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Influence of *Terminalia bellerica* Roxb. Fruit Extracts on Biochemical Parameters in Streptozotocin Diabetic Rats

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Abstract: *Terminalia bellerica* Roxb. is extensively used in Indian traditional systems of medicine to treat various diseases including diabetes mellitus. The present study investigated the antidiabetic effects of *Terminalia bellerica* fruit extracts and their protective effect in preventing the secondary complications of diabetes mellitus. Hexane, ethylacetate and methanol crude extracts of *Terminalia bellerica* fruits were administered at the dose of 200, 300 and 300 mg kg^{-1}, respectively, for 60 days to Streptozotocin induced diabetic male Wistar rats. It was found that the fruit extracts significantly ($p<0.05$) increased the plasma insulin, C-peptide and glucose tolerance levels compared to the diabetic control and the effect was more pronounced in methanol extract treated rats. In addition the plant extracts significantly ($p<0.05$) increased body weight and serum total protein and significantly decreased the serum levels of total cholesterol, triglycerides, low density lipoprotein cholesterol, urea, uric acid and creatinine in diabetic rats. Thus the results of this experimental study indicated that *Terminalia bellerica* fruit extracts restored all the biochemical parameters related to the patho-biochemistry of diabetes mellitus and prevented diabetic nephropathy, dyslipidemia and other diabetes-induced complications. These beneficial therapeutic effects of *Terminalia bellerica* fruits may be due to the synergistic action of more than one bioactive compound and due to the significantly increased C-peptide in extract treated diabetic rats.

Key words: *Terminalia bellerica,* Streptozotocin, antihyperglycemic, C-peptide, diabetic complications

INTRODUCTION

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. Globally the number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (King *et al*., 1998). By definition, diabetes mellitus is categorized as a metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Insulin lack at the metabolic level causes derangement of carbohydrate, protein and lipid metabolism which eventually leads to a number of secondary complications such as hyperlipidemia, coronary artery disease, renal failure, stroke, neuropathy, retinopathy and blindness (Chait and Brunzell, 1996). Despite considerable progress in therapies using expensive synthetic drugs, the search for indigenous anti-diabetic agents from medicinal plants is promising. Herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their relatively low costs and minimal side effects (Valiathan, 1998).

Terminalia bellerica Roxb. (Combretaceae) is a large deciduous tree which occurs widely in the moist valleys of India and its fruits are most commonly used in Indian traditional systems of medicine (Chopra *et al*., 1996). The fruit rind is used in different preparations, for example, as an ingredient in the popular Ayurvedic formula known as Triphala (three fruits), used for the treatment of fever, cough, diarrhea, dysentery, skin diseases and liver disorders (Kirtikar and Basu, 1933). Phytochemically, the fruits of *Terminalia bellerica* have been reported to contain beta sitosterol, gallic acid, ellagic acid, ethyl gallate, chebulagic acid, galloyl glucose, mannitol, glucose, galactose, fructose, rhamnose (Row and Murty, 1970), arjungenin, belleric acid, bellericoside (Nandy *et al*., 1989), cannogenol (Yadava and Rathore, 2001) and three lignans and one flavan (Valsaraj *et al*., 1997).

The fruit is reported to have hepatoprotective (Nadkarni, 1954; Anand *et al*., 1994, 1997; Anjana *et al*., 2007), purgative (Chakravarti and Tayal, 1947), choleretic (Siddiqui, 1963) and hypotensive effects (Srivastava *et al*., 1992). In a clinical study, *Terminalia bellerica* was found to possess antispasmodic, anti-asthmatic and anti-tussive effects (Trivedi *et al*., 1979). The fruit extracts of *Terminalia bellerica* have been evaluated for anti-mutagenic (Kaur *et al*., 2002), antimicrobial and anti-HIV-1 activity (Valsaraj *et al*., 1997). The plant is known to lower the levels of lipid in hypercholesterolemic animals and prevent the development of atherosclerosis

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and myocardial infarction (Tariq *et al*., 1977; Thakur *et al*., 1988; Shaila *et al*., 1995). Triphala and *Terminalia bellerica* reduced the serum glucose level and showed marked antioxidant properties in alloxan-induced diabetic rats (Sabu and Kuttan, 2002).

