

## Free radical scavenging effect of *Trichodesma amplexicaule* against renal vascular complications in diabetic Rats

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### Abstract

The aim of the present study was to investigate the antioxidant and renal protective effect of *Trichodesma amplexicaule* extract in streptozotocin-induced diabetic rats.

Wistar rats were randomly divided into five groups: Normal control, diabetic control and *Trichodesma amplexicaule* (100 and 200mg/kg) and glibenclamide treated groups. Blood glucose, Total Cholesterol, Triglycerides, Aminotransferases, kidney functions and kidney oxidant/antioxidants status were estimated. Streptozotocin effectively altered the biochemical, histoarchitecture, malondialdehyde and antioxidant enzyme levels, while *Trichodesma amplexicaule* ameliorated their levels in the treated group.

Oral treatment with methanolic extract for 4 weeks reduced significantly Blood glucose, Total Cholesterol, Triglycerides, Aminotransferases, kidney function parameter levels in diabetic animals. Furthermore, the biochemical findings were supplemented with histopathological examination of pancreas and kidney. Histological observation of kidney of diabetic rats showed degenerative changes in glomerulus and renal tubules and extract treatment rejuvenated kidney histoarchitecture. Results of this study suggest that *Trichodesma amplexicaule* offered significant protection against oxidative stress induced by Streptozotocin, as noted by increase in superoxide dismutase and catalase along with decrease in lipid peroxidation levels.

The present work provide scientific evidence of the preventive and therapeutic potential of *Trichodesma amplexicaule* against kidney disorder in diabetes and thus may provide a promising drug for managing diabetic kidney disorders and assumes that activity may be due to the prominent antioxidant property shown by *Trichodesma amplexicaule*.

**Keywords:** Antioxidant, *Trichodesma amplexicaule*, STZ, Diabetic Nephropathy.

### Introduction

Diabetic nephropathy is clinically defined as the progressive development of renal insufficiency in the setting of hyperglycemia. This disease is now the major single cause of end stage renal failure affecting about 15%–25% of type I diabetes patients and 30%–40% of patients with type II diabetes[1,2]. Even when diabetes is controlled, the disease can lead to chronic kidney disease and kidney failure. In chronic renal failure patients the prevalence of diabetic nephropathy was 30.3% followed by chronic interstitial nephritis (23%) and chronic glomerulonephritis (17.7%)[3]. Its complications are related to specific morphological and functional renal alterations. They include hyperplasia/hypertrophy of various cell types of the glomeruli and tubules producing changes of renal function, characterized by an increase in urinary albumin excretion in early stages and by proteinuria in advanced stage, which is considered as a decline in renal function [4,5]. Diabetes causes reduced nerve sensitivity including response to a filled bladder and the ensuing pressure can

damage kidneys. Urinary tract may also cause bacterial infection due to high sugar concentration in urine. Kimmelstiel-Wilson and kidney hypertrophy-hyper function syndromes are also well established in diabetic nephropathy [6].

Despite much research work, the diabetic kidney epidemic is increasing rapidly. Presently, researches to develop drugs that slow the progression of diabetic kidney damage with fewer side effects are being conducted, however, showing no significant outcome. The side effects and enormous cost of modern medicines indicate that alternative strategies are required for better management of diabetic nephropathy. Role of traditional herbs and medicines in the treatment of diabetic nephropathy needs attention especially in India, where several plant products have been used in the indigenous system of medicines to treat diabetes. A number of plant products/ herbs such as *Anacardium occidentale*, *Benincasa cerifera*, *Brassica oleracea* and *Terminalia chebula* etc. have been evaluated and confirmed to possess beneficial effects in diabetic nephropathy in preclinical and clinical studies[7]. Additionally antioxidant treatment could be a potential therapeutic procedure for

diabetic nephropathy as numerous reports have demonstrated that oxidative stress induced by diabetes plays an important role in the development and progression of diabetic vascular complications including nephropathy. Indeed, there is emerging evidence that the formation of reactive oxygen species (ROS) is a direct consequence of hyperglycemia [8-12].

