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RESEARCH ARTICLE

Antihyperglycemic and antihyperlipidemic effect of *Santalum album* in streptozotocin induced diabetic rats

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Abstract

Context: *Santalum album* Linn (Santalaceae), commonly known as Sandalwood is used traditionally for its antihyperlipidemic and diuretic activity. *Objective*: This study investigated the antihyperglycemic and antihyperlipidemic effect of long-term oral administration of the *Santalum album* pet ether fraction in streptozotocin induced diabetic rats. *Materials and methods*: Diabetes was induced by a single intraperitoneal injection of streptozotocin at 70 mg/kg body weight. Rats were treated with *Santalum album* pet ether fraction orally at a dose of 10 µg/ kg body weight twice daily for 60 days. Metformin (30 mg/kg body weight) was used as positive control. Lipid profile and glycated hemoglobin were estimated. HPLC profiling of *Santalum album* pet ether fraction was carried out. *Result and discussion*: Treatment of diabetic rats for 60 days demonstrated reduction in blood glucose level by 140 mg/dl. Metformin treated group showed a decrease in blood glucose by 70 mg/dl, as against an increase in diabetic control group by 125 mg/dl. Total cholesterol (TC), low density lipoprotein (LDL) and triglyceride (TG) levels were decreased by 22, 31 and 44%, respectively, in treated diabetic rats whereas, cardioprotective, high density lipoprotein (HDL) increased by 46%. In case of metformin, the values were 11, 29 and 15% respectively, while HDL increased by 7%. Significant improvement in atherogenic index from 267 to 139% was observed in treated rats. *Conclusion*: *Santalum album* pet ether fraction has potential antihyperlipidemic activity that can help in overcoming insulin resistance.

Keywords: Antidiabetic; metformin; atherogenic index

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is frequently associated with obesity (Roden et al., 1996). Plasma glucose levels increased in uncontrolled type 2 diabetes, along with increased plasma free fatty acids (FFAs) and altered lipid profile has long been recognized (Schalch & Kipnis, 1965). Therapeutic strategies with monotherapy or combination therapy have targeted these metabolic defects individually or in concert (Zangeneh et al., 2003). It includes insulin secretagogue, insulin sensitizers, insulin mimetic drugs and various enzyme inhibitors. Metformin, an insulin sensitizer obtained from *Galega officinalis* Linn. (Fabaceae) decreases blood glucose in streptozotocin induced diabetic rats through enhancement in β-endorphin secretion, decrease in the hepatic glucose production and reduces peripheral insulin resistance (Cheng et al., 2006). Several other plants also have been documented to possess good antihyperglycemic and antihyperlipidemic activity (Kasetti & Rajasekhar, 2010; Gupta et al., 2009; Bavarva & Narasimhacharya, 2008).

Santalum album Linn. (Santalaceae) is a mid-sized evergreen tree widely distributed in Indian subcontinent, Malaysia, and Australia; it is commonly known as sandalwood. The essential oil of sandalwood is usually prepared by steam distillation from chips and billets cut from the heartwood and are used in perfumes, cosmetics, and sacred unguents. Sandalwood oil has various biological activities, such as antiviral and chemopreventive effects (Kim et al., 2005). The analysis of odor components in East Indian Sandalwood oil (*Santalum album*) resulted in the identification of α-santalene, α-santalal, β-santalal, epi-β-santalal, α-santalol, β-santalol, (E)-β-santalol, α-bergamotol

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and spirosantalol (Nikiforov et al., 1988). Sandalwood and its oil has a long history of use without any reported adverse effects, therefore consumption of sandalwood oil as an added food ingredient is considered safe at present use levels (Burdock & Carabin, 2008). Diabetes is associated with a burning sensation of the hands and feet (Boulton et al., 2005). Traditionally sandalwood extracts are used as coolants to alleviate such symptoms, hence it was attempted to study the antidiabetic effect of *Santalum album*. The present study evaluates the antihyperglycemic and antihyperlipidemic activity of *Santalum album* on streptozotocin-induced diabetic rats.

Materials and methods

Chemicals

Streptozotocin and metformin were purchased from Sigma Chemicals, USA. Blood glucose was determined by standard glucometer (ACCUE CHECK active). Glycated hemoglobin (Hb) was measured from whole blood by using ion exchange resin kit purchased from Crest Biosystems, Goa, India. Insulin estimation using insulin Elisa kit, Calbio, Green Valley USA. Total triglycerides, high density lipoprotein (HDL) and total cholesterol kit were purchased from Biolab diagnostics, India, and very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated from total cholesterol and HDL cholesterol values.

Plant material

The plant material was obtained locally and verified by the Department of Botany, Shivaji University, Kolhapur. The plant material was powdered and 10 g powder was subjected to steam distillation followed by solvent extraction with petroleum ether. Pet ether is then evaporated by and residue was weighed. The yield of dry weight of fraction was 0.114 g (1.14%). The pet ether fraction was stored at 4°C temperature and used for further studies.

Experimental animals

Male Wistar rats weighing about 190–200 g were used in the experiment. All the animals were maintained under laboratory conditions and were allowed access to food (pellet) (Amruth, Pune) and water *ad libitum*. Experiments were carried out according to the guidelines of animal ethical committee of the institute and CPCSEA (Registration no. 233/CPCSEA).