The overall tonic effects of the fruit of *Terminalia bellerica* has been known for thousands of years in India and other Asian countries and even now, Ayurvedic practitioners recommend the dry fruits of *Terminalia bellerica* as a daily and preventive supplement to diabetic patients alone or in the triphala formula. But reports on the anti-diabetic activity of *Terminalia bellerica* are scanty and there is no detailed study on the beneficial role of this plant on prevention of secondary complications of diabetes mellitus. So, the following study was undertaken to evaluate the anti-diabetic and protective effects of *Terminalia bellerica* fruit extracts on certain biochemical parameters in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Preparation of plant extracts: The fruits of *Terminalia bellerica* Roxb (Combretaceae) were procured from authenticated Ayurvedic dealer and were identified and authenticated at the Rapinat herbarium of St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The fruits were dried in shade, chopped and coarsely powdered. The coarse powder was used for extraction with various solvents. The powdered form of fruit rind of *Terminalia bellerica* was subjected to successive solvent extraction with the following solvents in increasing order of polarity: hexane, ethylacetate and methanol in 1:3 ratios (Harborne, 1984; Trease and Evans, 1994). Extracts were filtered and the filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. The yields of the hexane, ethylacetate and methanol extracts were 1.2, 1.5 and 13.5%, respectively. A weighed portion of each extract was suspended in 0.5% aqueous carboxy methyl cellulose (CMC) prior to oral administration to animals (Daisy *et al*., 2008, 2009).

Experimental animals: Male albino rats of Wistar strain, weighing about 160±15 g bred in the animal house of King Institute, Chennai, Tamil Nadu and India were used for this study. The experimental protocol was approved by the Institutional Animal's Ethics Committee and by the regulatory body of the government (Reg.No.585/05/A/CPCSEA). The rats were kept in clean polypropylene cages and maintained at the local animal house conditions of temperature 24±2°C, humidity 45±5% and 12 h day and 12 h night cycle. The animals were fed with a standard pellet diet (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. After randomization into various groups, the rats were acclimatized to the laboratory conditions of temperature and photoperiod for a period of 1-2 weeks before initiation of the experiments.

Induction of diabetes: Diabetic mellitus was induced by single intraperitoneal injection of freshly prepared Streptozotocin (single dose of 50 mg kg^{-1} b.wt.) in 0.1 M Citrate buffers (pH-4.5) in a volume of 1 mL kg^{-1} body weight (Nakhoda and Wong, 1979). Diabetes was developed and stabilized in these STZ treated rats over a period of 7 days. The control animals were treated with citrate buffer (pH 4.5). After 7 days of STZ administration, plasma glucose levels of each rat were determined. Rats with a fasting plasma glucose level of more than 300 mg dL^{-1} were selected for the study.

Experimental design: In the experiment a total of 36 rats (6 normal; 30 STZ-diabetic rats) were used. The body weight and fasting plasma glucose levels of all the rats were determined before the start of the experiment. The rats were randomly divided into six groups of six rats each.

Group I : Normal rats given vehicle alone (0.5% CMC)
Group II : Diabetic control rats given vehicle alone (0.5% CMC)
Group III: Diabetic rats treated with hexane extract of *T. bellerica* (200 mg kg^{-1} b.wt.)
Group IV: Diabetic rats treated with ethylacetate extract of *T. bellerica* (300 mg kg^{-1} b.wt.)
Group V : Diabetic rats treated with methanol extract of *T. bellerica* (300 mg kg^{-1} b.wt.)
Group VI: Diabetic rats treated with Glibenclamide (0.6 mg kg^{-1} b.wt.)

The various crude extracts were suspended in 1mL of 0.5% CMC and administered (single dose per day) orally using intragastric tube for a period of 60 days. The optimum dose was selected on the basis of a preliminary study with various extracts at different doses (100, 200, 300 and 400 mg kg^{-1} b.wt.). Blood glucose levels and body weight of the animals were recorded every week. Glucose Tolerance Test was performed for all the rats on 45th day of treatment. After 60 days of treatment, rats were decapitated, blood was collected into plain and heparinized tubes and the serum and plasma were separated immediately by centrifugation at 3500 rpm for 15 min. Serum and plasma were assayed either immediately or stored at -20°C.