*Trichodesma amplexicaule* (Boraginaceae) reported as a medicinal herb widely used in traditional system of medicine to treat dysentery, snake bite and urinary diseases. It is useful in vitiated conditions of *Vata* and *Kapha*, arthralgia, inflammations, dyspepsia, diarrhoea, dysentery, leprosy and skin diseases [13-16]. The root extract of the plant is reported to possess several biological activities such as anti-inflammatory [17], antidiarrheal [18], analgesic, antipyretic [19], antispasmodic, lipoxygenase inhibitory activity [20] and antimicrobial [21]. The whole plant is evaluated for cough reflex induced by sulphur dioxide in animal model [22]. Phytochemical investigations of the plant have shown the presence of non-steroidal compounds; hexacosane, 21, 24-hexacosadienoic acid, Terpenoids, sitosterol, oleic, linoleic, palmitic, stearic and linolenic acid [23]. In our previous study [24], we have reported the antioxidant capacity of hydroalcohol extract of *Trichodesma amplexicaule* in different *in-vitro* models and also the plant extract was screened for *in-vitro* antidiabetic activity in L-6 cell lines. Based on these, in the present study we have investigated the antidiabetic and renal protective effect of *Trichodesma amplexicaule* extract in streptozotocin-induced diabetes in preclinical model.

## Materials and Methods

### Animals

Thirty male *Wistar* albino rats weighing 180-220g were used for the experimental studies. The rats were fed with standard laboratory chow and water before the experiment. They were divided in to 5 groups (n=6) and housed in cages. The animals were acclimatized to laboratory conditions for one week before commencement of experiment. The study protocol was approved by the Institutional Animal Ethics Committee (NIP/1330/AC/10/CPCSEA Dated 11<sup>th</sup> March), Nizam Institute of Pharmacy, (Hyderabad, Andhra Pradesh, India) and the rats were maintained in accordance with the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India guidelines for the care and use of laboratory animals.

### Plant Material and Preparation of Extract

The whole plant material was collected from Deshmukhi village of Nalagonda district and authenticated from Head, Department of Botany, Osmania University, Hyderabad (S.No.162, Voucher No: 0475). The collected plant material was cleaned, air dried and coarsely powdered. The coarse powder obtained was extracted exhaustively with methanol in Soxhlet apparatus and filtered. The extract was concentrated under reduced temperature and pressure to get dry residue and stored in a desiccators. The methanol

extract *Trichodesma amplexicaule* (META) was prepared using 2% (v/v) Tween-80 daily for oral administration.

### Chemicals

Streptozotocin and glibenclamide was purchased from Sigma Chemicals Co (St. Louis, Mo, USA), Glucometer and glucometer strips obtained from (Roche Diagnostic, USA). All other chemicals used in this study were of analytical grade and obtained from SRL Chemicals, Mumbai, India.

### Acute Toxicity Study

Healthy male *Wistar* albino rats (180-220g) starved overnight, were divided in to 4 groups (n=4). Group I-IV animals were orally fed META in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.w., while group-V (Untreated) served as control. The animals were observed continuously for the first 2h for any gross change in behavioral, neurologic and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6h and then again at 24 h, 48h and 72 h for any lethality or death. One tenth and one fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment [25].

### Induction of Experimental Diabetes

Overnight fasted rats were given a single intraperitoneal injection of 60 mg/kg STZ (Sigma, USA) dissolved in sodium citrate buffer (0.1 M, pH 4.5) to induce diabetes. Rats in the normal group were injected with the same amount of solvent. Fasting blood glucose (FBG) was measured on the third day after Streptozotocin injection using an Accu-Check Advantage Blood Glucose Monitor (Roche Group, Indianapolis, IN, USA). To prevent the hypo glycemia which occurred during the first 24 h following the Streptozotocin administration, 5% glucose solution was orally given to the diabetic rats.

