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Effect of the seeds of *Terminalia chebula* on blood serum, lipid profile and urine parameters in STZ induced Diabetic rats

Anurag Singh, Ragini Srivastav and Ajai Kumar Pandey

Abstract
Background: *Terminalia chebula* is a potent source of useful drugs for the treatment of diabetes and its associated complications like diabetic nephropathy. This plant has numerous therapeutic properties due to which it has been proved as safe and effective drugs over hundreds of years.
Objective: A large number of natural product are used by the tribal and folklore communities in many countries for the treatment of several aliments. The scope of the present study are to investigate the protective role of the seeds of *Terminalia Chebula* in the case STZ induced diabetic mice for Diabetic nephropathy.
Materials & Methods: The dose of *Terminalia chebula* seed methanolic extract (TCSME) 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg per day was given daily to diabetic rats for 13 weeks. Blood glucose, serum parameters such as albumin, creatinine, total protein, lipid profile and urine parameters such as glucose, urine protein and albumin were examined.
Result: TCSME significantly increases the level of serum creatinine, serum urea, and glucose. The results shows that this plant drugs are potent source of antioxidative phenolic compounds that counteract with ROS species due to which it shows protective role in the case of diabetic condition for diabetic nephropathy. After 13 weeks of treatment, in STZ-induced diabetic rats, severe hyperglycemia was developed, with marked increase in proteinuria and albuminuria. However, TCSME treatment significantly reduced proteinuria and albuminuria in diabetic rats.
Conclusion: The present study reveals that the seeds of *Terminalia chebula* showing protective role for diabetic nephropathy and improved the oxidative enzymatic condition by minimizing the oxidative stress. Thus, It might be conclude that the seeds of *Terminalia chebula* used as antidiabetic agents, reduce the risk of oxidative stress and ameliorate kidney damage.

Keywords: Diabetes; Diabetic Nephropathy; Herbal Drugs.

Introduction
Diabetes mellitus (DM) is a health problem affecting millions of individuals worldwide. The World Health Organization predicts that 300 millions of people will have diabetes mellitus by the year 2025 (Pradeepa R *et al*., 2002) [12]. Diabetes is a disease associated with glucose metabolism resulting from defects in insulin secretion and action. It is characterized by hyperglycemia, glycosuria and several microvascular and macro vascular complications (Brownlee M., 2001) [3]. The complications of diabetes are linked to oxidative stress induced by hyperglycemia which overcomes the body's natural anti-oxidant system (Anil Kumar *et al*., 2008). In the later stages of diabetes, lipid metabolism is affected and seen as hyperlipidemia and hypercholesterolemia which are risk factors in atherosclerosis (Schwartz *et al*., 2006) [2]. There is also possibility of liver damage in diabetes due to increased gluconeogenesis and ketogenesis.
The kidneys play a critical role in maintaining overall health. These twin organs filter waste products and excess water from the blood and also produce hormones that are essential for the optimal maintenance of bodily functions. Diabetic nephropathy is the condition that occurs when diabetes causes the kidneys to lose their ability to function properly. It is characterised by high levels of protein in the blood. High blood sugar caused by diabetes can cause problems in many parts of the body, including the kidneys. Kidneys contain many small blood vessels (Fuliang HU *et al*., 2005) [5]. With diabetes, these small blood vessels can be injured throughout the body, including the kidneys. Kidney cannot filter the blood properly and this causes the body to retain more water, salt and waste materials in the body.
High levels of blood sugars makes the kidney filter too much blood. After some time, they also start to leak useful proteins in the urine. Having small amount of protein in urine is called

microalbuminuria whereas secretion of larger amount of protein in the urine is called macroalbuminuria. In type-2 diabetes, a greater proportion of patients have microalbuminuria and over the nephropathy at or shortly after diagnosis of diabetes (Reboldi G *et al*., 2009) [13].

India has a rich tradition of plant-based knowledge on healthcare. A large number of plants are equally used by tribals for treatment of diabetic complications. These natural agents induce the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. These natural agencies are rich target for the development of alternatives to synthetic drugs. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine.

The main treatment of natural drugs is to lower blood pressure and prevent or slow the damage to the kidneys. These medicines include: Angiotensin-converting enzyme inhibitors, also called ACE inhibitors and Angiotensin II receptor blockers. As damage to the kidneys gets worse, the blood pressure rises along with cholesterol and triglyceride levels rise too (Huang HY *et al*., 2004) [7].

Factors important in the pathogenesis of diabetic nephropathy include hyperglycemia, hypertension, lipid abnormalities, albuminuria and proteinuria (Klag MJ *et al*., 1996) [8]. The clinical course of diabetic nephropathy includes an initial increase in glomerular filtration rate (GFR). As renal function declines, arterial blood pressure increases. The blood glucose level and blood pressure reduction will reduce the progression of nephropathy and cardiovascular complications.