Determination of plasma glucose, insulin and C-peptide: Fasting plasma glucose was estimated using glucose oxidase peroxidase method (Trinder, 1969). Plasma insulin

concentration was determined by radioimmunoassay kit (Diasorin, Saluggia, Italy). The kit included human insulin as standard and ^{125}I-labelled human insulin antibody, which cross reacts similarly with rat insulin. C-Peptide was determined using Rat C-Peptide RIA Kit from LINCO Research, Missouri, USA. The Millipore Rat C-Peptide assay utilized ^{125}I-labeled Rat C-Peptide antiserum to determine the level of C-Peptide in serum or plasma by the double antibody technique.

Measurement of serum total protein, urea, uric acid, creatinine and cholesterol levels: The serum total protein was estimated by the method described by Reinhold (1980). Serum urea, uric acid and creatinine were estimated using a commercial diagnostic kit (Ranbaxy Laboratories, New Delhi, India). Serum total cholesterol, triglycerides, HDL and LDL-cholesterol were determined using the diagnostic kits from Randox Laboratories Ltd., UK.

Oral glucose tolerance test: The oral glucose tolerance test was performed in overnight fasted normal, diabetic control and extract treated diabetic animals on 45th day of treatment. All animals received a load of 2.0 g of glucose kg^{-1} b.wt. Plasma glucose was measured in blood withdrawn from the tip of the tail, before load (t = 0) and 30, 60, 90, 120 and 150 min after glucose administration. Glucose was determined as mentioned above.

Statistical analysis: Statistical analysis was performed using SPSS software package Version 16.0. The values were analyzed by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). All the results were expressed as Mean±SD for six rats in each group. In this study, p-values <0.05 were considered significant.

RESULTS

Plasma glucose levels measured in normal and experimental rats after a single day and at the end of 3, 7, 15, 30 and 60 days of treatment are given in Table 1. The glucose levels of STZ-treated diabetic rats (diabetic control) remained significantly increased (>375 mg dL^{-1}) throughout the experimental period. Oral administration of various crude extracts of *Terminalia bellerica* for 60 days decreased the blood glucose level in STZ-induced diabetic rats. The decrease in blood glucose was greater in the methanol extract (92.93±8.93) treated diabetic rats than in ethylacetate (117.75±4.88), glibenclamide (124.28±6.26) and hexane (128.13±7.0) treated groups. Significant reduction were observed from third day onwards and fasting plasma glucose level reached near normal values by the end of 60 days.

On the 45th day, a glucose tolerance test was performed on all experimental groups. A typical normal response was seen both in the normal control and extract treated groups. It was observed that, plasma glucose levels reached maximum, 60 min after taking glucose and returned to the fasting glucose levels within 120 min of glucose administration. In untreated diabetic rats, the rise in the plasma glucose levels was greater than normal rats, peak was exaggerated and the glucose levels did not return back to the normal fasting level even after 120 min (Fig. 1).

Table 2 presents the effect of *Terminalia bellerica* fruit extract on body weight, plasma insulin and C-peptide in normal and diabetic rats. In untreated diabetic rats there was a significant ($p<0.05$) decrease in body weight, plasma insulin (5.43±1.16 μU mL^{-1}) and C-peptide (63.75±8.30 pmol L^{-1}) when compared to normal rats. Oral administration of hexane, ethylacetate and methanol extracts to diabetic rats significantly ($p<0.05$) increased body weight, plasma insulin and C-peptide when compared to untreated diabetic rats. The C-peptide levels of all the three extract treated groups were found to be more than the normal control rats and heterogeneously significant. Maximum serum C-peptide levels were observed in methanol extract treated rats (462.5±33.04 pmol L^{-1}) followed by ethylacetate (423.5±19.81 pmol L^{-1}) and hexane (350.5± 3.7 pmol L^{-1}).

Table 3 shows the levels of serum lipids in normal and experimental rats. There was a significant ($p<0.05$)

Table 1: Effect of *Terminalia bellerica* fruit extracts on plasma glucose levels in normal and STZ induced diabetic male Wistar rats

