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Antidiabetic activity of heart wood of *Pterocarpus marsupium* Roxb. and analysis of phytoconstituents†

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The crude powder, ethanolic extract and aqueous, chloroform, hexane and n-butanol soluble fractions of ethanolic extract of heart wood of *P. marsupium* showed marked improvement on oral glucose tolerance post sucrose load in normal rats. All these fractions except aqueous fraction showed improvement on oral glucose tolerance post sucrose load on streptozotocin (STZ)-induced diabetic rats. The crude powder, ethanolic extract and hexane and n-butanol fractions showed marked decline in blood glucose level on STZ-induced diabetic rats. The ethanolic extract (100 mg/kg body weight) when given to STZ-induced diabetic rats for 10 consecutive days declined blood glucose, improved OGTT and increased their serum insulin levels. The ethanolic extract also showed marked improvement on oral glucose tolerance on high fat-low dosed STZ-induced diabetic rats and neonatally STZ treated rats. The ethanolic extract of *P. marsupium* also showed marked antidyslipidemic effects on high fat diet fed Syrian golden hamsters. Altered renal and hepatic function markers and serum insulin levels of high fat diet fed-low dosed STZ-treated diabetic rats were also found towards normalization when these animals were treated with ethanolic extract of *P. marsupium* for 28 consecutive days. The four out of five phenolic C-glycosides isolated from n-butanol fraction of ethanolic extract of *P. marsupium* enhanced glucose uptake by skeletal muscle cells (C2C12) in a dose dependent manner. It may primarily be concluded that phenolic-C-glycosides present in *P. marsupium* heart wood are the phytoconstituents responsible for the antihyperglycemic activity and validate the claim of antidiabetic activity of heart wood of *P. marsupium.*

Keywords: Antidyslipidemic effect, Antihyperglycemic effect, Glucose uptake, High fat diet, *Pterocarpus marsupium* heart wood, Skeletal muscle cells

Pterocarpus marsupium Roxb. (Family Fabaceae) is a large tree that commonly grows in central, western and southern parts of India and Sri Lanka. It is also known as Bibla or Vijaysaar in Hindi, Sarfaka in Sanskrit and Indian kino in English. Kino is the dried exudation obtained by incising the trunk of the plant and is traditionally used as an astringent and anti diarrheal agent. Its gum is used for toothache; bark is used for heartburn and management of diabetes. The leaves of *P. marsupium* are used for boils, sores, and various skin diseases. Overnight water stored in tumblers made out of the heartwood of *P. marsupium* is used as traditional therapy for the patients of diabetes mellitus especially in state of Madhya Pradesh[1,2].

The antidiabetic and other pharmacological activities of various parts of the *P. marsupium* are reported[3]. An aqueous infusion along with ethanolic extract of *P. marsupium* heart wood is widely known for hypoglycemic activity[4-6]. It is postulated that antidiabetic activity of *P. marsupium* is the result of its ability to decrease glucose absorption from the gastrointestinal tract that leads to improve insulin and pro-insulin levels in the blood. *P. marsupium* has also been documented to help in regeneration of pancreatic β-cells[7,8]. The active antidiabetic ingredients in the aqueous extract has been identified as (-) epicatechin, a benzopyran which on administration to alloxan-induced diabetic rats increased insulin secretion and number of islets in the pancreas. Insulin like activity of (-) epicatechin has been reported[9]. (-) epicatechin isolated from the bark of *P. marsupium* was found to have protective and restorative effects in alloxan-induced diabetic rats though it could not be restored by Kolb *et al*[10]. Many of the phenolic compounds such as marsupin, pterosupin and pterostilbene have

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been shown to have significant antidiabetic activity in STZ-induced hyperglycemia in rats[11]. Heartwood of *P.marsupium* has also been tested clinically and found effective in non insulin dependent diabetes mellitus patients (Type 2DM)[12].

The present study is a confirmation towards establishing antidiabetic activity of aqueous and ethanolic extracts of the heart wood of *P. marsupium* in validated animal models of type 2 diabetes and analysis of the phytoconstituents of *P. marsupium*.

Materials and Methods

Materials—Streptozotocin, Dulbeco Minimum Essential Medium (DMEM), fetal bovine serum, pencillin G, streptomycin, gentamycin, amphoterecin B, sodium bicarbonate sodium hydroxide, HEPES, trypan blue, phosphate buffered saline, trypsin, EDTA, glucose free DMEM, insulin, dexamethasone, sodium chloride, potassium chloride, manganese sulphate, calcium chloride, 2-deoxy-glucose and metformin were procured from Sigma Chemical Company, St..Louis, MO (USA). 2-^{3}H-deoxy glucose was procured from Perkin Elmer, USA. Sucrose, gum acacia, scintillation cocktail were purchased from Sisco Research Laboratories, Mumbai. Primers used in this study were procured from Eurofins. All others chemicals and solvents used were of highest purity grade.