Induction of diabetes

Diabetes was induced by single intra-peritoneal injection of freshly prepared streptozotocin (70 mg/kg) in 0.1 M citrate buffer (pH 4.5) (Patil et al., 2011). After 14 days, blood was collected from rat tail puncture and the glucose level of each rat was determined. Rats with fasting blood glucose range of 240–350 mg/dl were considered diabetic and included in the study.

Oral glucose tolerance test (OGTT)

OGTT was carried out by modification of Adolfo Andrade-Cetto et al., (2005) method. After 12 h fasting, a 0 min blood sample was taken from rat tail; *Santalum album* pet ether fraction 10 µg/kg and 20 µg/kg, and metformin 30 mg/kg were given orally and after a gap of 10 min, glucose solution (3 mg/g body weight) was administered orally. Blood samples at intervals of 30 min for 120 min were taken and blood glucose levels were estimated. The dose showing optimum activity was used for long-term experiments.

Experimental design and treatment schedule

The diabetic rats were divided into three groups of six rats each as diabetic control, *Santalum album* pet ether fraction treated, and metformin treated, while one group of rats was considered as normal control. The normal and diabetic controls were administered 0.5% dimethyl sulphoxide (DMSO) orally as a vehicle, while 10 µg/kg body weight of *Santalum album* petroleum ether fraction and 30 mg/kg body weight of metformin dissolved in 0.5% dimethyl sulphoxide (DMSO) was administered twice daily to the diabetic rats for a period of 60 days. The body weight was recorded weekly. After 60 days treatment rats were sacrificed and blood was collected by cardiac puncture for measurement of different parameters.

Determination of serum parameters

Blood glucose was determined by glucose oxidase-peroxidase method. Glycated hemoglobin was measured from whole blood by using ion exchange method. 25 µL serum was used for insulin estimation by ELISA method. Results were compared with standard calibrator. Total triglycerides, HDL and total cholesterol were measured by diagnostic kit. VLDL and LDL were calculated from total cholesterol and HDL cholesterol values.

Atherogenic index

The atherogenic index serum (AIS) which is the measure of the extent of atherosclerotic lesions based on serum lipids is determined in all four groups. The atherogenic index is calculated using the formula AIS = TC/HDL (Balogun & Adebayo, 2007).

Statistical analysis

All the data obtained were expressed as mean ± SD. The differences of the means of the data between the test groups and diabetic control group were all analyzed statistically using ANOVA. Values of $p \leq 0.05$ were taken to imply statistical significance.

HPLC Profiling of Santalum album pet ether fraction

HPLC analysis was carried out (Water Model no. 2690) on C 8 Column (Symmetry 4.6 mm × 250 mm) by isocratic method with 10 min run time. The mobile phase was methanol with flow rate of 1 ml/min using UV detector (270 nm); 10 µl sample was manually injected.

Results

Oral glucose tolerance test of Santalum album pet ether fraction and metformin in streptozotocin-induced diabetic rats

Figure 1 shows effect of *Santalum album* pet ether fraction and metformin on blood glucose levels in oral glucose tolerance test. There was no reduction in the blood glucose level in diabetic control. It remains high from 320 to 550 mg/dl with 3 mg/g glucose load after 2 h. Dose of *Santalum album* oil 10 µg/ kg body weight changes blood glucose level from 286 to 330 mg/dl whereas dose of 20 µg/ kg body weight blood glucose level changed from 270 to 378 mg/dl. Although the blood glucose value was not reduced, it was less than the diabetic control values at the end of 2 h suggesting a glucose lowering action. Metformin reduces blood glucose from 353 to 265 mg/dl.

Effect of Santalum album pet ether fraction and metformin on body weight, blood glucose level, and glycated hemoglobin in diabetic rats

Table 1 shows that during the first two weeks, there was no significant difference in the average weights of the control and diabetic rats. However, later on, both the diabetic controls and metformin treated rats showed reduction in body weight. The body weight of diabetic rats was significantly reduced in these animals through out 7 weeks (Komolafe et al., 2009). A continuous increase in the body weight was seen in normal rats. The *Santalum album* pet ether fraction treated rats showed a weight loss for the first three weeks, but later demonstrated a slow gain in the body weight.

As regards the blood glucose levels it was found to decrease from 280 ± 16.5 to 140.5 ± 15.8 mg/dl in *Santalum album* pet ether fraction treated rats. Metformin treated

