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Full Length Research Paper

The effects of the aqueous extract of *Pterocarpus santalinus* heartwood and vitamin E supplementation in streptozotocin-induced diabetic rats

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Diabetic Nephropathy (DN) is a major complication of Diabetes Mellitus (MD) resulting in end-stage renal disease. Prevention or reversal of Diabetic Nephropathy is a major challenge in the current management of diabetes. The aim of the present study is to evaluate the hypoglycemic, antioxidant, hypolipidemic and nephroprotective effects of the aqueous extract of the *Pterocarpus santalinus* L (red sandalwood) alone, and in combination with vitamin E (α-tocopherol) supplementation in streptozotocin-induced diabetic rats. Twenty five healthy adult male Wistar rats were made diabetic by STZ-induction. At the end of 16 weeks of therapy, there were significant reductions in blood glucose ($p<0.001$), and improvements in glucose tolerance ($p<0.001$) as compared to untreated diabetic rats. Significant changes were also observed in lipid peroxidation (free radical activity), tissue and organ mass and cholesterol levels. The treatment caused significant lowering of blood sugar and improvement in glucose tolerance tests ($p<0.001$). The treatment also resulted in a significant reduction in serum lipids and body weight ($p<0.001$). A decrease was observed in HbA1c ($p<0.01$) on regular long-term control over blood glucose levels. The antioxidant effect of the red sandal wood extract was also evident, as it caused a reduction in malondialdehyde (MDA) in the brain, liver and muscle tissues. The extract also caused a decrease in the formation of lipid peroxidase, estimated by Thiobarbituric Acid Reactive Substance (TBARS) and increased antioxidants, Superoxide Dismutase (SOD), Catalase (CAT), glutathione peroxidase and gluthathione transferase in erythrocytes with $p<0.001$. Serum creatinine and urine albumin showed decreased levels after treatment and returned to control values ($p<0.05$). The kidneys were examined histologically for Diabetic Nephropathy and showed regression following treatment. Sixteen weeks combination therapy also resulted in decreases in LDL-C/ HDL-C, TC ($p<0.001$), TG ($p<0.001$) and an increase in HDL-C ($p<0.001$) of treated diabetic rats. The use of the aqueous extract of *P. santalinus* caused improvements in glycemia, lipid peroxidation and brain, liver and heart tissue masses.

Key words: *Pterocarpus santalinus* L, red sandal wood, antioxidant, diabetic nephropathy, vitamin E, malondialdehyde, lipid peroxidation, catalase, Thiobarbituric Acid Reactive Substance (TBARS), streptozotocin induction.

INTRODUCTION

Diabetes Mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia and associated metabolic dysregulation causes secondary pathophysiological changes in multiple organ systems. Diabetes Mellitus has attained epidemic proportions in most parts of the world, including developing countries. Approximately, 150 million people suffer from diabetes worldwide. Diabetes Mellitus is

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prevalent and obesity is a frequently observed associated complication (West, 1974). Metabolic disorders associated with this etiologically heterogeneous disease (including hyperglycemia, dyslipidemia and hyperinsulinemia) cause endothelial destruction and various micro- and macrovascular complications including coronary artery disease (Zargar et al., 1999). Experimental diabetic animal models have shown that oxidative stress causes persistent and chronic hyperglycemia, thereby depleting activities of the antioxidant defense system and otherwise promoting free radical generation (Eshrat, 2003; Bhor et al., 2004). Diabetic Nephropathy occurs in 30 to 40% of all diabetic patients and is also a major cause of increased morbidity and mortality (Parving et al., 1997). Although pathological classifications exist for several renal diseases, uniform classification for nephropathy, focal segmental glomerulosclerosis, and a Diabetic Nephropathy is lacking (Thijs et al., 2010).

The streptozotocin-induced diabetic rat model is widely used to simulate human Diabetic Nephropathy (Nicholas et al., 2004; Kalender et al., 2002). In rat models, mesangial expression and glomerular basement membrane thickening are the most frequently observed complications of STZ- induction; however, the exact mechanism of Diabetic Nephropathy is still not clearly understood. It has also been suggested that oxidative stress, may contribute to the pathogenesis of different diabetic complications (Ceriello, 2000; Schleicher and Friess, 2007).

A large number of herbal compounds are available on international markets, which are claimed to have hypoglycemic effects. However, the compounds cannot be put into clinical practice due to a lack of scientific studies on their efficacy and safety for human use. Many herbal products have been prescribed for the care of Diabetes Mellitus in ancient Ayurvedic literature in India. The World Health Organization (WHO) endorses the evaluation of the potential of plants as effective therapeutic agents, especially in areas where there is a lack of safe modern drugs. India has a rich heritage of medicinal plants of wide diversity which are used by the local population and traditional healers for the treatment of several diseases including diabetes (Eddouks et al., 2002). The present study was designed to see the hypoglycemic, hypolipidemic, antioxidant, and nephroprotective effects of the aqueous extract in streptozotocin induced diabetic rats.

Red sandalwood (*Pterocarpus santalinus*), part of the fabaceae plant family, contains santalic acid and has thus been used as a traditional medicine. Santalum - the mildly astringent Indian cooling tonic made from red sandal wood - is used to treat symptoms of diabetes. From the heartwood of *P. santalinus* a group of six closely related sesquiterpenes has been isolated which includes three new sesquiterpenes - namely isopterocarpolone, pterocarptriol and pterocarpdiolone. These sesquiterpenes include the known β-eudesmol, pterocarpol and cryptomeridiol. Their structures have been determined by spectral and chemical studies (Kumar et al., 1974; Nagaraju et al., 1991; Krishnamurthi et al., 1969). Methanolic extract of *P. santalinus* shown to exhibit anti-inflammations and anticancer effects shown cytotoxic activity (Kwon, 2006).