The present study thus attempts to explore the protective role of seeds of *Terminalia chebula* in Animal model for diabetic nephropathy. The pharmacological validation on seeds of *Terminalia chebula* to diabetic nephropathy is very limited and a large number of tribal people used these plants in kidney disorder and diabetes. The fruits of *T. chebula* are known for their antidiabetic properties but little is known on the medicinal values of *T. chebula* seeds. In the present study, the methanolic extract of the seeds of *T. chebula* was tested for its antidiabetic activity and protective role for their diabetic nephropathy in streptozotocin-induced diabetic rats. This work therefore attempts to make a validation of the traditional claims and development of safe and effective globally accepted herbal drugs for the treatment of diabetes and associated complications like diabetic nephropathy.

Materials & Methods

Collection and identification of plant materials

The seed of *Terminalia chebula* were collected from the faculty of Ayurveda, Banaras Hindu University, Varanasi.

Extraction of plant material

The plant material will be shade dried, pulverized and preserved in air tight containers and then subjected to methanolic extraction by using Soxhlet apparatus at 65^0C. The extracted materials was then kept in water bath to evaporate solvent totally and then kept on a rotary shaker at 190-220 rpm for 6 h to make the final volume one fourth of the original volume and stored at 4^0C in airtight bottles. The methanolic extract then further subjected to presence of phytochemical analysis and Diabetic Nephropathy activity.

Animals

All experiment were performed on male Wistar albino rats aged 2–3 months with an average weight of (182±1) g. The animals were individually kept under laboratory conditions at the Department of Biochemistry, Banaras Hindu University, Varanasi.

Induction of diabetes mellitus

The rats fasted for 12h (overnight) and diabetes was induced by giving intraperitonial streptozotocin (STZ) injection (50 mg/kg body weight) in cold 0.1M Na-citrate buffer, PH 4.5 for five consecutive days in the diabetic group of rats. The animals were confirmed for diabetes before the start of experiment. The fasting serum glucose level was measured by glucose oxidase-peroxidase method using glucose test kit (Span diagnostics Ltd., India). Only rats with fasting blood glucose level of 250 mg/dl and above were considered as diabetic and those with blood sugar level 130 mg/dl and below were considered as non-diabetic. These rats were used for the experiment further. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages, as per the guidelines of institutional animal ethics committee.

Drugs administration

To investigate the effects of TCSME, the animals were divided into six groups each consisting of six animals as Group 1:Untreated normal rats, Group 2: Untreated diabetic rats, Group 3: Diabetic rats treated with metformine at 50 mg/kg BW, Group 4: Diabetic rats treated with TCSME at 100 mg/kg BW, Group 5: Diabetic rats treated with TCSME at 200 mg/kg BW, Group 6: Diabetic rats treated with TCSME at 400 mg/kg BW After overnight fasting, The methanolic extract of the seeds of *Terminalia chebula* suspended in distilled water was fed to the group 4, 5, and 6 rats by gastric intubation using a force feeding needle. Group 1 and 2 rats were fed with water alone and group 3 rats were fed with standard drug Metformine daily orally for 13 weeks.

Biochemical analysis of blood & Urine

Blood samples were collected from the rats and used for the estimation of blood glucose carried out by glucose oxidase–peroxidase method (Kesari AN *et al*., 2005) [9]. Serum was used for the estimation of albumin, creatinine total protein and lipid profile. Pooled 24 h urine was evaluated for the estimation of glucose, total protein and albumin concentration. The estimation of the above mentioned parameters was carried out by using biochemical kits (from Avicon Biomedical Pvt. Ltd, VNS).

Statistical analysis

All values were expressed as Mean±SEM. The statistical analysis of the difference was carried out by using one way analysis of varience (ANOVA) followed by Dunnette's multiple comparison test and significant level was assumed at $p<0.05$.

Results

Effect of the seeds of *Terminalia chebula* on blood glucose level & body weight

At the end of 13 weeks treatment with TCSME and Metformine, the body weight of normal, TCSME, and metformine treated rats was found to increase significantly compared with the diabetic control group and the blood glucose level decreased significantly when compared with the untreated group (Table 1).

Table 1: Effect of methanolic extract of *Terminalia chebula* seeds on body weight and blood glucose level of normal and diabetic rats.