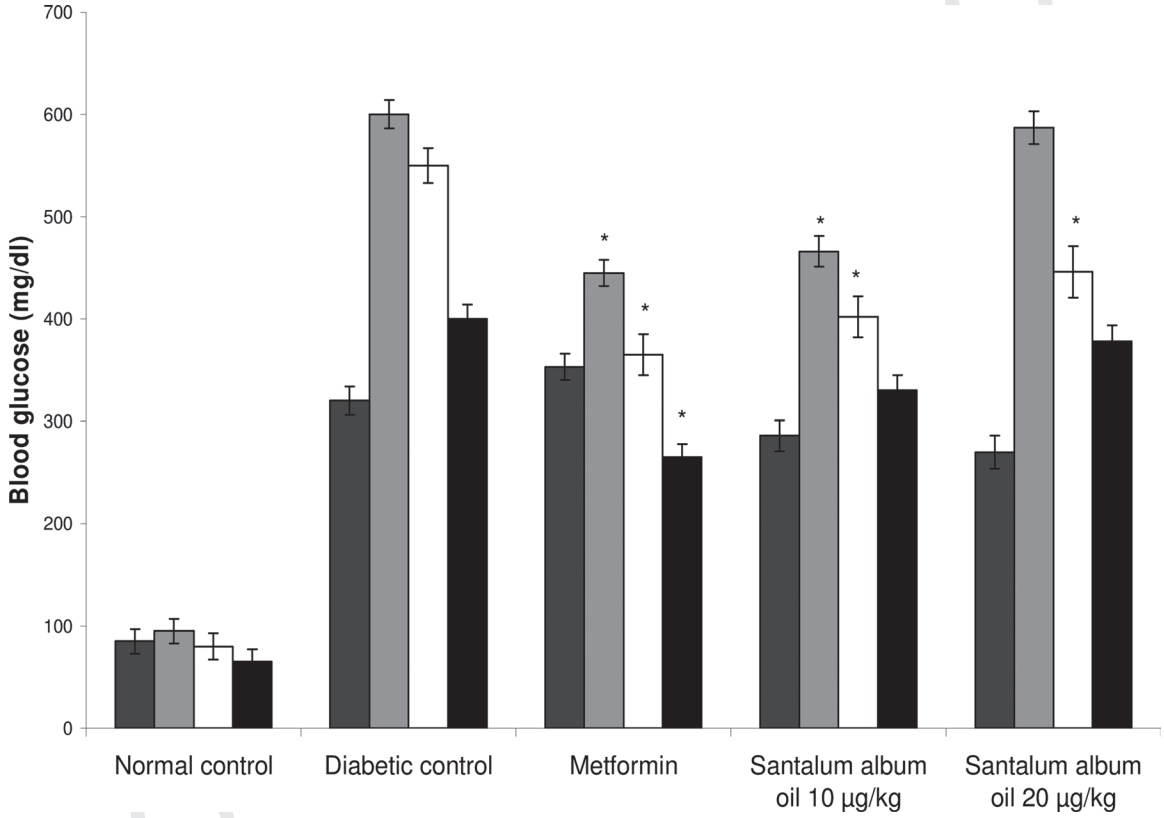

Figure 1. Oral Glucose tolerance test of *Santalum album* oil and metformin in streptozotocin induced diabetic rats.

Table 1. Effect of *Santalum album* oil and metformin on body weight during treatment for 60 days.

	Weight in grams			
Weeks	Normal control	Diabetic control	Diabetic + *Santalum album* oil 10 µg/kg	Diabetic + metformin 30 mg /kg
Week 1	200 ± 20	200 ± 20	200 ± 15	200 ± 20
Week 2	240 ± 15	180 ± 18	190 ± 17	180 ± 15
Week 3	270 ± 20	170 ± 15	180 ± 20	160 ± 17
Week 4	310 ± 17	150 ± 10	150 ± 16	160 ± 10**
Week 5	330 ± 14	150 ± 20	160 ± 18**	140 ± 20
Week 6	350 ± 20	130 ± 15	170 ± 20**	140 ± 15*
Week 7	370 ± 16	120 ± 17	180 ± 20**	130 ± 10*

Values are mean ± SD; $n = 6$. *The difference between treated and diabetic control values is significant at $P < 0.05$, ** The difference between treated and diabetic control values is significant at $P < 0.005$.

group showed decrease in blood glucose from 345 ± 12.5 to 285 ± 20.5 mg/dl. In diabetic control group, blood glucose level increased by about 85 mg/dl after 60 days. The level of Hb glycation was increased in the diabetic control group. *Santalum album* pet ether fraction treated group showed significant reduction in glycated Hb level. Comparatively the metformin treated group did not show much effect on glycated Hb (Table 2).

Effect of Santalum album pet ether fraction and metformin on serum lipids in diabetic rats

Table 3 demonstrates effect of *Santalum album* pet ether fraction and metformin on serum lipids in diabetic rats.

Table 2. *In vivo* effect of *Santalum album* oil and metformin on Glycosylated Hb and blood glucose level after treatment for 60 days.

Sr. No.	Groups	Glycosylated Hb in %	Blood glucose (mg/dL)
1.	Normal control	05.8 ± 0.3	80 ± 5.3
2.	Diabetic control	13.4 ± 0.4	323.7 ± 18.6
3.	Diabetic + *Santalum album* oil(10 µg/kg)	08.3 ± 0.2*	140.5 ± 15.8*
4.	Diabetic + Metformin (30 mg/kg)	15.6 ± 0.8*	285 ± 20.5*

Values are mean ± SD; $n = 6$. *The difference between treated and diabetic control values is significant at $P < 0.005$.

Santalum album pet ether fraction treatment showed improvement in serum lipid profile in hyperglycemic rats, reducing triglyceride, total cholesterol, and increasing HDL by 46%. The values are comparable to the normal controls. However in case of the diabetic controls there was gross increase in the triglyceride and LDL levels, while HDL was found to decrease. Metformin treatment led to a marginal improvement in the lipid profile. The overall atherogenic index was therefore found to improve significantly in the Santalum pet ether fraction treated animals. It can be seen that plasma insulin content did not improve both in case of metformin as well as S*antalum album* treated animals.

HPLC Profiling of Santalum album pet ether fraction

HPLC profile of *Santalum album* pet ether fraction shows major peak at 2.882 min with four minor peaks at 1.916, 3.35, 3.627, 3.748 min (Figure 2).