Recent studies have shown a correlation between a rise in the incidences of diabetes and obesity - thought to be the result of a large scale dietary shift - in developing countries (Anoop, 2010). In recent years, large deposits of adipose tissue (as seen in obese patients) has been implicated in the perturbation of numerous hormonal systems including the action of insulin of the cells of the body. Individuals with elevated body-mass indexes (BMIs) have been shown to exhibit abnormal blood glucose homeostasis and often demonstrate systemic resistance to secreted insulin, as well as other hormonal factors important for regulation of various systems (Lee, 2008). This overall dysregulation of hormonal systems is also thought to complicate insulin production, furthering a diabetic response (Miranda, 2008). Evidence linking inflammation to insulin resistance derives from both epidemiological studies and experimental data in humans and animal models (Pradhan, 2007). It is widely accepted that the prevalence of diabetes, obesity, and metabolic syndrome all increase with age (Ogden et al., 2006).

P. santalinus has been used for thousands of years in the treatment of Diabetes Mellitus (Warrier et al., 1995; Kasneswara, 2001). Many experimental studies in chemically-induced diabetes have been conducted with whole extracts of bark or heartwood of *P. santalinus*, which have shown its efficacy in reducing blood sugar levels (Kondeti et al., 2010; Maheswari et al., 1980; Nagaraju, 1991). The sandalwood extract has been found to increase antioxidant levels and also consequently prolong subject viability (Osawat et al., 2005). The present study aims to assess the effects of aqueous red sandalwood extract on diabetic nephropathy, lipid peroxidation and amelioration of systemic oxidative stress.

MATERIALS AND METHODS

Extraction of plant material

The *P. santalinus* L wood was dried and cut into small pieces and ground with a sterile mortar. The prepared extract was placed in sterile distilled water for 48 h. The mixture was thoroughly stirred with a glass rod until the extract had been fully dissolved. The mixture was then centrifuged at 2000 rpm for 10 min and the supernatant fluid was decanted, lyophilized and was stored at 4°C for the experimental study.

Animal ethics

The animal experimental committee approved the protocol, in accordance with the principles and procedures outlined in Jamia Hamdard, New Delhi, India. Approval was also obtained from the animal ethics committee (IEC) used in this study.

Induction of diabetes

Twenty- five healthy adult male albino rats of the Wistar strain of 150 to 200 g mass were used for the study. The animals were fed a pellet diet (Hindustan Lever. Ltd, Mumbai-India) and offered tap water and Lab Chow *ad libitum*.

Diabetes was induced in the subjects via the injection of 65 mg streptozotocin (STZ) per kilogram of body mass, purchased from Sigma (St. Louis, MO, US). The STZ was dissolved in citrate buffer, at pH 6.3, intraperitoneally. The method of Yotsumoto et al. (1997) was followed. Five rats were injected with the same volume of citrate buffer and used as non-diabetic healthy controls. All subjects except for the control group developed Diabetic Nephropathy after one-month.

Treatment

Each of the 25 total subjects (divided into the following five groups) were orally administered treatments based upon body mass of each subject:

1st group - Control; did not receive any treatment.
2nd group - Diabetic untreated.
3rd group - Received concentrated extract of *P. santalinus* at 250 mg/kg body mass daily for 16 weeks.
4th group - Received vitamin E at 544 mg per kilogram body mass daily for 16 weeks.
5th group – Received vitamin E and *P. santalinus*, according to the previously stated dosage levels for 16 weeks.

On the basis of preliminary data – that is albuminuria, increased urea and serum creatinine levels - suspected animals were examined to validate the early diagnoses of nephropathy. Baselines GTT, lipid profile, glycosylated hemoglobin, urine albumin, serum creatinine and blood urea, were the examined criteria to confirm the diagnosis. All of the suspected incidences of nephropathy were confirmed in this testing of the entire STZ-induced diabetic subject (total: 20). Body weights were also obtained for future comparison.

Histological determinations

For light microscopic examination, paraffin sections were prepared from 10% buffered formalin-fixed kidney tissue. The sections were deparaffinized in xylene and hydrated in a series of alcohol gradients (100, 90, 70, 50, 30% aqueous alcohol) for 2 min each. They were stained for 1 min in 0.5% hematoxylin and differentiated in 1% acid alcohol. The sections were stained for 30 s in eosin. After giving two changes in xylene for 2 min each, the sections were mounted with DPX and observed under a light microscope. The specimens from the diabetic-treated group were stained with hematoxylin. Eosin and periodic acid Schiff were examined by light microscopy. Microscopy showed an increase in mesangial matrix, thickening of glomerular basement membrane and Bowman capsule (haematoxylin and eosin stain).

Preparation for staining

Paraffin sections were made from 10% buffered formalin-fixed kidney tissue. The sections were deparaffinized and stained with haematoxylin and eosin and toludine blue.

Animal model protocol

The five groups were dosed by weekly urine albumin, serum creatinine and blood urea examinations. At the end of the 16 weeks trial, additional tests were performed, inducing a glucose tolerance test (GTT), glycosylated (HbA1c), lipid profile and body weight histological profile were determined via kidney biopsy.

Measurement of tissue enzymes

Activities of the enzymes superoxide dismutase (SOD), (CAT), (GSH-Px) and (GST) were measured by taking averages of the activities in supernatant fractions of the homogenate tissue from the liver, heart and brain after the termination of the subjects.