Animals—Sprague Dawley Strain of male albino rats weighing around 150 g body weight and male Syrian Golden hamsters weighing around 120 g body weight were procured from the animal colony of CSIR-Central Drug Research Institute, Lucknow. The animals were always housed in polypropylene cages in groups of 3 to 5. The following conditions were always maintained in the animal room: temperature 23 ± 2 C, relative humidity 50-60 %, light 300 Lx at floor level with regular 12:12 h light and dark cycles. The animals were provided pellet diet and tap water ad libitum unless stated otherwise.

Plant material—Heartwood of *P. marsupium* was purchased from the local market and its identity was ascertained by botanists of the Institute. The shade dried material was cut into fine pieces and powdered by a mechanical grinder, passed through 100 mesh sieve and the powder was stored in air-tight containers until used.

Extraction, fractionation and isolation of compounds—The powdered heart wood (2.6 kg) of *P. marsupium* was percolated in ethanol (10.0:1.0) for 48 h at room temperature. The percolate was collected and the process was repeated four times. The combined ethanolic extract was filtered and concentrated under reduced pressure at 55 C, afforded brown viscous liquid (200 g, 7.69 %). The part of extract (150 g) was suspended in water (2.0 L) and successively fractionated with hexane (500 mL × 15), chloroform (500 mL × 15) and n-butanol saturated with water (1 L × 10). The solvents were removed under reduced pressure to furnish corresponding fractions of hexane (10 g), chloroform (20 g), n-butanol (80 g) and aqueous (40 g). The n-butanol soluble fraction (80 g) was subjected to repeated flash chromatography over silica gel (230-240 mesh using chloroform: methanol (49:1) as solvent afforded pterosupol (10 mg), chloroform: methanol (19:1) afforded pteroside (15 mg), chloroform: methanol (9:1) pterocarposide (8 mg), chloroform: methanol (17:3) afforded pterosupin (7 mg), and marsuposide (8 mg), chloroform: methanol (4:1) afforded vijayoside (5 mg) and chloroform: methanol (4:1) afforded C-beta-D-glucopyranosyl-2,6-dihydroxyl benzene (11 mg). These compounds were characterized by comparison of the reported spectral data.

Assessment for improvement on glucose tolerance and blood glucose lowering activity

Normal rats—Male albino rats were grouped and acclimatized for 3 to 4 days in the polypropylene cages. The animals were starved overnight for determining their fasting blood glucose levels by glucometer using glucostrips (Roche) on the day of experiment. Animals showing blood glucose levels between 3.33-4.44 mM (60 to 80 mg/dL) were selected and regrouped each group having five to six animals. Rats of experimental groups were orally treated with standard drug metformin and the test samples i.e. crude powder of heartwood of *P. marsupium*, its ethanolic extracts and fractions thereof, respectively at doses specified in text and tables. Animals of control group always received an equal amount of 1.0% gum acacia. An oral sucrose load (10.0 g/kg) was given to each animal exactly after 30 min post administration of the test sample/ vehicle. Blood glucose profile of each rat was determined just before the administration of test sample (-30 min), sucrose (0 min) and thereafter at 30, 60, 90 and 120 min post administration of sucrose by glucometer using glucostrips. Food but not water was withdrawn from the cages during the course of experimentation. Quantitative inhibition on the rise of

postprandial hyperglycemia post sucrose load by the test sample was calculated by plotting blood glucose level and time on y and x axis, respectively, and determining area under curve (AUC) of each group using Prism Software. The percentage lowering in AUC of standard drugs/test sample treated groups compared to control group determined the percentage improvement on oral glucose tolerance (OGTT) post sucrose load.

Low dosed Streptozotocin-induced diabetic rats

(a) *Improvement on oral glucose tolerance*—The low dosed STZ-rat experimental protocol reveal functional, but impaired β-cell activity in rats treated with insulin secretagogues. The sucrose challenge is included to this as this increases the sensitivity of the model to chances of seeing the effect of test compound with alpha-glucosidase activity and may increase our chances of seeing effects on insulin sensitivity[13,14].