Group (n= 3)	Change in body weight (gm)	Blood Glucose (mg/dl)
Normal Control	178.57±3.67	104.66±1.77
Diabetic Control	135.12±4.73*	293.03±2.41*
Diabetic + Metformine (50 mg/kg)	164.69±3.01#	113±2.52#
Diabetic + TCSME (100 mg/kg)	147.80±4.16#	133.33±3.29#
Diabetic + TCSME (200 mg/kg)	151.21±7.05#	120±3.47#
Diabetic + TCSME (400 mg/kg)	165.34±3.76#	121.67±1.45#

- Significance between Normal control and Diabetic control; # Significance between Treatment group & Diabetic control;
- Level of significance was p<0.05 ; (post hoc test used for ANOVA was Dncan's Multiple Range Test)

Effect of TCSME on serum parameters in normal and STZ induced diabetic rats

In STZ-induced diabetic rats, after 13 weeks of treatment, the effect of TCSME on serum albumin, creatinine and total protein in all the experimental groups of rats were studied. The serum albumin, creatinine and total protein levels were significantly higher in diabetic control rats compared with those in normal rats. However, treatment of the diabetic rats with TCSME produced a significant reduction in serum albumin, creatinine and total protein levels when compared with diabetic control rats (Table 2).

Table 2: Effect of methanolic extract of *Terminalia chebula* seeds on serum parameters of normal and diabetic rats.

Gr oup (n= 3)	Albumin (mg/dl)	Creatinine (mg/dl)	Total Protein (mg/dl)
Normal Control	2.66±0.27	0.52±0.03	5.13±0.30
Diabetic Control	2.10±0.07*	1.19±0.10*	3.70±0.40*
Diabetic + Metformine (50 mg/kg)	2.79±0.13#	0.57±0.10#	5.30±0.39#
Diabetic + TCSME (100 mg/kg)	2.51±0.03#	0.67±0.01#	4.73±0.48#
Diabetic + TCSME (200 mg/kg)	2.61±0.13#	0.63±0.01#	5.17±0.41#
Diabetic + TCSME (400 mg/kg)	2.70±0.07#	0.59±0.02#	5.30±0.32#

- Significance between Normal control and Diabetic control; # Significance between Treatment group & Diabetic control;
- Level of significance was p<0.05 ; (post hoc test used for ANOVA was Dncan's Multiple Range Test)

Effect of TCSME on lipid profiles in normal and STZ induced diabetic rats

In STZ-induced diabetic rats, after 13 weeks of treatment, the effect of TCSME on serum triglycerides (TG), total cholesterol, LDL, VLDL, and HDL cholesterol in all the experimental groups of rats were studied. The serum triglycerides, total cholesterol, LDL, and VLDL cholesterol levels were significantly increased, while the HDL cholesterol levels decreased in the diabetic control rats when compared with normal rats. Treatment of the diabetic rats with TCSME caused a significant reduction in the serum triglycerides, total LDL, and VLDL cholesterol with a significant increase in HDL cholesterol levels when compared with diabetic control rats (Table 3).

Table 3: Effect of methanolic extract of *Terminalia chebula* seeds on serum lipid profile of normal and diabetic rats.

Group (n= 3)	Body Cholesterol	LDL	HDL (mg/dl)	VLDL	Triglycerides
Normal Control	68.21±0.35	25.53±0.58	65.28±0.47	18.72±0.31	102.37±0.63
Diabetic Control	88.65±0.58	89.39±0.45	41.76±0.51	26.67±0.42	143.23±0.38
Diabetic + Metformine (50 mg/kg)	71.38±0.25	37.18±0.38	44.62±0.55	19.67±0.63	106.38±0.48
Diabetic + TCSME (100 mg/kg)	85.54±0.32	54.81±0.42	58.47±0.45	22.12±0.28	123.87±0.61
Diabetic + TCSME (200 mg/kg)	81.47±0.41	48.13±0.30	61.73±0.52	20.65±0.67	119.45±0.28
Diabetic + TCSME (400 mg/kg)	79.26±0.21	43.17±0.65	63.51±0.48	17.26±0.34	113.29±0.44

Effect of TCSME on urine parameters in normal and STZ induced diabetic rats

In STZ-induced diabetic rats, after 13 weeks of treatment, the effect of TCSME on urine glucose, albumin, and total protein in all the experimental groups of rats were studied. The urine glucose, albumin, and total protein levels were significantly higher in diabetic control rats compared with those in normal rats. Treatment of the diabetic rats with TCSME produced a significant reduction in the albumin, creatinine, urea and total protein levels when compared with diabetic control rats (Table 4).