### Experimental Design

Normal and diabetic rats were divided into five groups (n=6) and were treated daily for 4weeks

Group I: Normal control received distilled water (2ml/kg/day, p.o.) for four weeks. Group II: Diabetic control received distilled water (2ml/kg/day, p.o.) for four weeks. Group III: Diabetic rats treated with META (100 mg/kg/ day, p.o) for four weeks. Group IV: Diabetic rats treated with META (200 mg/kg/ day, p.o) for four weeks. Group V: Diabetic rats treated with glibenclamide (5mg/kg/day, p.o.) for four weeks.

Body weight of rats was taken on pre and post treatment i.e. day 0, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks of post treatment. Fasting blood glucose level of rats were taken pre and post treatment i.e. 0, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks of post treatment. Glucose levels were estimated using a glucose oxidase peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA). At the end of

experimental period, all the rats were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging for 15 min and analyzed for various biochemical parameters. Antioxidant enzymes in kidney were estimated and a part of kidney was fixed in 10% formalin for histopathological studies.

### Biochemical Parameters

Diabetic nephropathy was evaluated by estimating Blood glucose, Lipid profile (Triglycerides and Cholesterol), Blood urea nitrogen, Creatinine, blood urea and urinary proteins. Diabetes has a higher incidence of Liver function test abnormalities so Aminotransferases (ALT and AST) levels were estimated [26].

### Evaluation of Oxidative stress

Right Kidney of individual rat was isolated and 10% kidney homogenate was prepared using 0.15m Potassium chloride, was used for antioxidant studies such as lipid peroxidation(MDA), Superoxide dismutase (SOD) and Catalase [27-29].

### Statistical analysis

All values were expressed as mean ± standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test. P values < 0.05 were considered to be significant.

### Results

Results of acute toxicity studies revealed the non-toxic nature of the methanol extract of *Trichodesma amplexicaule*. No mortality

was observed in the extract-treated rats and behavior of the treated rats also appeared normal. There was no lethality or toxic reaction found at any dose selected until the end of the study. In this study, the STZ treated rats developed diabetes and showed well developed signs like hyperglycemia, glycosuria, polyuria, increased water consumption and loss in body weight after 2 weeks of STZ administration when compared with control rats. Change in body weight of normal control and experimental groups are shown in Table 1.

**Table 1. Effect of META on body weight in STZ induced diabetic rats.**

Group	Body Weight Gain (g)		
	Day 1	2 week	4 week
Normal control	205± 10.1	212±12.4	222±12.4
Diabetic control	181± 5.5	171±9.9 <sup>a</sup>	181±9.9 <sup>a</sup>
Diabetic+ META(100mg/kg)	190±12.9 <sup>a</sup>	185±12.3 <sup>b</sup>	195±12.3 <sup>b</sup>
Diabetic+ META (200mg/kg)	188±15.5 <sup>a</sup>	182±11.4 <sup>b</sup>	192±11.4 <sup>b</sup>
Diabetic+ Glibenclamide (5mg/kg)	186±14.8 <sup>a</sup>	179±8.6 <sup>b</sup>	189±8.6 <sup>b</sup>

Values are mean±SEM for 6 rats in each group. a P<0.01 Vs Normal control, b P<0.01 Vs Diabetic control

However, META treatment appeared to protect the diabetic rats from body weight loss. High blood glucose level was observed in all the groups after injection with STZ. After 2 weeks, treatment with META showed a significant fall of blood glucose level in diabetic rats (Table 2).