Treatment

Assay of antioxidant enzymes

All the enzyme activities were measured in the supernatant fraction of homogenate tissue separately from the liver, heart and brain and the numerical average of the three measurements was considered. The activity of total (SOD) was measured according to the methods of Beers (1952) and Poliodoro et al. (1984). The assay of (CAT) was performed according to the methods outlined by Aebi (1983). One unit of CAT activity was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 in 1 min. Activity of GSH-Px and GST activities were estimated by the methods of Lawrence and Burk (1976). Lipid (LPO) was determined by using the thiobarbituric acid reaction (Ohkawa et al., 1979). The standard for thiobarbituric acid reaction (TBARS) in this study was (MDA). The MDA concentration in tissue homogenates was normalized with protein concentration, which was measured by the Lowry method (Lowry et al., 1951). The effects of body mass were measured on the same day. The amounts of MDA in the brain, liver, cardiac and muscle tissue were estimated. Erythrocyte CAT and GPX, were also measured before treatment and following combined therapy.

Estimation of biochemical parameters

Glucose and glucose tolerance test (GTT)

Glucose was estimated at the beginning and at the end of the experiment (16 weeks apart) by the glucose oxidase method, using the kit from Ranbaxy Lab, New Delhi India. Standard oral glucose tolerance test was carried out as described by (Halim, 2003) at the beginning and at the end of 16 weeks of the experiment.

Glycosylated hemoglobin (HbA1c)

The glycosylated hemoglobin kit was purchased from Stangen Immunodiagnostics, Hyderabad, India. All other chemicals were of the highest purity locally available, produced by using ion-exchange

Table 1. Comparative effect of aqueous extract of *P. santalinus L.* (250 mg/kg/day) and vitamin E (544 mg daily) on blood glucose level in control, and streptozotocin – induced diabetic rats at the end of 16 weeks.

Group	Plasma glucose (mg/dl)	
	0 weeks	16 weeks
Non-diabetic control	105.2 ± 19.06	92.0 ± 18.0
Diabetic untreated	250.3 ± 23.47	360.4 ± 2.23
Diabetic treated *P. santalinus. L*	220.5 39.9	93.7 ± 8.0[a]
Diabetic + vitamin E	163.4 ± 36.67[a]	134.6 ± 17.5[a]
Diabetic + vitamin E and *P. santalinus*	200.6 ± 41.47	90.8 ± 12.3[a]

Values are mean ± S.D of five animals in each group. The significant comparisons of each experimental group are shown [a]*P < 0.001* when compared with diabetic untreated.

resin (Willey et al., 1984).

Protein estimation

Protein was estimated according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard, at 660 ppm.

Lipid profile

Total cholesterol (TC), high-density lipoprotein in cholesterol (HDL-C) and triacylglycerol (TG) were estimated by enzymatic methods employing kits from Orthodiagnostics Systems. Very low-density lipoprotein cholesterol (VLDL) and Low-density lipoprotein (LDL) levels were calculated by the standard formula as described. Low-density lipoprotein (LDL) cholesterol was estimated by using the following formula: LDL in mg% = total cholesterol–(HDL-C-1/5triglycerides). Cholesterol concentrations were measured using enzyme and calculated according to Friedewald (1972). HDL-C was estimated after precipitating low-density lipoprotein cholesterol (LDL-C) and VLDL-C from serum, using the precipitating reagent polyethylene glycol (PEG) provided with the kit. LDL-C and VLDL-C levels were calculated by subtracting HDL-C from TC. Glycosylated hemoglobin was estimated by using the kit from Stangen Immunodiagnostics.

Assessment of hypolipidemic effect

Subjects that received treatment were divided into two groups of 5 each. One group was orally administered a single dose of *P. santalinus* at 250 mg/kg body wt/day for 16 weeks. FBG and GTT were determined once a week to follow improvement in these two parameters. Serum lipid profile and glycosylated hemoglobin was determined before starting treatment and after 16 weeks of treatment. Lipid profile, blood sugar level and glycosylated hemoglobin levels were also determined in the healthy control group.

Effect on glucose tolerance

Results of the glucose tolerance test conducted on normal rats fed with plant extract *P. santalinu*s are shown in Table 1. Levels of catalase and superoxide dismutase were estimated by the method of Wendell (1981). Glutathione peroxidase (GPX) and glutathione-S-transferase (GST) activities were estimated by the method of Gupta et al. (1999).

Lipid peroxidation assay

The serum peroxidative status was assessed by measuring maximum absorption at 532 ppm after reacting malondialdehyde (MDA) with TBARS to form the absorption adduct. Absorption measurements were obtained on a Jason V-530 UV/ VIS spectrophotometer. The tissues were mixed with thiobarbituric acid solution and incubated for 60 min at 95°C. Absorbing compounds were extracted and measured as presented by Misra and Friedewald (1972).

Protein assay

The protein estimations were determined by the method of Lowry et al. (1951). Estimation of urinary albumin by the method of Doumas and Biggs (1972) and creatinine levels by the methods of Friedewald et al. (1972).

Statistical analysis

Results are presented as mean standard deviation (SD) for all group of n = 5 independent experiments. From the biochemical data, comparisons of antioxidant activity of the plant *P. santalinus* to vitamin E were made. The significance of the difference between data pairs was evaluated by the analysis of variance (ANOVA) followed by the Dunnet, student t-test. Results significance was set at $P < 0.001$.

RESULTS

P. santalinus combined with vitamin E reduced blood glucose levels in diabetic group during the16 week testing according to the Table 1. The patterns

Table 2. Comparative effects of aqueous wood extract of *P. santalinus* L and vitamin E on glucose tolerance after 16 weeks in control and STZ induced diabetic rats.