Male albino rats were used. STZ was dissolved in 100 mM citrate buffer *p*H 4.5 and calculated amount (45 mg/kg) of the fresh solution was injected i.p. to overnight fasted rats. Fasting blood level of each animal was checked after 48 h by glucometer using glucostrips and animals showing blood glucose levels between 144-216 mg/dL (8.0 to 12 mM) were finally included in the experiments and termed as diabetic animals. These diabetic animals were divided into groups each consisted of 6 animals. Rats of experimental groups were orally treated with standard antidiabetic drug metformin and test samples i.e. crude powder of heartwood of *P. marsupium*, its ethanolic extract and fractions thereof, respectively at doses specified in text and tables. Animals of control group were always given an equal amount of 1.0 % gum acacia. A sucrose load of 2.5 g/kg body weight was given to each rat 30 min after the administration of test sample/standard drugs/vehicle. The blood glucose profile of each animal was checked just before the administration of test sample/standard drug/vehicle (-30 min), sucrose load (0 min) and thereafter at 30, 60, 90, 120, 180, 240, 300 and 1440 min, respectively, by glucometer using glucostrips. Animals not found diabetic at 1440 min were not considered as diabetic and omitted from the calculations and termed as non-responders. The animals which did not show any fall in blood glucose profile in a group while the others in that group showed fall in blood glucose profile were also considered as non responders. Food but not water was withdrawn from the cages during the experimentation. The blood glucose profile was plotted against time and the AUC of each group determined. The percentage lowering in AUC of experimental/standard drug treated groups compared to control group determined the percent improvement on oral glucose tolerance (OGTT) post sucrose load.

(b) *Blood glucose lowering effect*—Diabetes was induced in male albino rats (Sprague Dawley strain) by injecting STZ intraperitoneally as described earlier[15]. The animals showing fasting blood glucose over 270 mg/dL on day 3rd post STZ injection were finally included in the experiment. The animals were divided into groups each consisted of 6 animals. The animals of experimental groups were treated with metformin, and the test samples, respectively at doses specified in text and tables. The animals of control group always received an equal amount of 1.0 % gum acacia (the vehicle used for preparing the suspensions of standard antidiabetic drugs metformin and test samples i.e. crude powder of heartwood of *P. marsupium*, its ethanolic extract and fractions thereof) for administration to rats. The blood glucose level of each animal was determined just before the administration of standard drug and test samples (0 min) and thereafter at 30, 60, 90, 120, 180, 240, 300 and 1440 min. Food but not water was withdrawn from the cages during 0 to 300 min. The average lowering in blood glucose level between 0 to 300 min and 0 to 1440 min was calculated by plotting the blood glucose level on y-axis and time on x-axis and the AUC determined. Comparing the AUC of experimental group with that to control group determined the percent lowering of blood glucose level during the period.

High fructose enriched diet fed and low dosed streptozotocin treated diabetic rats—Rats of Sprague Dawley strain were fed with homemade high fructose high fat diet (comprising of 60% fructose, 13 % saturated fat, 22 % protein, 1.0 % salt mixture, 1.0 % minerals mixture and traces of vitamins) for 4 weeks. The blood of each rat was withdrawn from their retro-orbital plexus for the estimation of blood glucose and serum lipid profiles after 4 weeks. The rats showing their serum triglyceride levels ≥ 300 mg/dL and total cholesterol levels ≥ 150 mg/dL were considered as hyperlipidemic. Half of these hyperlipidemic rats were injected STZ intraperitoneally at the dose of 45mg/kg, whereas the rest half were kept as such and

termed hyperlipidemic rats. The STZ-treated hyperlipidemic rats with the fasting blood glucose level ≥ 280 mg/dL after 48 h were considered as diabetic and hyperlipidemic and selected for further pharmacological studies. The hyperlipidemic and hyperglycaemic hyperlipidemic STZ treated rats in each group were randomly divided into 3 subgroups. The rats of subgroup I were fed with high-fructose high-fat diet + STZ (diabetic control, received 1.0% gum acacia), subgroup II were fed with high-fructose high-fat diet + STZ (experimental group, received test sample at 100 mg/kg body weight dose) and subgroup III were fed with high-fructose high-fat diet + STZ (experimental group, received standard drug metformin at 100 mg/kg body weight dose). Biochemical estimations i.e. blood glucose, serum Insulin, triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were carried out on 0 day and 7^{th}-14^{th} days after the treatment. The rats were allowed to continue to feed on their respective diets until the end of the study[16]. Plasma insulin levels were assayed using an enzyme–linked immunosorbant assay kit (Mercodia, Uppsala, Sweden). OGTT of the animals of each group was done as earlier post glucose load.

Neonatally-streptozotocin treated diabetic rats—Two-day-old pups of Sprague Dawley strain (weighing 5-8 g), were injected 80 mg/kg STZ prepared in citrate buffer 0.1 M, *p*H 4.5 and left along with their mothers for 4 weeks. The rats separated from mothers were further kept for 3 months in polypropylene cages and given pellet diet and water ad libitum. These animals showed signs of polydypsia, polyurea and abnormal OGTT at the end of the period. These rats were randomly divided into three groups of 6 animals. Group I (diabetic control group) received 1% gum acacia**,** Group II (experimental group) received test sample i.e. ethanolic extract of heartwood of *P. marsupium* at 100 mg/kg body weight dose and Group III (standard group) received standard oral hypoglycemic drug metformin at 100 mg/kg body weight dose. Blood glucose, serum insulin and OGTT post glucose load of these animals were followed at weekly intervals for three weeks.