Discussion

It can be observed that while treatment with *Santalum album* pet ether fraction does not show significant reduction in the blood glucose values in an OGTT, prolonged treatment for 60 days showed a significant lowering of the blood glucose level. Glycated hemoglobin is known to

Table 3. *In vivo* effect of *Santalum album* oil and metformin on serum lipid profile level after treatment for 60 days.

	Normal rat	Diabetic rat	Diabetic+ *Santalum album* oil (10 µg / kg twice daily)	% Change (compare to diabetic rats)	Diabetic + metformin 30 mg/kg twice daily	% Change(compare to diabetic rats)
Total cholesterol(mg/dl)	70.93 ± 3.5	75.30 ± 2.6	58.15 ± 3.2*	↓ 22	67.40 ± 3.0*	↓ 11
Total triglyceride (mg/dl)	73.53 ± 10.2	157.33 ± 11.2	89.48 ± 2.4*	↓ 44	133.34 ± 4.0*	↓ 15
HDL (mg/dl)	53.0 ± 3.3	28.20 ± 2.7	41.69 ± 5.2*	↑ 18	30.31 ± 0.10	↑ 7
LDL (mg/dl)	14.73 ± 0.3	28.50 ± 4.5	19.56 ± 2.2*	↓ 43	20.14 ± 1.5*	↓ 29
VLDL (mg/dl)	14.70 ± 2.0	31.47 ± 2.2	17.89 ± 1.7*	↓ 43	30.73 ± 1.1	↓ 2
Plasma Insulin (µIU)	12.50 ± 1.5	4.50 ± 2.0	6.00 ± 1.3	↑ 33	5.4 ± 0.3	↑ 20
Atherogenic index (%)	133	267	139	↓ 36	222	↑ 2

Values are mean ± SD; $n = 6$. * The difference between treated and diabetic control values is significant at $P < 0.005$.

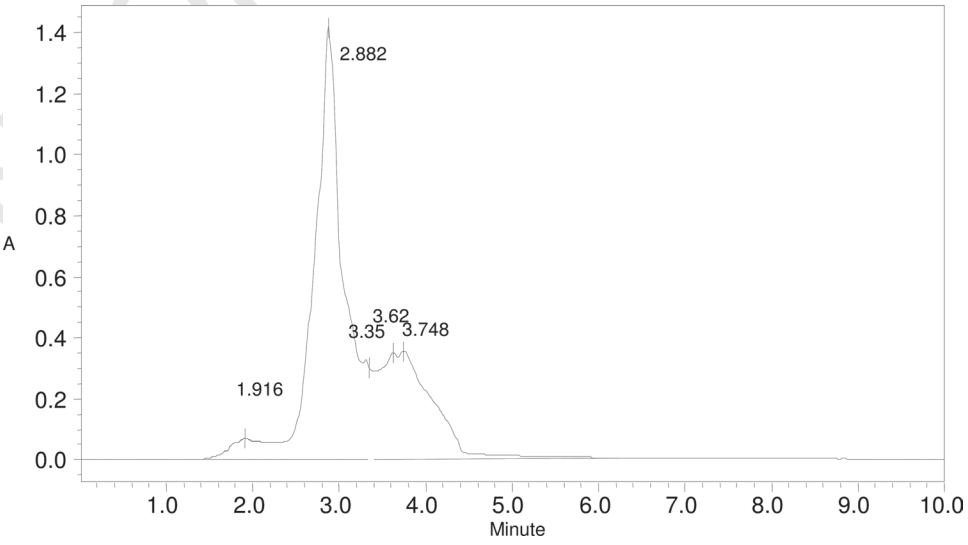

Figure 2. Reverse phase HPLC profiling of *Santalum album* pet ether fraction at 270 nm.

increase in patients with diabetes mellitus (Koenig et al., 1976), and the increase has been found to be directly proportional to the fasting blood glucose level (Jackson et al., 1979). The significant reduction in glycated hemoglobin of *Santalum album* pet ether fraction treated diabetic rats indicates its efficiency in glycaemic control. No significant increase in the levels of insulin has been observed compared to diabetic control in case of *Santalum album* pet ether fraction and metformin treated groups.

Premature and extensive arteriosclerosis involving renal, peripheral, and cardiovascular vessels remain the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes increasing the risk of coronary heart disease. A reduction in serum cholesterol, triglycerides and VLDL fractions should be considered as being beneficial in long term prognosis of patients (Yadav et al., 2008). It is widely held that excessive lipid metabolism, achieved either by high-fat feeding or intravenous infusion of lipid emulsion to raise circulating free fatty acid (FFA) levels, leads to decreased insulin stimulated glucose uptake (Hevener et al., 2002). Elevation in the plasma lipid content is known to cause insulin resistance by inhibition of glucose transport and its phosphorylation, which leads to NIDDM (Roden et al., 1996). It was observed in the present study that the dose of 10 μg/kg body weight of the extract lowered the blood glucose, TC, TG, and LDL levels significantly and enhanced the cardio protective lipid HDL after 60 days of treatment. This would definitely reduce the incidence of coronary events (Lipid Research Clinics Programs, 1984). Similar observations are reported for several plant components. Eremanthin isolated from *Costus speciosus* was shown to act as an antihyperglycemic and antilipidemic at 10 and 20 mg/kg (Eliza et al., 2009). Likewise, the aqueous extract of *Aegle marmelos* seeds at 250 mg/kg (Kesari et al., 2006) has demonstrated similar results to our observations. However in both these studies as well as in several other plants the dosage generally varies from 5–500 mg/kg as against the 10 μg/kg dose of pet ether extract used in the present study demonstrating the potency of *Santalum album*. Metformin has been reported to have beneficial effects on circulating lipids linked to increased cardiovascular risk (Silveira et al., 2008). However, in spite of being a purified drug the effect of *Santalum album* pet ether extract was better.