**Table 2: Effect of META on blood glucose (mg/dL) levels in STZ- induced diabetic rats.**

Treatment Dose (mg/kg)	0 Day	After Streptozotocin Induced	1st Week After Treatment	2 <sup>nd</sup> Week	3 <sup>rd</sup> Week	4 <sup>th</sup> Week
Normal Control ( 2ml/kg)	87.47 ±1.62	85.20 ±1.22	85.51 ±1.15	86.72 ± 1.26	88.16 ± 1.07	87.19±1.13
Diabetic Control (60mg/kg)	92.14 ±1.55	188.26 ± 2.78 <sup>a</sup>	226.51 ±2.29 <sup>a</sup>	259.19 ± 3.15 <sup>a</sup>	374.05 ±4.76 <sup>a</sup>	380.12±4.82 <sup>a</sup>
META (200mg/kg)	93.34 ± 1.43	175.64 ± 3.05 <sup>b</sup>	229.43 ± 2.10	204.72 ± 1.92 <sup>b</sup>	169.25 ±2.14 <sup>b</sup>	135.22±2.56 <sup>b</sup>
META (400mg/kg)	91.24±1.33	186.53±3.07 <sup>b</sup>	194.34±2.09	138.67± 1.23 <sup>b</sup>	117.32±1.31 <sup>b</sup>	105.23±1.35 <sup>b</sup>
Glibenclamide (5mg/kg)	92.84 ± 1.32	167.18 ± 2.15 <sup>b</sup>	130.26 ±1.74 <sup>b</sup>	144.59 ± 1.22 <sup>b</sup>	139.26 ± 1.32 <sup>b</sup>	121.43±1.65 <sup>b</sup>

Values are mean±SEM for 6 rats in each group. a P<0.01 Vs Normal control, b P<0.01 Vs Diabetic control



**Table 3: Effect of four weeks Treatment of META on Renal Functional Parameters**

Treatment	Serum Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)	Urine Protein (mg/dl)	Blood Urea (mg/dl)
Normal Control	0.97±0.13	17.42 ± 1.26	18.2± 0.20	31± 0.70
Diabetic Control	2.13 ±0.58 <sup>a</sup>	34.27 ± 3.26 <sup>a</sup>	32.54± 0.21 <sup>a</sup>	53±0.62 <sup>a</sup>
META(200mg/kg)	1.33 ± 0.31 <sup>b</sup>	22.64± 3.45 <sup>b</sup>	20.56± 2.22 <sup>b</sup>	35±0.70 <sup>b</sup>
META (400mg/kg)	1.18± 0.28 <sup>b</sup>	21.32±3.35 <sup>b</sup>	21.45±2.89 <sup>b</sup>	35±0.67 <sup>b</sup>
Glibenclamide (5mg/kg)	1.35±0.32 <sup>b</sup>	20.44± 3.53 <sup>b</sup>	17.12 ±3.20 <sup>b</sup>	30±0.60 <sup>b</sup>

Values are mean±SEM for 6 rats in each group. a P<0.01 Vs Normal control, b P<0.01 Vs Diabetic control

BUN, serum creatinine, Urine protein (24 hour) and blood urea were measured, and the results are shown in Table 3.