	Plasma glucose levels (mg dL^{-1})					
Groups	0th day	3rd day	7th day	15th day	30th day	60th day
Normal	85.3±2.9	83.6±2.9^{a}	85.4±4.8^{a}	85.4±3.7^{a}	86.1±4.0^{a}	85.9±4.4^{a}
Diabetic	342.3±13.0	355.4±13.8^{d}	373.2±9.8^{e}	400.9±11.6^{d}	416.8±4.8^{d}	431.4±9.5^{c}
Diabetic+hexane	342.3±16.4	308.7±7.9^{c}	252.8±11.3^{d}	179.3±18.6^{b,c}	149.0±7.6^{c}	128.1±7.0^{b}
Diabetic+ethyl Acetate	337.3±12.0	294.6±10.2^{b,c}	216.4±13.9^{c}	179.7±9.5^{b,c}	147.0±13.8^{c}	117.8±4.9^{b}
Diabetic+methanol	340.2±16.0	279.3±23.3^{b}	187.5±20.9^{b}	165.4±19.1^{b}	111.5±5.3^{b}	92.9±8.9^{a}
Diabetic+ glibenclamide	330.7±23.5	287.9±12.2^{b}	253.9±16.0^{d}	190.2±18.1^{c}	155.2±6.3^{c}	124.3±6.3^{b}

Each value is mean±SD for six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

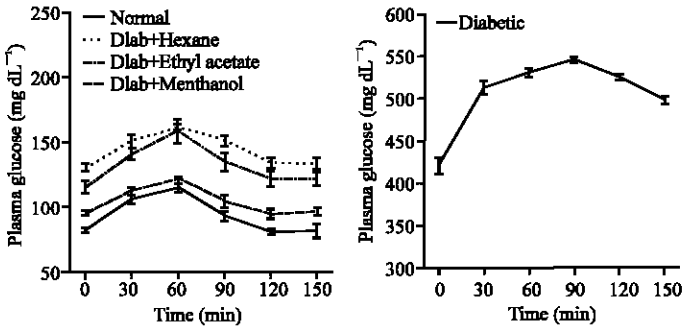

Fig. 1: Effect of *Terminalia bellerica* fruit extracts on glucose tolerance test in normal and STZ induced diabetic male Wistar rats

Table 2: Effect of *Terminalia bellerica* fruit extracts on body weight, plasma levels of insulin and C-peptide in normal and STZ induced diabetic male Wistar rats

	Body weight (g)			
Groups	Initial	Final	Insulin (μU mL^{-1})	C-peptide (pmol L^{-1})
Normal	159.00±10.0	198.50±7.37^{c}	15.63±1.12^{c}	260.25±11.00^{e}
Diabetic	157.00±7.75	128.50±6.19^{a}	5.43±1.16^{a}	63.75±18.30^{a}
Diabetic+hexane	158.75±9.29	186.50±5.51^{b}	15.28±1.16^{c}	350.50±3.70^{d}
Diabetic+ethylacetate	157.00±7.07	194.25±6.85^{b,c}	14.35±0.50^{b,c}	423.50±19.81^{e}
Diabetic+methanol	155.50±7.77	197.75±6.02^{c}	15.48±0.81^{c}	462.50±33.04^{f}
Diabetic+glibenclamide	157.75±8.18	186.25±5.91^{b}	13.13±0.50^{b}	159.50±9.88^{b}

Each value is Mean±SD for six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

Table 3: Effect of *Terminalia bellerica* fruit extracts on serum levels of total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol in normal and STZ induced diabetic male Wistar rats

	Total cholesterol	Triglycerides	LDL cholesterol	HDL cholesterol
Groups	(mg dL^{-1})			
Normal	79.85±5.03^{a}	73.20±6.33^{a}	37.05±1.51^{a}	30.70±3.21^{d}
Diabetic	137.13±12.66^{c}	139.60±9.51^{c}	86.25±4.65^{d}	15.98±2.81^{a}
Diabetic+hexane	69.25±3.69^{a}	77.95±3.98^{a}	34.38±6.22^{a}	26.75±2.46^{c,d}
Diabetic+ethylacetate	77.65±4.07^{a}	93.05±6.54^{b}	44.85±3.83^{b}	21.95±2.17^{b}
Diabetic+methanol	71.40±5.46^{a}	91.55±7.82^{b}	36.58±5.21^{a}	25.03±2.78^{b,c}
Diabetic+glibenclamide	90.88±6.73^{b}	100.13±5.96^{b}	54.80±7.69^{c}	21.70±2.47^{b}

Each value is Mean±SD for six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

Table 4: Effect of *Terminalia bellerica* fruit extracts on serum levels of total protein, creatinine, uric acid and urea in normal and STZ induced diabetic male Wistar rats