Group	Plasma glucose (mg/dl), mean ± S.D				
	0 h	0.5 h	1 h	1.5 h	2 h
Non-diabetic control	95.50 ± 25.8	145.40 ± 3.02	147.20 ± 6.39	118.40 ± 2.61	110.00 ± 2.55
Diabetic untreated	185.60 ± 42.99	255.60 ± 14.92	276.00 ± 18.20	283.60 ± 16.40	276.00 ± 16.96
Diabetic + treated *P. santalinus*	89.30 ± 24.19	112.00 ± 4.00	118.7 ± 7.84	105.40 ± 0.06	88.60 ± 0.72
Diabetic+ vitamin E	85.20 ± 23.09	113.00 ± 3.78 [b]	116.20 ± 2.56	105.20 ± 2.40	89.20 ± 0.52
Diabetic + vitamin E + *P. santalinus*	75.20 ± 21.99	95.00 ± 0.48[a]	83.30 ± 0.59 [a]	88.80 ± 0.48[a]	75.80 ± 0.74 [a]

Values are mean ± S.D of the five (n = 5) animals in each group. The significant comparisons of each experimental group are shown for diabetic untreated. [a]P < 0.001 when compared with diabetic treated.

demonstrate that only the combination of both substances lead to the improved condition of the diabetic rats (Table 1).

While there is not a significant difference in glucose levels at the end of the trial period between the efficacies of vitamin E as compared to the red sandalwood, there was a significant discrepancy between the GTT results of vitamin E administered in tandem with red sandalwood and the single treatment approach. The GTT for the group that received vitamin E changed from 85.20 ± 23.09 mg/dl at t = 0 h to 89.20 ± 0.52 mg/dl at t = 2.0 h. The group that received *P. santalinus* changed from 89.30 ± 24.19 mg/dl to 88.60 ± 0.72 mg/dl from t = 0 h to t = 2.0 h. The group that received combined treatment exhibited a similar response, from 75.20 ± 21.99 mg/dl at t = 0 h to 75.80 ± 0.74 mg/dl. However, over time, the intervals between these two measurements (t = 0.5,1.0 and 1.5 h) of glucose levels peaked at lower points, exhibiting less variance than in the groups that received only red sandalwood or vitamin E (Table 2).

Table 3 shows the comparative effects of aqueous extract of *P. santalinus* wood alone and combination with vitamin E on lipid profiles. Reduction in total cholesterol TC, LDL-C, TG, VLDL-C was significant at P < 0.005 and significant increases were observed in HDL-C (at P < 0.001) on 16 weeks treatment with *P. santalinus* and vitamin E either alone or in combination. The effect was more pronounced with therapies combined rather than single therapies. There was a significant decrease in HbA1c only in combination therapy and no significant decrease with the individual administration of red sandalwood or vitamin E.

Significant decreases were present in congruence with the application of the combined approach in LDL-C levels. Further, HDL-C levels were highest in the group that received mixed treatment. Total cholesterol and haemoglobin A1C levels were also lowest in the mixed-treated group. In each measured category, the combination of vitamin E and red sandalwood was significantly more effective than either substance individually.

Great potential is seen in *P. santalinus* L. when combined with antioxidants. Healthy controls show lower levels of LPO and higher levels of antioxidant-related erythrocyte enzymes, as compared to the diabetic-untreated group. The effect of treatment after 16 weeks was also evident in the diabetic subjects. *P. santalinus* alone yields marginal results. The plant extract was, however, able to decrease TBARS. Potency of the red sandalwood was again observed to increase in tandem with vitamin E. Combination therapy led to significant decreases in LPO, SOD, GSH-PX and GST, when compared to the control, individual vitamin E or red sandal wood groups (Table 4)

There was again significant effect of combination therapy on body (210.00 ± 2.84 g), muscle (135.6 ± 0.18 g), and liver (3.35 ± 0.19 g) mass. As compared to untreated diabetes, there was no effect of combination therapy on kidney (1.50 ± 0.03 g) or heart (0.62 ± 0.01 g) mass as compared to untreated diabetic.

Table 5 shows a general improvement on overall levels as determined by organ and body mass when applying a combination-based treatment instead of an individual approach of vitamin E or red sandal wood.

Amounts of malondialdehyde (MDA) in subject organs in control and STZ-induced diabetic groups at the end of 16 weeks. Significant decreases in MDA presence in brain, liver and muscle tissue in subjects at the end of the 16 week period was observed. Significant decrease in the amount of MDA was seen, as a result of combination therapy in brain (231.32 ± 6.84 mg/g), liver (110.62 ± 7.29 mg/g) and muscle (183.00 ± 11.52 mg/g) tissues as compared to untreated diabetic. This treatment prevented severe and acute increases in MDA levels in the combination-treated group that witnessed the diabetic-untreated specimens (Table 6).

Significant decreases in urea (29.05 ± 0.59 g/100), albumin (3.00 ± 0.16 g/100), and serum creatinine (1.00 ±

Table 3. Comparative effect of aqueous extract of *P. santalinus* wood alone and in combination with vitamin E on lipid profile in control, diabetic untreated and diabetic treated groups subjects at the end of 16 weeks.