Atherogenic index has been proposed as a marker of plasma atherogenecity as it is found to be enhanced in patients suffering from cardiovascular disorders (**Tan** et al., 2004). The higher the atherogenic index the bigger the risk for organs such as the heart, liver and kidney for oxidative damage (Subramaniam et al., 2010). Atherogenic index was significantly reduced in *Santalum album* pet ether fraction treated rats compared with diabetic control. It reduced from 267 to 139%. Although metformin showed reduction in atherogenic index changes from 267 to 222% it was not as effective as *Santalum album* extract. Reduction in fat accumulation is known to prevent beta cell apoptosis and thereby prevent NIDDM (Shimabukuro et al., 1998). HPLC profile of *Santalum album* pet ether fraction showed major peak at 2.882 min. which may be a mixture of two primary sesquiterpene alcohols, α-santalol and β-santalol which is about 90% of oil fraction (Sindhu et al., 2010).

In conclusion *Santalum album* pet ether fraction at a very low concentration of 10 μg/kg body weight can effectively improve the lipid profile in diabetic rats. The improvement in the atherogenic index is even better than metformin at 30 mg/kg body weight, lowering the risk of cardiovascular damage in diabetics. Although the result from these studies did not demonstrate immediate lowering of blood glucose, as in case of a secretagogue or insulin action, prolonged treatment appears to alleviate diabetes through lowering of blood glucose and an improved glycated hemoglobin level. It is likely that this effect could be due to its lipid lowering action that could prevent development of insulin resistance and allow better glycemic control through existing insulin content and preventing further damage to the beta cells.

***Declaration of interest*:**.The authors declare that they have no conflicts of interest to disclose.

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Research Article

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Antihyperglycemic and antihyperlipidemic effect of *Santalum album* in streptozotocin induced diabetic rats

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Abstract

Context: *Santalum album* Linn (Santalaceae), commonly known as Sandalwood is used traditionally for its antihyperlipidemic and diuretic activity. *Objective*: This study investigated the antihyperglycemic and antihyperlipidemic effect of long--term oral administration of the *Santalum album* pet ether fraction in streptozotocin induced diabetic rats.

Materials and methods: Diabetes was induced by a single intraperitoneal injection of streptozotocin at 70 mg/kg body weight. Rats were treated with *Santalum album* pet ether fraction orally at a dose of 10 µg/ kg body weight twice daily for 60 days. Metformin (30 mg/kg body weight) was used as positive control. Lipid profile and glycated h~~a~~emoglobin were estimated. HPLC profiling of *Santalum album* pet ether fraction was carried out. *Result and discussion*: Treatment of diabetic rats for 60 days demonstrated reduction in blood glucose level by

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140 mg/dl. Metformin treated group showed a decrease in blood glucose by 70 mg/dl, as against an increase in diabetic control group by 125 mg/dl. Total cholesterol (TC), low density lipoprotein (LDL) and triglyceride (TG) levels were decreased by 22, 31 and 44%, respectively, in treated diabetic rats whereas, cardioprotective, high density lipoprotein (HDL) increased by 46%. In case of metformin, the values were 11, 29 and 15% respectively, while HDL increased by 7%. Significant improvement in atherogenic index from 267 to 139% was observed in treated rats.

Conclusion: *Santalum album* pet ether fraction has potential antihyperlipidemic activity that can help in overcoming insulin resistance.

Keywords: ~~antidiabetic~~Antidiabetic,~~,~~ metformin,~~,~~ atherogenic index~~,~~

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is frequently associated with obesity (Roden et al., 1996). Plasma glucose levels increased in uncontrolled type 2 diabetes, along with increased plasma free fatty acids (FFAs) and altered lipid profile has long been recognized (Schalch & Kipnis, 1965). Therapeutic strategies with monotherapy or combination therapy have targeted these metabolic defects individually or in concert (Zangeneh et al., 2003). It includes insulin secretagogue, insulin sensitizers, insulin mimetic drugs and various enzyme inhibitors. Metformin, an insulin sensitizer obtained from *Galega officinalis* Linn. (Fabaceae) decreases blood glucose in streptozotocin induced diabetic rats through enhancement in β-endorphin secretion, decrease in the hepatic glucose production and reduces peripheral insulin resistance (Cheng et al., 2006). Several other plants also have been documented to possess good antihyperglycemic and antihyperlipidemic activity (Kasetti & Rajasekhar, 2010; Gupta et al., 2009; Bavarva & Narasimhacharya, 2008).