High fructose fed male Syrian golden hamsters—Male Syrian Golden hamsters weighing around 120 ± 10 g were used. The animals were given the home made high fructose high fat diet (comprising of 60% fructose, 13% saturated fat, 22% protein, 1.0 % salt mixture, 1.0 % minerals mixture and traces of vitamins) for 4 weeks. The animals showing serum triglyceride levels ≥ 400 mg/dL and total-cholesterol ≥ 250 mg/dL were considered as hyperlipidemic. The hyperlipidemic hamsters were randomly divided into two groups of 6 animals in each. The hamsters of Group I were fed with high-fructose high-fat diet (dyslipidemic control, received 1.0% gum acacia), whereas, Group II were fed with high-fructose high-fat diet (experimental group, received test sample i.e. ethanolic extract of crude powder of heartwood of *P. marsupium* at 100 mg/kg body weight dose). Blood from each hamster was withdrawn from retro-orbital plexus in EDTA tubes on 14^{th} day post treatment. Serum was separated for immediate analysis of total cholesterol, TG, HDL-C and LDL-C on Cobas Integra 400 automated analyser.

Effect of compounds isolated from P. marsupium on 3H-2-deoxyglucose (2-DG) *uptake by C2C12 skeletal muscle cells*—Mouse muscle cells (C2C12) grown in 24 well plates (6 × 104 cells/well) were employed for studying the effect on 2-deoxyglucose uptake as reported by Klip *et al*[17]. Briefly, C2C12 myotubes were incubated with 10 µM/mL varying concentrations of isolated phenolic C-glycosides from n-butanol fraction of ethanolic extract of heartwood of *P. marsupium*, respectively, for 24 h with final 3 h in serum-deprived medium and a sub-set of cells was stimulated with 100 nM insulin for 20 min. Glucose uptake was assessed for 5 min in HEPES-buffered saline [140 mM NaCl, 20 mM HEPES, 5 mM KCl, 2.5 mM $MgSO_4$, 1 mM $CaCl_2$ (*p*H 7.4)] containing 10 µM 2-DG (0.5 µCi/mL 2-[H3] DG) at room temperature. After the period, radioactive solution was rapidly aspired, and the cell mono layers were washed three times with ice-cold HEPES buffered saline solution. Cell associated radioactivity was determined by cell lysis in 0.05 N NaOH, followed by scintillation counting (Beckman Coulter, USA). All assays were performed in triplicates and normalized to total protein, was expressed as fold change with respect to control.

Statistical analysis—Each biochemical parameter was expressed as mean ± S.E. Statistical comparisons between groups were made by Student's *t* test. The results were considered significant if *P* values are 0.5 or less.

Results

Antihyperglycemic activity of crude powder of heart wood of P. marsupium, its ethanolic extract and fractions of ethanolic extract on normal and streptozotocin-induced diabetic rats—Table 1 presents the effect of crude powder, ethanolic extract and fractions of ethanolic extract and standard drug metformin on % improvement of glucose tolerance post sucrose load in normal and STZ-induced diabetic rats, and decline in blood glucose level of STZ-induced diabetic rats. It is evident from the results that all the samples i.e. crude powder, ethanolic extract and fractions of ethanolic extract of *P. marsupium* heart wood showed significant improvement on oral glucose tolerance post sucrose load on normal as well as STZ-induced diabetic rats at the selected dose i.e. 250 mg/kg. Except the aqueous and chloroform fractions of ethanolic extract, all the samples also showed significant decline in blood glucose levels of STZ-induced diabetic rats. On comparison, none of the samples of *P. marsupium* showed better effect than the standard drug metformin.

Multiple dose effects of ethanolic extract of heartwood of P. marsupium on streptozotocin-induced diabetic rats—Table 2 presents the multiple dose effects of ethanolic extract of *P. marsupium* heart wood and metformin on fasting blood glucose level and on improvement of oral glucose tolerance of STZ-induced diabetic rats. It is evident from the results that the effect of both on these parameters was dose dependent as the effect increased with days of treatment.

Table 3 presents the effect of ethanolic extract of heart wood of *P. marsupium* and metformin on total triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) of STZ-induced diabetic rats. It is evident from the results that both lowered TG, TC and LDL-C and increased HDL-C levels of STZ-induced diabetic rats, however, the effect was not found dependent on time. It seems that the maximum effect was achieved on day 7.

Table 4 presents the effect of ethanolic extract of heart wood of *P. marsupium* and metformin on the kidney function markers in STZ-induced diabetic rats.