Group	Parameter						
	HbA_{1c} %	Total protein (g/L)	TC (%)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	TC (mg/dl)
1. Non-diabetic control	2.7 ± 0.18	75.20 ± 0.84	162.00 ± 2.86	83.5 ± 2.69	50.6 ± 4.2	1.45 ± 0.02	115.60 ± 1.04
2. Diabetic untreated	5.7 ± 0.13	60.00 ± 0.64	245.00 ± 3.2	186.8 ± 2.03[a]	33.5 ± 1.78	3.80 ± 0.04	210.12 ± 1.28
3. Diabetic+*P. santalinus*	3.2 ± 0.07	78.0 ± 0.74[a]	173.00 ± 0.25[a]	92.18 ± 3.37[a]	55.7 ± 1.43[a]	2.10 ± 0.09[a]	130.00 ± 2.5[a]
4. Diabetic + vitamin E	3.4 ± 0.06	69.0 ± 0.70[a]	164.72 ± 7.30[a]	97.5 ± 3.85[a]	42.7 ± 4.16	2.53 ± 0.01[a]	125.40 ± 8.63[a]
5. Diabetes + *P.santalinus + vitamin E*	2.9 ± 0.0.1[a]	88.00 ± 0.83[a]	140.20 ± 5.64[a]	42.6 ± 1.64[a]	58.7 ± 2.05[a]	1.84 ± 0.08[a]	100.50 ± 8.47[a]

Values are mean ± S.D of the five (n=5) animals in each group. The significant comparisons of each experimental group are shown for diabetic untreated [a]P < 0.001 when compared with diabetic treated. LDL-Low –density lipoprotein, HDL-high – density, Lipoprotein, VLDL – very low –density lipoprotein, HDL-C _HDL-cholesterol, TC- total cholesterol.

Table 4. Effect of treatment with aqueous extract (250 mg/kg/b.w/ day) of P. santalinus, and Vitamin E (544 mg daily) alone or in combination with lipid peroxidation products as TBARS (LPO) and antioxidants (SOD), CAT glutathione peroxidase andantioxidants (GSH-Px) and glutathione transferase (GST) activities in erythrocytes in control, diabetic untreated and diabetic treated groups at the end of 16 weeks.

Treatment	LPO	SOD	CAT	GSH-Px	GST
Non-diabetic control	270.6 ± 0.86	382.4 ± 8.5	265.3 ± .179	32.6 ± 0.34	30.4 ± 0.39
Diabetic untreated	398.6 ± 1.18	300.2 ± 1.24[a]	200.7 ± 0.60[a]	19.8 ± 0.32[a]	20.5 ± 0.34[a]
Diabetic + *P. santalinus*	262.3 ± 1.28[a]	345.8 ± 0.72	220.4 ± 2.38[a]	32.8 ± 2.4[a]	38.0 ± 0.67
Diabetic vitamin E	142.1 ± 8.06[a]	334.5 ± 0.40[a]	224.6 ± 8.64[a]	29.1 ± 1.66	25.5 ± 0.97[a]
Diabetic + vitamin E + *P. santalinus*	190.8 ± 17.93[a]	300.8 ± 0.34 [a]	257.3 ± 0.45[a]	31.0 ± 0.64[a]	33.6 ± 0.38[a]

Values are mean ± S.D of the five (n= 5) animals in each group. The significant comparisons of each experimental all group are shown for diabetic untreated, [a]P< 0.001 when compared with diabetic treated. Values are expressed as Lipid (n peroxides (nm) of TBA reactants /mg protein; SOD – Units /mg protein CAT – nmol of H_2O_2/mg protein, GPX – nmol of glutathione oxidized/ min /mg protein; LPO – nmol of malonaldehyde /mg, and GST- µmole of H_2O_2 utilized / min /mg protein.

0.08 g/100) were seen in combination therapy, as compared to the untreated-diabetic group. There was a decrease in albuminuria as a result of combination therapy. Albuminuria is a well known risk factor for cardiovascular status (Agewall, 1997). Comparative assessment of the histological features of the kidney of the diabetic-untreated and diabetic-treated with *P. santalinus* was performed. There was histological response after 16 weeks of treatment of diabetic subjects, as evident on microscopic findings in untreated-diabetic and treated-diabetic subject kidney specimens.

On light microscopy, diabetic rats treated with combination therapy for 16 weeks showed less mesangial expansion and less thickening of basement membranes in the glomeruli. The above-mentioned features are characteristic of Diabetic Nephropathy with amelioration indicating nephroprotective effect of the combination treatment. Histomorphologically the glomeruli

Table 5. Effect of experimental diabetes and therapy on general parameters in control, diabetics and diabetic treated rats at the end of 16 weeks.

Treated	Body wt (g)	Muscle (g)	Heart wt (g)	Kidney (g)	Liver (g)
Non-diabetic control	224.10 ± 3.23	136.00 ± 1.9	0.70 ± 0.04	1.45 ± 0.06	3.37 ± 0.25
Diabetic untreated	110.20 ± 0.3	120.00 ± 5.19	0.50 ± 0.01	2.0 ± 0.35	5.4 ± 0.11
Diabetic + *P. santalinus L*	*156*.00 ± 1.99[a]	135.6 ± 0.27[a]	0.66 ± 0.03	1.89 ± 0.22	5.2 ± 0.24
Diabetic + Vitamin E	168.90 ± 2.19[a]	132.0 ± 0.09[a]	0.59 ± 0.04	1.24 ± 0.008	2.50 ±0. 03[a]
Diabetic + Vitamin E + P. *santalinus L*	210.00 ± 2.84 [a]	135.6 ± 0.18[a]	0.62 ± 0.01	1.50 ± 0.03	3.35 ± 0.19[a]

Values are mean ± S.D of the five animals in each group. The significant comparisons of each experimental group are shown for diabetic untreated. [a]P < 0.001 when compared with diabetic treated.

Table 6. Amounts of malondialdehyde (MDA) in rat organs, control and STZ-induced diabetic rats at the end of 16 weeks.