	Total protein	Creatinine	Uric acid	Urea
Groups	(mg dL^{-1})			
Normal	6.75±0.42^{b}	0.96±0.07^{a}	1.88±0.25^{a}	24.65±3.09^{a}
Diabetic	4.08±0.25^{a}	2.16±0.16^{c}	2.95±0.29^{b}	53.20±4.14^{c}
Diabetic+hexane	6.23±0.35^{b}	1.15±0.21^{a}	2.10±0.29^{a}	32.93±2.98^{b}
Diabetic+ethylacetate	6.53±0.46^{b}	1.10±0.18^{a}	2.15±0.26^{a}	32.05±4.32^{b}
Diabetic+methanol	6.50±0.50^{b}	1.00±0.18^{a}	1.78±0.33^{a}	33.82±4.15^{b}
Diabetic+glibenclamide	6.45±0.51^{b}	1.45±0.23^{b}	1.88±0.18^{a}	31.78±4.11^{b}

Each value is Mean±SD for six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

decrease in the level of serum HDL-Cholesterol and increase in the levels of total cholesterol, triglycerides and LDL-Cholesterol in diabetic rats when compared to normal rats. Administration of various crude extracts of *Terminalia bellerica* fruit brought back the levels of serum lipids to near normal values in diabetic rats. Among the three solvent extracts, hexane extract produced maximum significant reduction in total cholesterol, triglycerides and LDL-Cholesterol and increase in HDL-Cholesterol in diabetic rats.

Serum total protein was significantly ($p<0.05$) reduced and serum urea, creatinine and uric acid levels were significantly elevated in STZ-diabetic rats when compared to the normal rats. Administration of various crude extracts of *Terminalia bellerica* for 60 days to diabetic rats significantly ($p<0.05$) increased serum total protein and significantly ($p<0.05$) lowered all the three Non-Protein Nitrogenous (NPN) substances. All the three extracts showed similar activity as they were homogeneously significant (Table 4).

DISCUSSION

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. After treatment with a low dose of STZ there should be many surviving beta-cells and regeneration is also possible (Gomes *et al*., 2001). From the results of the present study, it appears that still insulin-producing cells are functioning and the stimulation of insulin release and the increase in C-peptide could be responsible for most of the metabolic effects. Daily administration of various crude extracts of *Terminalia bellerica* fruits produced gradual decrease in the blood glucose level and significant inhibition of STZ induced diabetes and its complications in albino rats. Maximum significant reduction in the serum glucose level was found in methanol extract treated diabetic rats followed by ethylacetate and hexane extract. The increase in plasma C-peptide levels also followed the same pattern.

C-peptide and insulin are the products of the enzymatic cleavage of pro-insulin and secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion rather than insulin alone (Doda, 1996). In the present study, the antihyperglycemic activity caused by glibenclamide in STZ-induced diabetic rats is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells. The significant increase in plasma insulin and more importantly the C-peptide levels of extract treated rats as compared to the glibenclamide groups indicate that the crude extracts of *Terminalia bellerica* fruits augmented the conversion of pro-insulin to insulin in addition to the stimulation of the remnant beta cells.

The antidiabetic effect of crude extracts of *Terminalia bellerica* fruits may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. Preliminary phytochemical analysis of *Terminalia bellerica* fruit extracts revealed phenolic compounds and tannins as major constituents. Fruit contains 23.60 to 37.36% tannins such as Chebulinic acid (C_{41} H_{32} O_{27}); Chebulagic acid (C_{41} H_{30} O_{27}); 1,3,6-Trigalloylglucose and 1,2,3,4,6-pentagalloyl glucose; Glucogallin (C_{13} H_{16} O_{10}); Ellagic acid; Gallic acid; etc., (Row and Murty, 1970). The hypoglycemic activity of *Terminalia bellerica* fruit extracts might be due to the presence of polyphenolic compounds that suppress the increase in plasma glucose (Iwai, 2008), tannin molecules which were found to be responsible for the insulin-like glucose transport stimulatory activity and more specifically the presence of gallotannins such as Pentagalloyl Glucose (PGG), found to be more potent and efficacious in Insulin Receptor (IR) binding, IR activation and glucose transport induction (Klein *et al*., 2007).

In the glucose tolerance test, the plasma glucose levels of extract treated rats returned back to the fasting levels within 120 min of glucose administration indicating increased glucose tolerance due to augmented glucose transport and utilization. Maximum glucose tolerance was observed in methanol extract treated group followed by ethylacetate and hexane treated groups. The C-peptide levels of these groups also followed the same pattern suggesting the possible role of C-peptide in promoting glucose transport and its utilization. C-peptide was found to promote glucose utilization in diabetic patients (Johansson *et al*., 1992). Therefore, the results of the present study suggest that the possible mechanism of action of *Terminalia bellerica* fruit extracts were stimulation of insulin secretion from remnant beta cells, augmenting the conversion of pro-insulin to insulin, improving insulin sensitivity and promoting glucose transport and its utilization.

