ability of METGF to decrease HbA1c levels in diabetic rats showed its potentiality to prevent the diabetic associated complication.

Liver necrosis in STZ-induced diabetic rats increases the activities of SGPT and SGOT in plasma by leakage of these enzymes from liver cytosol into the blood stream. The metabolic waste such as urea, uric acid, creatinine and ions removed by kidney and thus body fluid chemicals homeostasis is maintained. But, uncontrolled diabetes also associated with renal diseases or damage leads to increase in the above metabolites levels in blood. The creatinine levels are considered as significant markers of renal dysfunction and clinical end point to detect treatment related toxic effect of compounds on the kidney in experimental animals[28,29]. In the present study, the significant increase in serum SGOT, SGPT and creatinine levels were observed in STZ-induced diabetic rats represents liver and renal damage compared to control rats. Oral administration of METGF reduced

the elevated levels of SGOT, SGPT and creatinine which supports its protective nature on liver tissue and in diabetes associated early renal changes.

Diabetes causes a profound alteration in skeletal muscle protein catabolism rather than a decrease in protein synthesis due to the insulin deficiency[30]. The imbalance between synthesis and catabolism of protein leads to drastic alteration in the metabolism of many tissues such as gut, skeletal muscle and heart. The protein loss was observed in diabetic rats is due to the absence or deficiency of insulin in the chemically induced diabetes[31]. The significant increase in serum protein levels were observed after the treatment of METGF in diabetic rats may also accounts for its antidiabetic activity. The end product of protein catabolism is urea and enhanced breakdown of liver and plasma protein in experimental diabetes leads to the accumulation of urea nitrogen[32]. The METGF treated diabetic rats showed significant reduction in the elevated levels of serum urea indicating the preventive role of METGF in protein catabolism. The diabetic vascular complication is mediated by oxidative stress. Uric acid has endogenous antioxidants action in the body. Therefore, the elevated levels of serum uric acid in diabetes may be a protective mechanism of body to scavenge free radicals. In oxidative stress, uric acid preserves the ability of vascular dilatation of the endothelium and prevents alteration of endothelial enzymes levels[25]. In our investigation, METGF treatment in diabetic rats showed significant reduction in uric acid representing indirectly its antioxidant activity.

Lower-extremity arterial disease, coronary heart disease and cerebrovascular disease are frequent vascular complications in diabetes. The atherogenic process occurrences in vascular diseases are proceeding at a more rapid rate in diabetic than in nondiabetic subjects[33]. The vascular disease accounts for more than 60% of the morbidity and mortality of diabetes that includes both micro and macrovascular diseases, and is common in both type of

diabetic patients^[34]. The elevated cholesterol, triglycerides levels and decreased HDL levels as well as LDL particles of altered composition were reported in diabetic patients^[35]. In this study, administration of METGF significantly reduced elevated total cholesterol, triglycerides, VLDL and LDL levels in diabetic rats. Also, increased level of HDL was observed in diabetic rats treated with both the doses of METGF and glibenclamide compared to diabetic control rats. This action of METGF supports its lipid lowering activity in diabetic condition and therefore it helps to prevent diabetic associated complication.

Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses. The increased oxidative stress has key role in the development and progression of diabetes associated complications. The various mechanisms involved in the formation of reactive oxygen-free radicals and glucose oxidation is believed to be the primary source of free radicals. The generation of superoxide anion radicals by glucose oxidization and its dismutation to hydrogen peroxide, which if not scavenged by CAT or GPx, leads to the formation of reactive hydroxyl radicals^[36,37]. Also, superoxide anion radicals react with nitric oxide to form reactive peroxynitrite radicals^[38,39]. Hyperglycemia promotes lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway^[40–50]. Moreover, formation of advanced glycation endproducts (AGEs) activate the transcription factor nuclear factor kappa B (NF- κ B) promoting up-regulation of various NF- κ B controlled target genes and it enhances production of nitric oxide, which is believed to be mediator of islet beta cell damage^[51,52]. In present study, elevated TBARS levels and decreased SOD, CAT, GPx and reduced GSH levels were observed in STZ-induced diabetic rats compared to control rats. These changes may be due to the glucose oxidation, formation of AGEs mediated free radical generation and NO donor property of STZ^[22]. Administration of METGF significantly reduced TBARS and increased SOD, CAT, GPx and reduced GSH levels in diabetic rats. The action of the METGF to restore the altered antioxidant enzymes in STZ-induced diabetic rats indicates its free radical scavenging potential and hence it has the ability to prevent diabetic associated complication. The present study clearly concluded that methanol extract of *T. grandis* flower possess ability to control blood glucose in diabetes. It's antihyperlipidemic and free radical scavenging property has potential to prevent diabetic associated complications. Our current investigation supports the traditional use of *T. grandis* flower in the treatment of diabetes.

Conflict of interest

The authors declare that there is no any conflict of interest to disclose.

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