Table 4: Effect of methanolic extract of *Terminalia chebula* seeds on urine parameters of normal and diabetic rats

Group (n= 3)	Glucose (mg/dl)	Albumin (mg/dl)	Total Protein (mg/dl)
Normal Control	0.26±0.04	2.38±0.64	5.58±1.32
Diabetic Control	2.68±0.45*	14.51±1.72*	16.82±2.16*
Diabetic + Metformine (50 mg/kg)	1.20±0.19#	5.07±0.58#	5.61±1.09#
Diabetic + TCSME (100 mg/kg)	2.29±0.15	7.79±0.35#	8.33±0.70#
Diabetic + TCSME (200 mg/kg)	1.65±0.48#	6.91±0.23#	6.88±0.78#
Diabetic + TCSME (400 mg/kg)	1.77±0.18#	6.34±0.37#	6.76 ±0.71#

- Significance between Normal control and Diabetic control; # Significance between Treatment group & Diabetic control;
- Level of significance was p<0.05 ; (post hoc test used for ANOVA was Dncan's Multiple Range Test)

Discussion

In this study, the elevated blood glucose level clearly indicate the persistent of hyperglycemic condition in the STZ-induced diabetic rats, which is as a result of the destruction of the beta cells of the pancreas by STZ. However, administration of the methanolic extract of *Terminalia chebula* seed at a dosage of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg in diabetic rats tended to lower the blood glucose levels toward near normal levels.

This finding indicates that *Terminalia chebula* seeds act as an antihyperglycemic agent rather than a hypoglycemic agent. It has been reported that the seeds of *Terminalia chebula* altered the hyperglycemic condition of streptozotocin-induced diabetic rats to normal (Nalamolu Koteswara Rao *et al*. 2006). They suggested that the seeds might contain substances that mimic the action of insulin. It has been reported that the seeds of *Terminalia chebula* contains triterpenes arjun glucoside 1, arjungenin, chebulosides 1&2, tannins up to 30%, chebulic acid 3-5%, chebulinic acid 30%, anthraquinone, flavonoids like luteolin, rutins, and quercetin etc. Methanolic extracts of *Terminalia chebula*, *Terminalia belerica*, *Emblica officinalis* and their combination named 'Triphala' (equal proportion of the above three plant extracts) are being used extensively in Indian system of medicine for atherosclerosis, cancer, diabetes and liver cirrhosis. They were found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals *in vitro*.

This study concludes that *Terminalia chebula* seed is effective in controlling hyperglycemia to a significant extent in STZ induced diabetic rats. It may be beneficial in reducing diabetic complications arising due to insulin deficiency and metabolic perturbations such as hypercholesterolemia and LDL increment in diabetic rats. The results of the present study showed that an elevation in total serum cholesterol and LDL in diabetic rats.

Diabetic nephropathy is mainly associated with excess urinary albumin excretion, abnormal renal function as represented by an abnormality in serum creatinine. Clinically, diabetic nephropathy is characterized by a progressive increase in proteinuria and decline in GFR, hypertension, and a high risk of cardiovascular morbidity and mortality. Increased glomerular capillary pressure occurs early in diabetes and is associated with hyperfiltration at the glomerulus. The glomerular mesangium expands, initially by cell proliferation and then by cell hypertrophy. Increased mesangial stretch and pressure can stimulate this expansion, as can high glucose levels. The extract due to its significant hypoglycemic activity may have inhibited the formation of advanced glycosylation end products (Kumar KV *et al*., 2002) [10].

The results from the current study showed that TCSME reduces the development of diabetic nephropathy by increasing serum parameters such as albumin and total protein, but a reduction in serum creatinine and urea as well as urine albumin and total protein in treated rats as compared with diabetic control rats (Zhu HW., *et al* 2005) [14]. Hence, current study confirmed that TCSME-treated diabetic rats showed significant improvement in renal functions such as proteinuria and albuminuria. Treatment of the diabetic rats with TCSME caused a significant reduction in the serum triglycerides, LDL and VLDL cholesterol with a significant increase in HDL cholesterol levels when compared with diabetic control rats (Evans JL *et al*., 2002) [4]. Administration of the TCSME significantly attenuated diabetes induced fiber derangement and increase in number of mesangial cells as a marker of histopathological alterations when compared with diabetic control group (Varma SB *et al*., 2010) [15]. Hence, further studies are needed to isolate the principle components of *Terminalia chebula* and clarify the possible mechanisms of their action in the diabetes condition.

Conclusions

The present study clearly conclude that the administration of TCSME reduces the risk factors of diabetic copmplications like diabetic nephropathy. It has been remarked by the study that TCSME has therapeutic or preventive effects on several pharmacological targets in the complicated pathological mechanism of diabetic nephropathy. Thus, it is worthwhile to be further investigated for its potential pharmacological effects in diabetic nephropathy and to screen out their potential phytoconstituents.

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