Treatment	Brain (MDA mg/g)	Liver (MDA mg/g)	Muscle (MDA mg/g)
Non-diabetic control	220.9 ± 13.12	100.10.±6.64	168.85 ± 10.08
Diabetic untreated	285.42 ± 11.28	150.17 ± 5.74	225.80 ± 8.8
Diabetic+ vitamin E+ *P. santalinus L*	231.32 ± 6.84	110.62 ± 7.29	183.00 ± 11.52*

Values are mean ± S.D of the five animals in each group. The significant comparisons of each experimental group are shown for diabetic untreated. *P < 0.001 when compared with diabetic-treated.

Table 7. The effect of experimental diabetes and parameters in control, diabetics and diabetic treated rats at the end of 16 weeks**.**

Treatment	Urea (g/100ml)	Urine albumin (g/100 ml)	Serum creatinine (g/100 ml)
Non-diabetic control	30.00 ± 1.0	3.00 ± 0.12	1.08 ± 0.04
Diabetic untreated	40.00 ± 1.0	5.70 ± 0.08	2.50 ± 0.01
Diabetic - vitamin E + *P. santalinus*	29.05 ± 0.59*	3.00 ± 0.16*	1.00 ± 0.08*

Values are mean ± S.D for the five animals in each group. The significant comparisons of each experimental group are shown with the diabetic-untreated *P < 0.05 when compared with diabetic treated.

showed significant increases in the mesangial matrix. There was thickening of glomerular basement membrane and Bowman capsule treatment of *P. santalinus* L and vitamin E in the subjects (Table 7).

Toxicity

The possible toxicity of *P. santalinus* was invested in this study. Subjects which received the 250 mg/kg body mass treatment were monitored for any related symptoms. No indications of negative impacts of the treatment were observed. Toxicity was investigated up to the administration of 3500 mg/kg body mass per day, which did not result in any indication of lethality within the subjects.

Histopathological changes

The kidney sections of the diabetic-untreated and treated -diabetic groups were examined for structural changes under a light microscope. In diabetic subjects kidney biopsies revealed thickening of the basement membrane (Figure 1). We have studied Ayurvedic medication on five streptozotocin-induced diabetic rats. Aqueous extract of *P. santalinus* L from wood (traditional Ayurvedic medicine) and vitamin E supplementation in streptozotocin-induced diabetic rats. Desirable control of blood sugar, lipid peroxidation before and after treatment ($P<0.05$) and improvement of renal functions were observed. Vitamin E has been shown to ameliorate Diabetic Nephropathy and various possible mechanism induce reduction oxidative stress, TGF modulation and

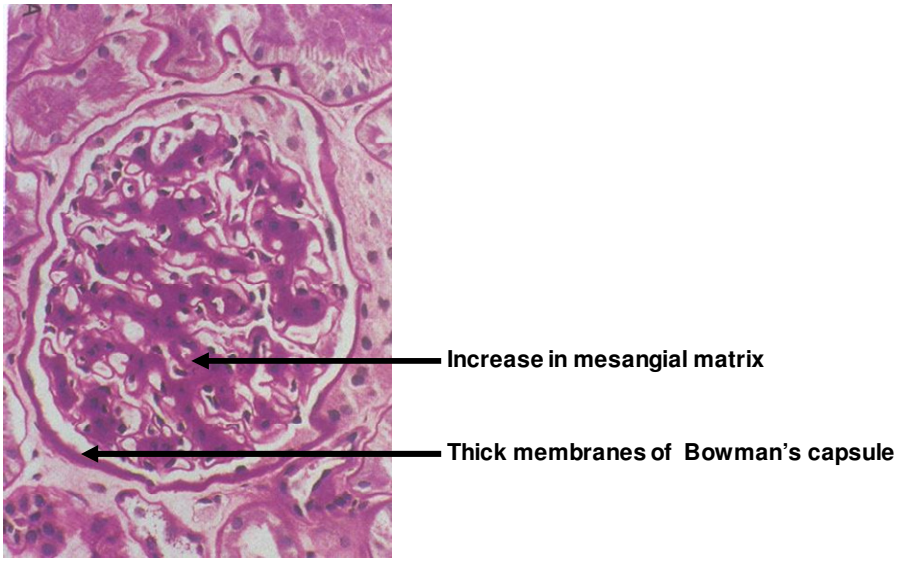

Figure 1. Diabetes Mellitus with glomerulosclerosis.

protein kinase-C modulation (Cojocel et al., 2005). Diabetic Nephropathy can be successfully prevented by using *P. santalinus* wood (traditional Ayurvedic medicine) and vitamin E supplementation can give better therapeutic efficacy for better management. Table 1 revealed that after 16 weeks of treatment with *P. santalinus* subjects showed very good activity, which was comparable to the groups that were receiving vitamin E individually. Highly significant results were obtained by using a combination of *P. santalinus* and vitamin E in diabetic–treated groups.

Glucose tolerance tests after 16 weeks of treatment revealed that a combination of vitamin E and *P. santalinus* were highly effective. The percentage drops of blood glucose level by combination of vitamin E with *P. santalinus* was 72.4%. Table 3, serum lipid profile of diabetic rats before and after 16 weeks of treatment, demonstrated that the extract lowered TC and LDL/HDL-C significantly.

Morphologically, the development of Diabetic Nephropathy is characterized by progressive thickening of the glomerular basement membrane and by expansion of the mesangial matrix which correlates to glomerular filtration function. Besides the classical clinical chemical parameters for evaluation of renal function, the measurement of urinary albumin excretion is now widely used for detection of developing diabetic nephropathy. Since diabetes causes glomerular and tubular changes, tubular marker proteins may be used to detect early renal damage (Lehmann and Schleicher, 2000).