Table 1—Effect of crude powder, ethanolic extract and fractions of ethanolic extract of heart wood of *P. marsupium* on normal and streptozotocin-induced diabetic rats.
[Values are mean ± SE of 3 independent experiment from 6 animals in each group]

Sample and dose (mg/kg)	Normal rats	Streptozotocin-induced diabetic rats	
	% improvement in OGTT	% improvement in OGTT	% decline in blood glucose
Crude Powder (250)	17.1***	22.2***	13.4*
Ethanolic Extract (250)	18.3***	14.8*	19.6***
Aqueous Fraction (250)	13.3***	13.2^{NS}	9.89^{NS}
Chloroform Fraction (250)	12.3***	16.5*	0.02^{NS}
Hexane Fraction (250)	21.5***	16.7*	20.6*
Butanol Fraction (250)	23.6***	24.4***	21.4*
Metformin (100)	35.8***	30.9***	28.6**

P values:*<0.05, **<0.01, ***<0.001

Table 2—Multiple dose effects of ethanolic extract of *P. marsupium* heartwood and metformin on fasting blood glucose and oral glucose tolerance of streptozotocin-induced diabetic rats

[Values are mean ± SE of 3 independent experiment from 6 animals in each groups, figures in parenthesis are % change from sham treated control]

Groups	Assessment parameters					
	Fasting blood glucose (mg/dl)			Oral glucose tolerance (0-120 min) (AUC)		
	0 day	7^{th} day	14^{th} day	0 day	7^{th} day	14^{th} day
Sham treated (1.0 % Gum acacia)	349±28.3	365±20.9	370±47.4	54580±1220	54520±1307	53510±1734
P. marsupium treated (100 mg/kg)	352±14.6	$314±41.4^{NS}$(13.9)	287±26.9*(22.4)	54700±1991	42180±1696**(22.6)	37080±2244**(30.7)
Metformin treated (100 mg/kg)	347±27.9	256±12.7*(29.8)	202±5.30**(45.4)	54550±1435	38900±2675**(28.7)	34440±1786**(35.6)

P values:*<0.05, **<0.01, ***<0.001

The results reveal that both the ethanolic extract and metformin decreased the elevated levels of serum urea, uric acid and creatinine levels of STZ-induced diabetic rats and effect was found dose dependent as the effect increased with duration of treatment.

Table 5 presents the effect of ethanolic extract of *P. marsupium* and metformin on liver function markers i.e. AST, ALT and bilrubin in sera of STZ-induced diabetic rats. Metformin caused significant decline in both AST and ALT levels whereas, the ethanolic extract of *P. marsupium* lowered AST level significantly. STZ-induced diabetic rats did not show any significant elevation on serum bilrubin levels and neither of metformin or ethanolic extract of *P. marsupium* caused significant effect on serum bilrubin level.

Antihyperglycemic and antidyslipidemic effects of ethanolic extract of P. marsupium on high-fructose high-fat fed streptozotocin induced diabetic rats—

Table 3—Effect of ethanolic extract of *P. marsupium* heartwood and metformin on serum lipid profile of streptozotocin-induced diabetic rats

[Values are mean ± SE of 3 independent experiment from 6 animals in each groups, figures in parenthesis are % change from sham treated control]

Groups	Serum lipid profiles (mg/dl)											
	Triglycerides (TG)			Total-cholesterol (Chol)			HDL-Cholesterol			LDL-Cholesterol		
	0 day	7th day	14th day	0 day	7th day	14th day	0 day	7th day	14th day	0 day	7th day	14th day
Sham treated (1.0% gum acacia)	123.9 ±22.3	154.1 ±24.9	162.5 ±24.1	84.2 ±6.80	97.2 ±7.70	89.1 ±1.60	32.9 ±1.50	30.0 ±1.40	32.0 ±1.50	64.3 ±2.80	75.3 ±1.90	75.9 ±1.80
P. marsupium treated (100 mg/kg)	122.9 ±2.80	99.2 ±2.40* (35.6)	94.7 ±1.90* (41.7)	71.4 ±1.50	62.8 ±0.50** (35.3)	58.9 ±2.90 ** (33.8)	33.8 ±0.30	39.4 ±1.10* (+31.2)	41.1 ±0.90 ** (+30.3)	69.5 ±2.00	54.3 ±1.00** (27..8)	52.7 ±0.89** (30.5)
Metformin treated (100 mg/kg)	122.1 ±2.90	97.4 ±2.60* (36.8)	89.6 ±1.06* (44.8)	82.2 ±2.80	60.6 ±1.30** (37.7)	55.9 ±1.50 ** (37.2)	30.8 ±0.80	41.7 ±2.50 ** (+38.9)	45.8 ±3.20 ** (+43.1)	69.4 ±1.4	53.2 ±1.6 ** (29.3)	49.8 ±2.00** (34.3)

P values:*<0.05, **<0.01, ***<0.001

Table 4—Effect of ethanolic extract of *P. marsupium* heartwood and metformin on Kidney function markers of streptozotocin-induced diabetic rats