Santalum album Linn. (Santalaceae) is a mid-sized evergreen tree widely distributed in Indian subcontinent, Malaysia, and Australia; it is commonly known as sandalwood. The essential oil of sandalwood is usually prepared by steam distillation from chips and billets cut from the heartwood and are used in perfumes, cosmetics, and sacred unguents. Sandalwood oil has various biological activities, such as antiviral and chemopreventive effects (Kim et al., 2005). The analysis of odor components in East Indian Sandalwood oil (*Santalum album*) resulted in the identification of α-santalene, α-santalal, β-santalal, epi-β-santalal, α-santalol, β-santalol, (E)-β-santalol, α-bergamotol and spirosantalol (Nikiforov et al., 1988). Sandalwood and its oil has a long history of use without any reported adverse effects, therefore consumption of sandalwood oil as an added food ingredient is considered safe at present use levels (Burdock & Carabin, 2008). Diabetes is associated with a burning sensation of the hands and feet (Boulton et al., 2005). Traditionally sandalwood extracts are used as coolants to alleviate such symptoms, hence it was attempted to study the antidiabetic effect of *Santalum album*. The present study evaluates the antihyperglycemic and antihyperlipidemic activity of *Santalum album* on streptozotocin-induced diabetic rats.

Materials and methods

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Chemicals

Streptozotocin and metformin were purchased from Sigma Chemicals, USA. Blood glucose was determined by standard glucometer (ACCUE CHECK active). Glycated hemoglobin (Hb) was measured from whole blood by using ion exchange resin kit purchased from Crest Biosystems, Goa, India. Insulin estimation using insulin Elisa kit, Calbio, Green Valley USA. Total triglycerides, high density lipoprotein (HDL) and total cholesterol kit were purchased from Biolab diagnostics, India, and very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated from total cholesterol and HDL cholesterol values.

Plant material

The plant material was obtained locally and verified by the Department of Botany, Shivaji University, Kolhapur. The plant material was powdered and 10 g powder was subjected to steam distillation followed by solvent extraction with petroleum ether. Pet ether is then evaporated by and residue was weighed. The yield of dry weight of fraction was 0.114 g (1.14%). The pet ether fraction was stored at 4°C temperature and used for further studies.

Experimental animals

Male Wistar rats weighing about 190–200 g were used in the experiment. All the animals were maintained under laboratory conditions and were allowed access to food (pellet) (Amruth, Pune) and water *ad libitum*. Experiments were carried out according to the guidelines of animal ethical committee of the institute and CPCSEA (Registration no. 233/CPCSEA).

Induction of diabetes

Diabetes was induced by single intra-peritoneal injection of freshly prepared streptozotocin (70 mg/kg) in 0.1 M citrate buffer (pH 4.5) (Patil et al., 2011). After 14 days, blood was collected from rat tail puncture and the glucose level of each rat was determined. Rats with fasting blood glucose range of 240–350 mg/dl were considered diabetic and included in the study.

Oral glucose tolerance test (OGTT)

OGTT was carried out by modification of Adolfo Andrade-Cetto et al., (2005) method. After 12 h fasting, a 0 min blood sample was taken from rat tail; *Santalum album* pet ether fraction 10 µg/kg and 20 µg/kg, and metformin 30 mg/kg were given orally and after a gap of 10 min, glucose solution (3 mg/g body weight) was administered orally. Blood samples at intervals of 30 min for 120 min were taken and blood glucose levels were estimated. The dose showing optimum activity was used for long--term experiments.

Experimental design and treatment schedule

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The diabetic rats were divided into three groups of six rats each as diabetic control, *Santalum album* pet ether fraction treated, and metformin treated, while one group of rats was considered as normal control. The normal and diabetic controls were administered 0.5% dimethyl sulphoxide (DMSO) orally as a vehicle, while 10 µg/kg body weight of *Santalum album* petroleum ether fraction and 30 mg/kg body weight of metformin dissolved in 0.5% dimethyl sulphoxide (DMSO) was administered twice daily to the diabetic rats for a period of 60 days. The body weight was recorded weekly. After 60 days treatment rats were sacrificed and blood was collected by cardiac puncture for measurement of different parameters.

Determination of serum parameters

Blood glucose was determined by glucose oxidase-peroxidase method. Glycated hemoglobin was measured from whole blood by using ion exchange method. 25 µL serum was used for insulin estimation by ELISA method. Results were compared with standard calibrator. Total triglycerides, HDL and total cholesterol were measured by diagnostic kit. VLDL and LDL were calculated from total cholesterol and HDL cholesterol values.

Atherogenic index

The atherogenic index serum (AIS) which is the measure of the extent of atherosclerotic lesions based on serum lipids is determined in all four groups. The atherogenic index is calculated using the formula AIS = TC/HDL (Balogun & Adebayo, 2007).

Statistical analysis

All the data obtained were expressed as mean ± SD. The differences of the means of the data between the test groups and diabetic control group were all analyzed statistically using ANOVA. Values of $p \leq 0.05$ were taken to imply statistical significance.

HPLC Profiling of *Santalum album* pet ether fraction

HPLC analysis was carried out (Water Model no. 2690) on C 8 Column (Symmetry 4.6 mm ~~x~~ x 250 mm) by isocratic method with 10 min run time. The mobile phase was methanol with flow rate of 1 ml/min using UV detector (270 nm); 10 µl sample was manually injected.