The results also revealed that aqueous extract of *P. santalinus* and vitamin E treatment in combination for 16 weeks reduced LPO, increased SOD, CAT, GPX and GST significantly. Significant improvement in body mass, muscle, heart, kidney, and liver size was recorded after 16 weeks of combined treatment with vitamin E and *P. santalinus*. Combination of aqueous extract of *P. santalinus* and vitamin E also exhibited improved total protein and hemoglobin blood levels. Simultaneously, it also reduced glycosylated hemoglobin A1C levels significantly. Malondialdehyde levels in brain, liver and muscle tissue of diabetic rats was found to be significantly reduced after 16 weeks of treatment with *P. santalinus* and vitamin E. *P. santalinus* and vitamin E-treated diabetic subjects showed reduced concentrations of urea, albumin, and serum creatinine in urine after 16 weeks of treatment. Histomorphologically, the glomeruli showed significant increases in mesangial matrix. There was thickening of glomerular basement membrane and Bowman capsule (as shown in Figure 1). Following 16 weeks treatment with *P. santalinus* and vitamin E combination the glomeruli showed decrease in mesangial matrix. The glomerular basement membrane and Bowman capsule appeared as shown in Figures 2 and 3).

DISCUSSION

The pathogenesis of type 2 Diabetes Mellitus involves insulin resistance, as well as insulin secretion from the pancreatic β cell. Diabetic Nephropathy is a major complication of type 1 and type 2 diabetes result of end – stage renal disease. Diabetic Nephropathy is the most frequent cause of terminal kidney failure in developed

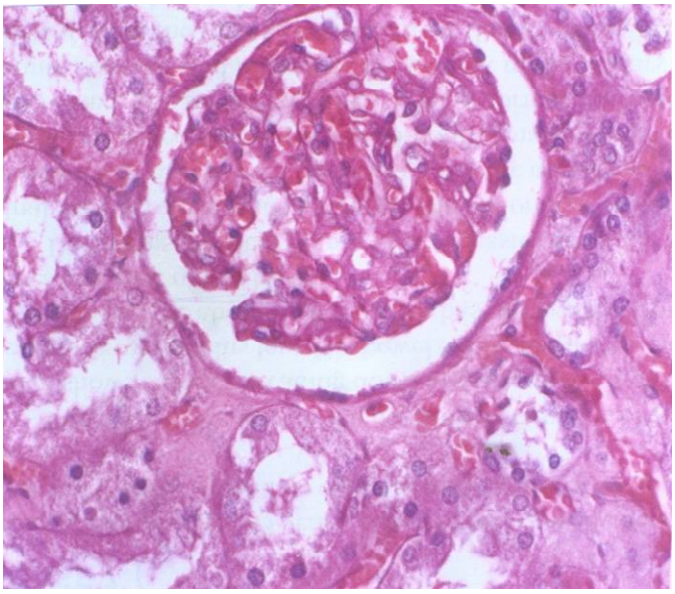

Figure 2. Decrease in mesangial matrix and thickness of capsule following treatment of Pterocarpus santalinus L and vitamin E.

countries. The manifestation of Diabetic Nephropathy is associated with a poor prognosis of affected patients. (Nawroth, 2010). It is a major health concern in both ruraland urban populations of the Indian subcontinent (Basavanagowdapa et al., 2005).

Although, pathologic classifications exist for several renal diseases, including nephropathy, focal segmental glomerulosclerosis Diabetic Nephropathy is lacking, (Terrvaert et al., 2010). Four major molecular mechanisms have been implicated in hyperglycemia–mediated vascular damage, including increased polyol pathway flux, hexosamine pathway flux, advanced glycation end product (AGE) formation and activation of protein kinase C. In a recent study, antiglycation activity was positively correlated with total phenolic content and free radical scavenging activity and reduced malonyldiadehyde levels (after oxidation of low-density lipoprotein) while simultaneously being negatively correlated with a time lag during the formation of conjugated dienes (Cory, 2010). Development of novel therapeutic agents inhibiting the aforementioned factors is of particular interest as they represent potential treatments for the prevention of diabetic complications (Harbilas, 2009). Several clinical trials and studies have shown that improved glycemic control is strongly associated with decreased development or regression of diabetic complications in both type I and II DM and glomerulosclerosis with other clinical or pathologic evidence that sclerosis is attributable to Diabetic Nephropathy (Surya, 2010). However, reports show the beneficial effect of bodyweight reduction, diet and physical activity changes on high glomerular filtration rates. The rate also showed improvement in parameters like bodyweight and lipid profiles and histopathologies. The body mass reduction stops the events caused by glomerular hyperfiltration and its eventual and possible progression toward renal damage (Plamen, 2009).

Scientists report that aboriginal communities in Canada, such as the Cree Nation of Eeyou Istchee, have been identified as a population that falls in the high-risk category. Limited treatment options are constrained by their culture, specifically concerning complications resulting in peripheral neuropathy (Cory, 2008). Progression of complications with diabetes is now primarily considered to be the manifestation of oxidative stress, which plays a major role in the end organ complications of DM (Baynes and Thorpe, 1999; DeAngelis, 2000). Diabetic Nephropathy is a major complication of DM along with the lethal tetrad of diabetes: hyperglycemia, dyslipidemia, oxidative stress and endothelial dysfunction (Yu and Lyons, 2005). Accordingly, strategies to reduce oxidative stress in DM may exert favorable effects on the progression of DM (Bhor et al., 2004; Vasavada, 2005). Nephropathy could be prevented via the decrease of oxidative stress by *P. santalinus* and vitamin E (α-tocopherol), which has been shown to ameliorate diabetic nephropathy. Vitamin E may also induce reduction in oxidative stress, Tumour Growth Factor (TGF) modulation and protein kinase-C modulation. Vitamin E has also been shown to reduce