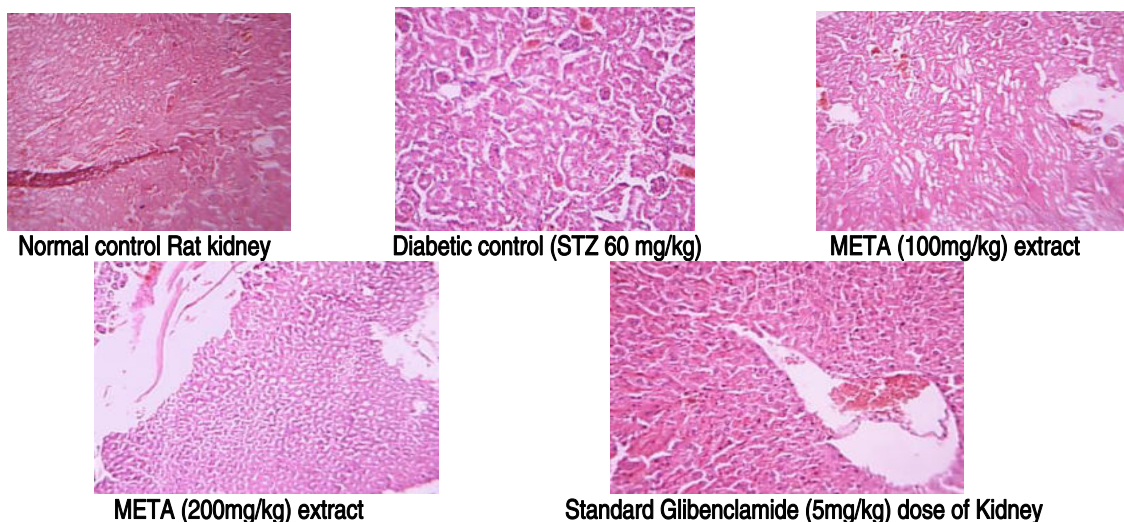
The levels of BUN, 24 h urea protein, blood urea and creatinine were significantly increased in STZ-induced diabetic rats when compared with those of normal control rats and rats treated with META. Administration of extract at 200mg/kg to diabetic rats tends to bring the values to near normal after 4 weeks of treatment. The levels of blood urea nitrogen, 24 h urea protein, blood urea and creatinine were significantly decreased. Effects of treatment with META to diabetic rats on serum lipids (TC and TG) and Aminotransferases (ALT and AST), are presented in Table 4.

**Table 4: Effect of four weeks Treatment of META on Biochemical Parameters**

Treatment	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	ALT (IU/L)	AST (IU/L)
Normal Control	45.4 ± 2.3	64.7 ± 6.2	62.1±3.4	45.6±4.4
Diabetic Control (STZ 60mg/kg)	112.6 ± 12.5 <sup>a</sup>	144.2± 30.4 <sup>a</sup>	133.4±8.1 <sup>a</sup>	118.3±6.8 <sup>a</sup>
META (200mg/kg)	70.3± 5.0 <sup>b</sup>	84.6 ± 14.7 <sup>b</sup>	93.4±4.5 <sup>b</sup>	98.7±1.1 <sup>b</sup>
META (400mg/kg)	61.3± 6.3 <sup>b</sup>	79.9 ± 26.3 <sup>b</sup>	82.5±1.3 <sup>b</sup>	84.3±8.6 <sup>b</sup>
Glibenclamide(5mg/kg)	59.7 ± 6.6 <sup>b</sup>	71.6 ± 25.3 <sup>b</sup>	74.6±5.3 <sup>b</sup>	81.5±5.6 <sup>b</sup>

Values are mean±SEM for 6 rats in each group. a P<0.01 Vs Normal control, b P<0.01 Vs Diabetic control

As shown in Table 4 the levels of serum TC, TG, ALT and AST were significantly increased in diabetic rats when compared with those of normal control rats. The serum lipids like TC, TG, and Aminotransferases ALT and AST were significantly decreased in diabetic rats treated with META when compared with those of diabetic control rats. The biochemical parameters were supplemented with histopathological studies of pancreas (Figure 1) and kidney (Figure 2).