Results

Oral glucose tolerance test of *Santalum album* pet ether fraction and metformin in streptozotocin-induced diabetic rats

Figure 1 –shows effect of *Santalum album* pet ether fraction and metformin on blood glucose levels in oral glucose tolerance test. There was no reduction in the blood glucose level in diabetic control. It remains high from 320 to 550 mg/dl with 3 mg/g glucose load after 2 h. Dose of *Santalum album* oil 10 µg/ kg body weight

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changes blood glucose level from 286 to 330 mg/dl whereas dose of 20 µg/ kg body weight blood glucose level changed from 270 to 378 mg/dl. Although the blood glucose value was not reduced, it was less than the diabetic control values at the end of 2 h suggesting a glucose lowering action. Metformin reduces blood glucose from 353 to 265 mg/dl.

Effect of *Santalum album* pet ether fraction and metformin on body weight, blood glucose level, and glycated haemoglobin in diabetic rats

Table 1 shows that during the first two weeks, there was no significant difference in the average weights of the control and diabetic rats. However, later on, both the diabetic controls and metformin treated rats showed reduction in body weight. The body weight of diabetic rats was significantly reduced in these animals through out 7 weeks (Komolafe et al., 2009). A continuous increase in the body weight was seen in normal rats. The *Santalum album* pet ether fraction treated rats showed a weight loss for the first three weeks, but later demonstrated a slow gain in the body weight.

As regards the blood glucose levels it was found to decrease from 280 ± 16.5 to 140.5 ± 15.8 mg/dl in *Santalum album* pet ether fraction treated rats. Metformin treated group showed decrease in blood glucose from 345 ± 12.5 to 285 ± 20.5 mg/dl. In diabetic control group, blood glucose level increased by about 85 mg/dl after 60 days. The level of Hb glycation was increased in the diabetic control group. *Santalum album* pet ether fraction treated group showed significant reduction in glycated Hb level. Comparatively the metformin treated group did not show much effect on glycated Hb (Table 2).

Effect of *Santalum album* pet ether fraction and metformin on serum lipids in diabetic rats

Table 3 demonstrates effect of *Santalum album* pet ether fraction and metformin on serum lipids in diabetic rats. *Santalum album* pet ether fraction treatment showed improvement in serum lipid profile in hyperglycemic rats, reducing triglyceride, total cholesterol, and increasing HDL by 46%. The values are comparable to the normal controls. However in case of the diabetic controls there was gross increase in the triglyceride and LDL levels, while HDL was found to decrease. Metformin treatment led to a marginal improvement in the lipid profile. The overall atherogenic index was therefore found to improve significantly in the Santalum pet ether fraction treated animals. It can be seen that plasma insulin content did not improve both in case of metformin as well as S*antalum album* treated animals.

HPLC Profiling of *Santalum album* pet ether fraction

HPLC profile of *Santalum album* pet ether fraction shows major peak at 2.882 min with four minor peaks at 1.916, 3.35, 3.627, 3.748 min (Figure 2).

Discussion

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It can be observed that while treatment with *Santalum album* pet ether fraction does not show significant reduction in the blood glucose values in an OGTT, prolonged treatment for 60 days showed a significant lowering of the blood glucose level. Glycated h~~a~~emoglobin is known to increase in patients with diabetes mellitus (Koenig et al., 1976), and the increase has been found to be directly proportional to the fasting blood glucose level (Jackson et al., 1979). The significant reduction in glycated h~~a~~emoglobin of *Santalum album* pet ether fraction treated diabetic rats indicates its efficiency in glycaemic control. No significant increase in the levels of insulin has been observed compared to diabetic control in case of *Santalum album* pet ether fraction and metformin treated groups.

~~-~~Premature and extensive arteriosclerosis involving renal, peripheral, and cardiovascular vessels remain the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes increasing the risk of coronary heart disease. A reduction in serum cholesterol, triglycerides and VLDL fractions should be considered as being beneficial in long term prognosis of patients (Yadav et al., 2008). It is widely held that excessive lipid metabolism, achieved either by high-fat feeding or intravenous infusion of lipid emulsion to raise circulating free fatty acid (FFA) levels, leads to decreased insulin stimulated glucose uptake (Hevener et al., 2002). Elevation in the plasma lipid content is known to cause insulin resistance by inhibition of glucose transport and its phosphorylation, which leads to NIDDM (Roden et al., 1996). It was observed in the present study that the dose of 10 µg/kg body weight of the extract lowered the blood glucose, TC, TG, and LDL levels significantly and enhanced the cardio protective lipid HDL after 60 days of treatment. This would definitely reduce the incidence of coronary events (Lipid Research Clinics Programs, 1984). Similar observations are reported for several plant components. Eremanthin isolated from *Costus speciosus* was shown to act as an antihyperglycemic and antilipidemic at 10 and 20 mg/kg (Eliza et al., 2009). Likewise, the aqueous extract of *Aegle marmelos* seeds at 250 mg/kg (Kesari et al., 2006) has demonstrated similar results to our observations. However in both these studies as well as in several other plants the dosage generally varies from 5–~~–~~500 mg/kg as against the 10 µg/kg dose of pet ether extract used in the present study demonstrating the potency of *Santalum album*. Metformin has been reported to have beneficial effects on circulating lipids linked to increased cardiovascular risk (Silveira et al., 2008). However, in spite of being a purified drug the effect of *Santalum album* pet ether extract was better.