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus. High levels of total cholesterol and more importantly LDL-cholesterol in blood are major coronary risk factors (Tchobroutsky, 1978). Insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue which results in increased production of cholesterol rich LDL particle and dyslipidemia. In the present study, treatment with *Terminalia bellerica* fruit extracts improved the lipid profile by reducing the serum levels of total cholesterol, triglycerides and LDL-Cholesterol and at the same time increased HDL-Cholesterol. The hypolipidemic and cardioprotective activity of *Terminalia bellerica* fruit extracts in hypercholesterolemic rats have been reported by Tariq *et al.* (1977), Thakur *et al.* (1988) and Shaila *et al.* (1995) which might be due to the presence of beta sitosterol, as plant sterols are well known for its cardioprotective properties (Jones *et al*., 1997). Further, C-Peptides were found to effectively prevent and even reverse cardiovascular disease in diabetic rats (Ido *et al*., 1997) and improve blood flow in the heart of diabetic patients (Hansen *et al*., 2002). Therefore, the normal lipid profile in extract treated diabetic rats might be due to the significant increase in their C-peptide level.

Insulin plays an important role in the maintenance of protein balance, since it not only stimulates the uptake of amino acid and protein synthesis but also inhibits protein degradation (Jasra and Talesara, 1986). Significant reduction in serum total protein observed in diabetic rats may be due to decreased protein synthesis, increased

protein catabolism (Almdal and Vilstrup, 1988), microproteinuria and albuminuria, which are important markers of diabetic nephropathy (Mauer *et al*., 1981). The diabetic hyperglycemia induces elevation of the serum levels of urea, uric acid and creatinine which are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). The results of the present study demonstrated that *Terminalia bellerica* fruit extracts treated diabetic rats showed significant increase in body weight and serum total protein and significant reduction in the levels of serum urea, creatinine and uric acid indicating the protective role of *Terminalia bellerica* fruit extract in preventing diabetic nephropathy and weight loss. In the kidneys of diabetic rats, C-peptide were found to exert beneficial renal effects by reducing glomerular hyper filtration, albuminuria, glomerular hypertrophy and mesangial matrix expansion and prevent diabetic nephropathy (Johansson *et al*., 2000; Samnegård *et al*., 2005). Significant increase in plasma C-peptide concentration in extract treated diabetic rats explains the possible role of this C-peptide in preventing diabetic nephropathy and other complications in diabetic rats.

The beneficial effects of the crude extracts of *Terminalia bellerica* fruits might be due to the synergistic action of some polyphenolic compounds that have glucose lowering activity (Iwai, 2008), antioxidant property (Latte and Kolodziej, 2004) and more specifically due to the presence of gallotannins such as PGG which augments IR activation and glucose transport (Klein *et al*., 2007). Recent research have shown that C-peptide promotes glucose utilization (Johansson *et al*., 1992) and exerts beneficial therapeutic effects on many complications associated with diabetes mellitus (Marques *et al*., 2004), such as diabetic nephropathy (Johansson *et al*., 2000; Samnegård *et al*., 2005), neuropathy (Kamiya *et al*., 2006) and other diabetes-induced ailments. In the present study, *Terminalia bellerica* fruit extracts were found to increase the insulin and C-peptide levels, prevent hyperglycemia, diabetes induced complications such as weight loss, dyslipidemia and diabetic nephropathy and improves the regenerative and reparative capacity of the pancreas, liver and kidney in STZ induced diabetic rats. All these beneficial effects were more pronounced in methanol extract treated rats, which had the maximum plasma C-peptide levels. Therefore it may be concluded that hypoglycemic and protective role of *Terminalia bellerica* fruit extract in preventing secondary complications of uncontrolled diabetes mellitus may be due to the highly significant increase in plasma C-peptide levels in extract treated diabetic rats.

Studies are in progress in our laboratory to elucidate the many possible mechanisms of action through which this plant seems to act, as well as identify its bioactive constituents, so that the active compounds of *Terminalia bellerica* fruit extract can be used for the development of drugs to treat diabetes-induced complications.

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