**Figure 1. Histopathology of Kidney**



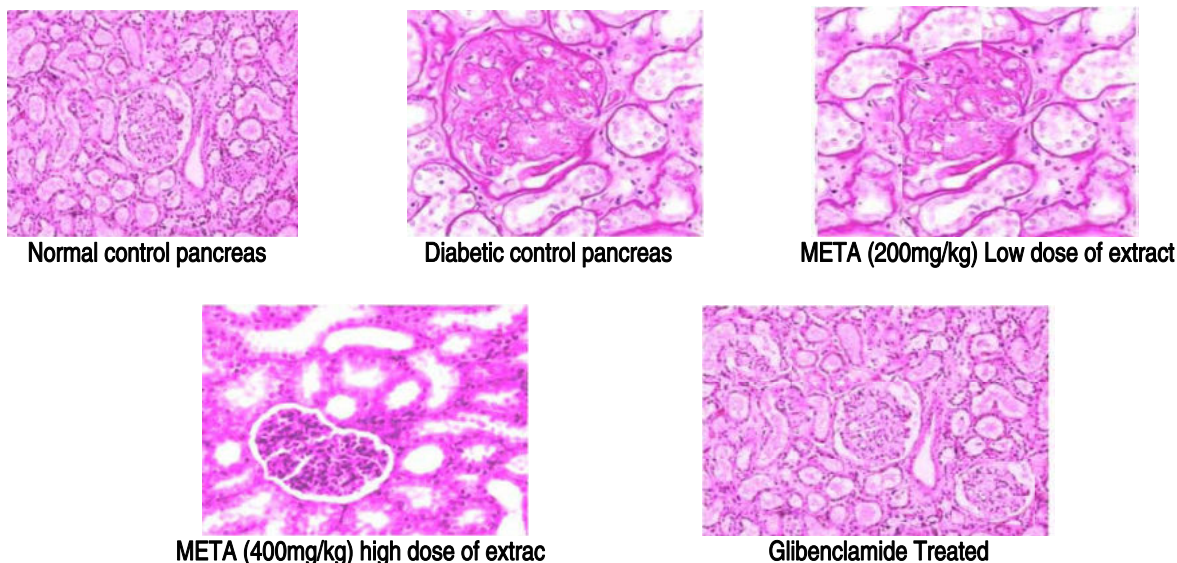


Figure 2; Histopathology of Pancreas

Diabetes produced oxidative stress as a result there was a significant ( $P < 0.01$ ) increase in renal MDA, SOD and decrease Catalase levels. The treatment of META for 4 weeks (100 and 200mg/kg) showed decrease in MDA, SOD and increase in Catalase level when compared to diabetic control (Table 5). In the streptozotocin diabetic untreated rats, the islets of langerhans showed diffused necrotic changes of moderate to marked degree as a result of which they were significantly reduced in size and number. The META extract treated group of rats showed moderate degree of necrosis of the islets of langerhans. The pancreatic damage observed in glibenclamide and META treated diabetic animals was milder than that found in the untreated diabetic control group. The kidney specimen of the diabetic group showed markedly severe destruction in glomerular and tubulointerstitial lesions such as glomerular sclerosis atrophy, interstitial expansion, and interstitial cellular infiltration. General morphology of glomerulus and tubulointerstitial lesions were much improved and showed quite normal appearance in META treated groups.

### Discussion

Studies with type I or type II diabetes have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy, and nephropathy [30, 31]. These observations indicate that hyperglycemia is the major risk factor for vascular complications of diabetes. There is emerging evidence that the generation of reactive oxygen species (ROS) is one major factor in the development of diabetes and its complications. There is much evidence from experimental studies

Table 5. Effect of META on kidney Lipid peroxidation, SOD and Catalase in STZ induced diabetes

Group	MDA (nmoles/mg)	SOD Units/mg of protein	Catalase $\mu$ mole of $H_2O_2$ consumed/ min / mg of protein
Normal Control	14.3 $\pm$ 0.05	5.55 $\pm$ 1.47	88.71 $\pm$ 4.72
Diabetic Control (STZ 60mg/kg)	41.23 $\pm$ 0.10 <sup>a</sup>	8.69 $\pm$ 1.09 <sup>a</sup>	31.78 $\pm$ 2.4 <sup>a</sup>
META (200mg/kg)	29.53 $\pm$ 0.11 <sup>b</sup>	6.53 $\pm$ 1.15 <sup>b</sup>	62.26 $\pm$ 3.51 <sup>b</sup>
META (400mg/kg)	25.68 $\pm$ 2.8 <sup>b</sup>	5.89 $\pm$ 17.12 <sup>b</sup>	51.26 $\pm$ 3.51 <sup>b</sup>
Glibenclamide(5mg/kg)	20.20 $\pm$ 0.17 <sup>b</sup>	4.41 $\pm$ 1.28 <sup>b</sup>	48.65 $\pm$ 4.85 <sup>b</sup>