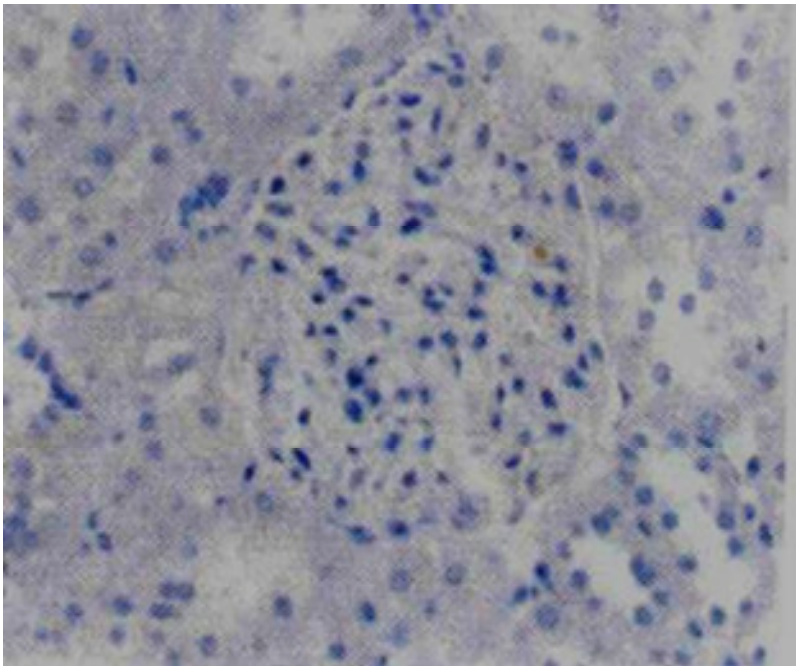

Figure 3. Decrease in mesangial matrix and thickness of capsule following treatment of Pterocarpus santalinus L and vitamin E in same animals.

somatic insulin resistance (Patrick et al., 2004 and Marles et al., 1995).

STZ-induced DM was characterized not only by impaired glucose tolerance and hyperglycemia but also by low antioxidant activity. The PS has strong antioxidant activity which has been demonstrated via different *in vitro* assays, as well as by using liver-slice slides. Although the β-cell cytotoxic effect of STZ-induction is not fully understood, it is thought to result in the inhibition of free radical scavenger–enzyme production. Oxidative stress has been shown to be responsible, at least in part, for tissue damage and β-cell dysfunction (Kröncke et al., 1995).

Extensive research has been done on this combination as an antidiabetic agent; however, no work of the present kind has ever been reported (Maheswari et al., 1980). We observed an increase in HDL and reduction in LDL, TC has observed in combination of vitamin E and *P. santalinus*, compared to when *P. santalinus* and vitamin E were administered separately to each group of subjects. Body, muscle, heart, kidney and liver weight were all seen to benefit from the combinational approach. Weight loss due to excessive breakdown of tissue proteins is also a complication of Diabetes Mellitus (Chatterjea and Shinde, 2002). Weight loss in treated groups either with *P. santalinus* or vitamin E alone or in combination was less significant, as compared to the untreated-diabetic group, which lost a considerable amount of weight. The combined treatment with *P. santalinus* and vitamin E showed promising results. We observed an improvement of body weight, lipid profile, glucose tolerance, biochemical parameters such as urea, creatinine and histological reversal of the nephropathy and activity of antioxidant enzymes. These results are most likely the result of the combination of antidiabetic activity of *P. santalinus* and the antioxidant action of vitamin E, which is responsible for the potentiating effect of this combination. From this, it is evident that body weight and reduced blood glucose level can be improved. The aqueous extract of *P. santalinus* to have beneficial effects in animals as well as in humans. The aqueous extract was also studied for the possible management of metabolic syndrome.

Oxidative stress has been implicated in the pathology of many diseases and conditions, including diabetes and cardiovascular disease. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease (Youdim, 2001). Many indigenous peoples have long employed the use of these products for treatment of diabetes, demonstrated by the low incidences of diabetic disease in their populations. Treatments such as these – if properly standardized –may have limitless potential for world-wide

application (Letitia et al., 2002). To date, over 1200 antidiabetic plant species used around the world have been identified for the treatment of type 2 diabetes (T2D) symptoms, with more than 80% of 295 species reviewed, showing strong activity in hypoglycaemic analyses.

In DM, morphologically, the glomeruli showed significant increases in mesangial matrix. This phenomenon is observed in Figures 1 to 3. There is a thickening of the glomerular basement membrane and Bowman capsule. Following 16 weeks treatment with *P. santalinus* and vitamin E in combination, the glomeruli showed decrease in mesangial matrix and reduction in glomerular basement membrane thickness (Figures 2 and 3).

Conclusion

The progression of DM in the subjects is largely attributed to free radical generation. The impacts of these free radicals are observed as lipid peroxidation and its resulting complications. In the diabetic subjects, the plasma and tissue lipid peroxidation products exhibited significantly higher concentrations than in the diabetic-treated groups. The levels of MDA – the end products of lipid peroxidation – were lower in the subjects treated with Pterocarpus santalinus which can be attributed to an increase in superoxide dismutase activity, preventing free radical activity. The primary free radicals effecting the observed lipid peroxidation are superoxide (O_2^-), hydroxyl (OH), and peroxyl (LOO). These free radicals may all play a role in DNA damage, glycation, protein modification reactions and in lipid oxidation modification in DM (Hunt et al., 1990).

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