[Values are mean ± SE of 3 independent experiment from 6 animals in each groups, figures in parenthesis are % change from sham treated control]

Groups	Kidney function tests								
	Serum-urea (mg/dl)			Serum-uric acid (mg/dl)			Serum-creatinine (mg/dl)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Sham treated (1.0 % gum acacia)	115 ±8.07	143 ± 8.98	151 ±8.62	3.76 ±0.21	4.95 ±0.21	5.24 ±0.23	0.49 ±0.02	0.64 ±0.04	0.65 ±0.03
P. marsupium treated (100 mg/kg)	120 ±5.23	83.9 ±6.48* (41.3)	72.1 ±5.11** (52.2)	4.16 ±0.21	2.76 ±0.20* (44.2)	2.41 ±0.09** (54.0)	0.48 ±0.03	0.36 ±0.02* (43.7)	0.34 ±0.02** (47.6)
Metformin treated (100 mg/kg)	112 ±10.1	71.2 ±7.69** (50.2)	59.4 ±1.72** (60.7)	3.80 ±0.48	1.73 ±0.18** (65.1)	1.14 ±0.08** (78.2)	0.50 ±0.02	0.32 ±0.02** (50.0)	0.25 ±0.01** (61.5)

P values:*<0.05, **<0.01, ***<0.001

Table 5—Effect of ethanolic extract of *P. marsupium* heartwood and metformin on liver function markers of streptozotocin-induced diabetic rats

[Values are mean ± SE of 3 independent experiment from 6 animals in each groups, figures in parenthesis are % change from sham treated control]

Groups	Liver function tests		
	Serum aspartate amino transferase (S-AST) (U/l)	Serum alanine amino transferase (S-ALT) (U/l)	Serum T-Bilrubin (mg/dl)
	Day 14 PT	Day 14 PT	Day 14 PT
Sham treated (1.0 % gum acacia)	90.3± 3.9	88.5± 3.9	0.33 ± 0.03
P. marsupium treated (100 mg/kg)	59.6± 3.8** (33.9)	61.1± 5.3 NS (30.8)	0.29± 0.05 NS (12.1)
Metformin treated (100 mg/kg)	55.2± 3.9** (38.9)	59.0± 2.0** (33.3)	0.24± 0.02 (27.3)

P values:*<0.05, **<0.01, ***<0.001

Fig. 1a and b show the effect of ethanolic extract of *P. marsupium* heart wood and metformin on oral glucose tolerance test (OGTT) of high fructose high fat fed low dosed STZ-induced diabetic rats on day 7th and 14th post treatment, respectively. The ethanolic extract of *P. marsupium* heart wood improved OGTT to the tune of 21.6% (P<0.05) and 28.3% (P<0.01), respectively on day 7 and 14, whereas metformin improved OGTT around 25.4% (P<0.01) and 32.2 % (P<0.01), respectively, on day 7th and 14th, respectively.

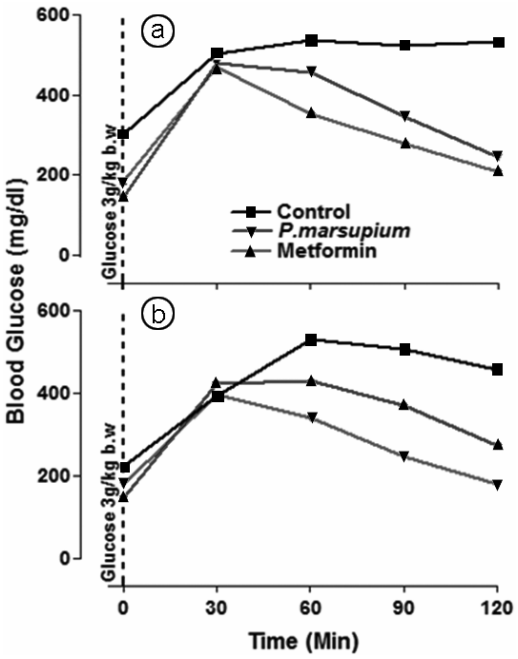

Fig. 1—Effect of ethanolic extract of *P. marsupium* heartwood and metformin on oral glucose tolerance (OGTT) of high-fructose high-fat fed low dosed STZ- induced diabetic rats on day 7 and 14th post treatment

Table 6 shows the effect of ethanolic extract of *P. marsupium* heart wood and metformin on serum TG, TC, LDL-C, HDL-C and insulin levels of high fructose high fat fed low dosed STZ-induced diabetic rats. The results demonstrate that treatment with ethanolic extract for 14 days caused decline in serum TG, TC, LDL-C and insulin levels of high fructose high fat fed low dosed STZ-induced diabetic rats. However, these rats did not show any significant effect on serum HDL-C levels.