Values are mean $\pm$ SEM for 6 rats in each group. a  $P < 0.01$  Vs Normal control, b  $P < 0.01$  Vs Diabetic control

that the formation of ROS is a direct consequence of hyperglycemia [32]. Various types of vascular cells including endothelial and renal cells are able to produce ROS under hyperglycemic conditions. Streptozotocin-induced diabetes is characterized by a severe loss in body weight [33] and decrease in body weight in diabetic rat's shows the degradation of structural proteins is due to diabetes, and structural proteins are known to contribute to the body weight [34]. In the present study when diabetic rats were treated with META for 4 weeks the weight loss

was recovered, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control. In this study, the glucose level of diabetic nephropathy rats showed significant increase; however, META inhibited this increase dose-dependently. The present investigation showed that META resulted in reduction of the elevated blood glucose concentration in diabetic rats, an effect that was attributed in former studies to META enhancement of glucose uptake in L-6 cell lines [24]. In addition, an increase in lipids, e.g., total cholesterol and triglyceride, whose abnormal metabolism has been proven to play a role in the pathogenesis of diabetic nephropathy [35], was all improved by administration of the META. Therefore, it is suggested that META shows a positive effect on blood glucose and lipid metabolic abnormalities. The results of the study presented demonstrate that diabetic nephropathy rats showed significant increases in the BUN, Creatinine, blood urea and urinary protein excretion rate, compared with normal rats, representing a decline in renal function. However, the META treatment positively affected these parameters. These data suggest that META may improve the typical parameters under the development of diabetic nephropathy. The increase in aminotransferases levels may be due to the cellular damage in the kidney caused by STZ-induced diabetes. It had been shown by Rogers *et al.* (1986) that mitochondrial activity was decreased and cytoplasmic AST activity was increased in STZ diabetic rats [36]. Voss *et al.* (1988) proposed that STZ in hyperglycemic animals caused a time dependent rise in AST and ALT levels [37]. It is suggested that this may be due to the inactivation of cytosolic AST in the diabetic rat tissues by a glycation reaction, accompanied by impairment in glucose utilization in STZ induced diabetes. AST and ALT levels were decreased very significantly in groups treated with META when compared with diabetic control rats. The possible reason for decrease in ALT levels is achieved due to hypoglycemic effect of the extract. Lipid peroxidation is a free radical mediated process which gives rise to malondialdehyde. Oxidative stress was found to be inhibited by META as assessed by decrease in renal MDA level. Diabetic rats exhibited elevated levels of antioxidant enzymes SOD

and reduced Catalase. META reversed these changes by scavenging the oxidative stress. Some bioactive compounds isolated from plants like terpenoids and Lupeol were reported to affect pancreatic beta-cells and stimulate insulin secretion with antioxidant activities [38]. Since oxidative stress and free radicals injure or destroy pancreatic  $\beta$  cells in diabetes, *Trichodesma amplexicaule* extracts is able to increase the secretion of insulin via its antioxidant actions [39, 40]. Since streptozotocin is known to destroy pancreatic  $\beta$  cells, *Trichodesma amplexicaule* extract may also act extra-pancreatic and thus influencing glucose uptake and utilization by different tissues [41]. Furthermore the results of our previous studies in *in-vitro* models of antioxidant and antidiabetic are in accordance with the results obtained in *in vivo* study [24].

## Conclusion

In conclusion, our observations presented here suggest that *Trichodesma amplexicaule* has a beneficial effect on diabetic nephropathy via suppressing hyperglycemia, their related oxidative stress and also pathological states. It effectively prevents renal dysfunction associated with diabetes by attenuating the oxidative stress in renal tissues. Further studies will be needed to determine the active components in META and their role in controlling nephropathy complications in diabetes, and to reveal the exact underlying mechanism(s).

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## Disclosure Statement

No competing financial interests exist

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