Atherogenic index has been proposed as a marker of plasma atherogenecity as it is found to be enhanced in patients suffering from cardiovascular disorders (Tan et al., 2004). The higher the atherogenic index the bigger the risk for organs such as the heart, liver and kidney for oxidative damage (Subramaniam et al., 2010). Atherogenic index was significantly reduced in *Santalum album* pet ether fraction treated rats compared with diabetic control. It reduced from 267 to 139~~-~~%. Although metformin showed reduction in atherogenic index changes from 267 to 222~~-~~% it was not as effective as *Santalum album* extract. Reduction in fat accumulation is known to prevent beta cell apoptosis and thereby prevent NIDDM (Shimabukuro et al., 1998). HPLC profile of *Santalum album* pet ether fraction showed major peak at 2.882 min. which may be a mixture of two primary sesquiterpene alcohols, α-santalol and β-santalol which is about 90% of oil fraction (Sindhu et al., 2010).

In conclusion *Santalum album* pet ether fraction at a very low concentration of 10 µg/kg body weight can effectively improve the lipid profile in diabetic rats. The improvement in the atherogenic index is even better than metformin at 30 mg/kg body weight, lowering the risk of cardiovascular damage in diabetics. Although the result from these studies did not demonstrate immediate lowering of blood glucose, as in case of a secretagogue

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or insulin action, prolonged treatment appears to alleviate diabetes through lowering of blood glucose and an improved glycated haemoglobin level. It is likely that this effect could be due to its lipid lowering action that could prevent development of insulin resistance and allow better glycemic control through existing insulin content and preventing further damage to the beta cells.

Declarations

Conflict of interest

The author(s) declare(s) that they have no conflicts of interest to disclose.

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Figure 1. Oral Glucose tolerance test of *Santalum album* oil and metformin in streptozotocin induced diabetic rats.

Figure 2. Reverse phase HPLC profiling of *Santalum album* pet ether fraction at 270 nm.

Table 1. Effect of *Santalum album* oil and metformin on body weight during treatment for 60 days.

~~w~~Weeks	Weight in grams

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	Normal control	Diabetic control	Diabetic + *Santalum album* oil 10 µg/kg	Diabetic + ~~M~~metformin 30 mg /kg
Week 1	200 ± 20	200 ± 20	200 ± 15	200 ± 20
Week 2	240 ± 15	180 ± 18	190 ± 17	180 ± 15
Week 3	270 ± 20	170 ± 15	180 ± 20	160 ± 17
Week 4	310 ± 17	150 ± 10	150 ± 16	160 ± 10**
Week 5	330 ± 14	150 ± 20	160 ± 18**	140 ± 20
Week 6	350 ± 20	130 ± 15	170 ± 20**	140 ± 15*
Week 7	370 ± 16	120 ± 17	180 ± 20**	130 ± 10*

Values are mean ± SD; *n* = 6. *The difference between treated and diabetic control values is significant at $P < 0.05$, ** The difference between treated and diabetic control values is significant at $P < 0.005$.

Table 2.~~:~~ *In vivo* effect of *Santalum album* oil and metformin on Glycosylated Hb and blood glucose level after treatment for 60 days.

Sr. No.	Groups	Glycosylated Hb in %	Blood glucose (mg/~~dl~~dL)
1.	Normal ~~C~~control	05.8 ± 0.3	80 ± 5.3
2.	Diabetic ~~C~~control	13.4 ± 0.4	323.7 ± 18.6
3.	Diabetic + *Santalum album* oil (10 µg/kg)	08.3 ± 0.2*	140.5 ± 15.8*
4.	Diabetic + Metformin (30 mg/kg)	15.6 ± 0.8*	285 ± 20.5*

Values are mean ± SD; *n* = 6. *The difference between treated and diabetic control values is significant at $P < 0.005$.

Table 3.~~:~~ *In vivo* effect of *Santalum album* oil and metformin on serum lipid profile level after treatment for 60 days.

	Normal ~~R~~rat	Diabetic ~~R~~rat	Diabetic+ *Santalum album* oil (10 µg / kg twice daily)	% Change (compare to diabetic rats)	Diabetic + ~~M~~metformin 30 mg/kg twice daily	% Chang (compar to diabeti rats)
Total cholesterol (mg/dl)	70.93 ± 3.5	75.30 ± 2.6	58.15 ± 3.2*	↓ 22	67.40 ± 3.0*	↓ 11
Total triglyceride (mg/dl)	73.53 ± 10.2	157.33 ± 11.2	89.48 ± 2.4*	↓ 44	133.34 ± 4.0*	↓ 15
HDL (mg/dl)	53.0 ± 3.3	28.20 ± 2.7	41.69 ± 5.2*	↑ 18	30.31 ± 0.10	↑ 7

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LDL (mg/dl)	14.73 ± 0.3	28.50 ± 4.5	19.56 ± 2.2*	↓ 43	20.14 ± 1.5*	↓ 29
VLDL (mg/dl)	14.70 ± 2.0	31.47 ± 2.2	17.89 ± 1.7*	↓ 43	30.73 ± 1.1	↓ 2
Plasma Insulin (µIU)	12.50 ± 1.5	4.50 ± 2.0	6.00 ± 1.3	↑ 33	5.4 ± 0.3	↑ 20
Atherogenic index (%)	133	267	139	↓ 36	222	↑ 2

Values are mean ± SD; $n = 6$. * The difference between treated and diabetic control values is significant at $P < 0.005$.

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