Effect of ethanolic extract of P. marsupium heart wood on neonatally-Streptozotocin treated diabetic rats—Table 7 shows the effect of ethanolic extract of *P. marsupium* heart wood and metformin on fasting blood glucose, OGTT and serum insulin levels of neonatally-STZ treated rats. The results clearly indicate that both the ethanolic extract and metformin decline fasting blood glucose level, improve OGTT and increase serum insulin levels and the effect was found dependent on duration of treatment.

Effect of ethanolic extract of P. marsupium heart wood on high fat fed male Syrian golden hamsters—Fig. 2 (a to d) shows the effect of ethanolic extract of *P. marsupium* heart wood on serum TG, TC, LDL-C and HDL-C levels of high fructose fed male Syrian golden han ers. The results clearly indicate that ethanolic extract cause decline in the serum levels of TC, TG and LDL-C and increase HDL-C of high fat fed dyslipidemic golden hamsters when given for 14 consecutive days at 100 mg/kg dose levels.

Effect of phenolic-C-glycosides of P. marsupium heart wood on glucose uptake by mouse skeletal muscle cells (C2C12) —Figs. 3-7 present the effect of five phenolic-C-glycosides i.e. vijayoside, pteroside,

Table 6—Antihyperglycemic and antidyslipidemic effect of ethanolic extract of *P. marsupium* heartwood and metformin on serum insulin and lipid profile of high fructose enriched diet fed and low dosed streptozotocin-induced diabetic rats

[Values are mean ± SE of 3 independent experiment from 6 animals in each groups, figures in parenthesis are % change from sham treated control]

Groups	Serum profiles									
	Triglycerides (TG) (mg/dl)		Total Cholesterol (T-Chol) (mg/dl)		HDL-Cholesterol (HDL-C) (mg/dl)		LDL-Cholesterol (LDL-C) (mg/dl)		Serum-insulin (ng/l)	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Sham treated (1.0 % gum acacia)	326.9±6.70	343.1±4.50	178.6±1.30	180.2±2.30	36.2±7.50	35.6 ±1.20	73.1±13.6	75.3±1.40	2900 ±305	2883±297
P. marsupium treated (100 mg/kg)	324.2±7.30	245.4±9.70* (28.5)	156.5±2.10	133.2±1.70* (26.1)	34.2±2.50	38.5 ±0.60^{NS} (+8.14)	72.1±10.2	60.2±0.50^{NS} (20.0)	2800 ±182	1100±154**
Metformin treated (100 mg/kg)	325.3±1.20	237.4±1.70* (30.8)	169.3±1.60	125.1±3.40* (30.5)	34.1±4.20	36.5 ±6.10^{NS} (+2.52)	71.3±2.30	57.6±7.70^{NS} (23.5)	2733 ±270	1800±281*

P values:*<0.05, **<0.01, ***<0.001

Table 7—Effect of ethanolic extract of *P. marsupium* heartwood and metformin on fasting blood glucose, oral glucose tolerance test and serum insulin profile of neonatally-streptozotocin-treated diabetic rats

[Values are mean ± SE of 3 independent experiment from 6 animals in each groups, figures in parenthesis are % change from sham treated control]

Groups	Assessment Parameters								
	Fasting blood glucose (mg/dl)			Oral Glucose tolerance (0-120 min) (AUC)			Serum-insulin (ng/l)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Sham treated (1.0 % gum acacia)	146 ± 4.55	143 ± 5.56	149 ± 5.10	42479 ± 103.3	44090 ± 167.9	48940 ± 307.8	30 ± 4.08	50 ± 10.8	22.5± 4.78
P. marsupium treated (100 mg/kg)	149 ± 2.21	124 ± 2.63 ^{NS} (13.2)	113 ± 3.40* (24.1)	42461 ± 118.1	38020 ±114.7** (13.8)	35930 ±102.9** (26.6)	52.5 ± 11.1	90 ± 7.07 ^{NS}	160 ± 31.3*
Metformin treated (100 mg/kg)	147 ± 5.64	116 ± 2.95* (18.8)	104 ± 5.11** (30.2)	42463 ± 122.8	33990 ±126.3** (22.9)	32050 ± 143.8** (34.5)	42.5 ± 13.1	120 ± 24.8*	190 ± 43.0**

P values:*<0.05, **<0.01, ***<0.001

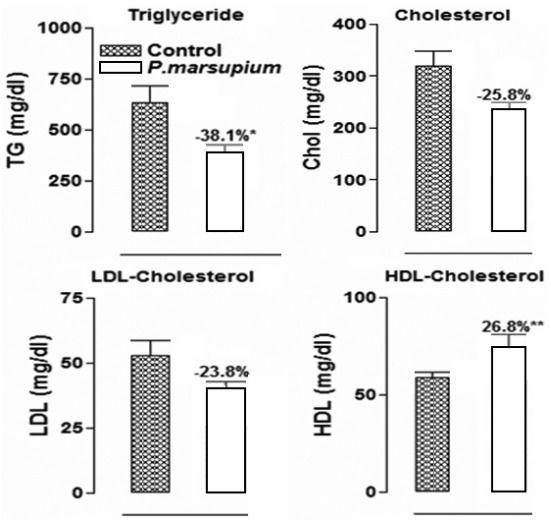

Fig. 2—Effect of ethanolic extract of *P. marsupium* heartwood on serum lipid levels of high fructose fed male Syrian golden hamsters [Values are mean ± SE]

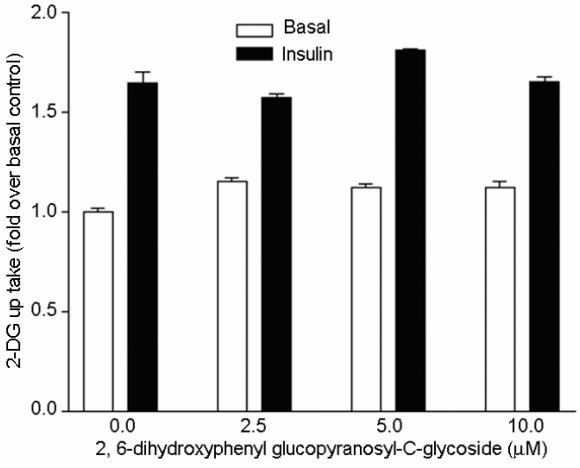

Fig. 3—Concentration-dependent effect of 2, 6-dihydroxyphenyl glucopyranosyl-C-glycoside on 2-deoxyglucose uptake by C2C12 cells

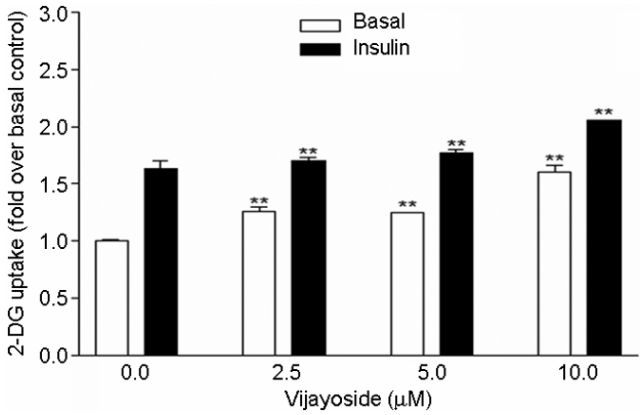

Fig. 4—Concentration-dependent effect of vijayoside on 2-deoxyglucose uptake by C2C12 cells

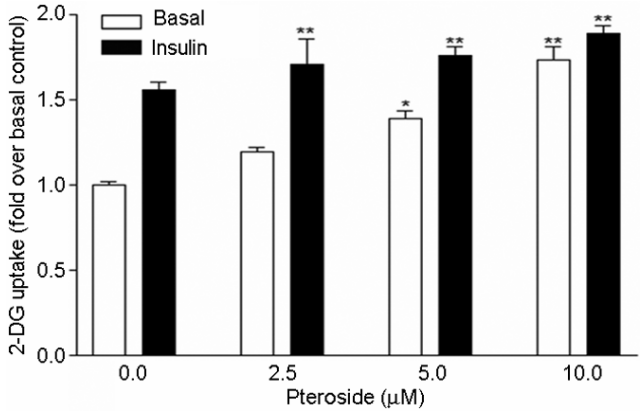

Fig. 5—Concentration-dependent effect of pteroside on 2-deoxyglucose uptake by C2C12 cells

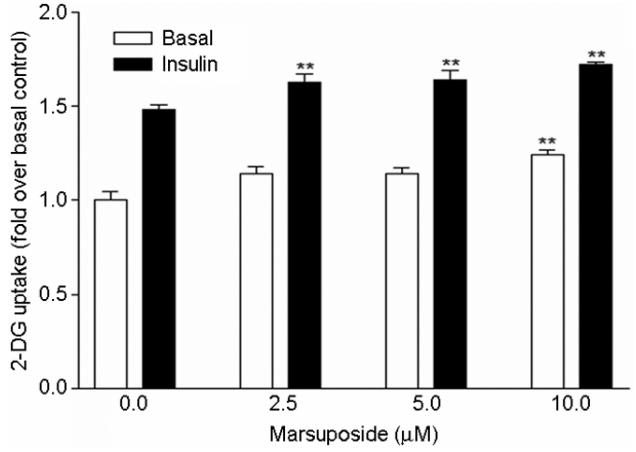

Fig. 6—Concentration-dependent effect of marsuposide on 2-deoxyglucose uptake by C2C12 cells

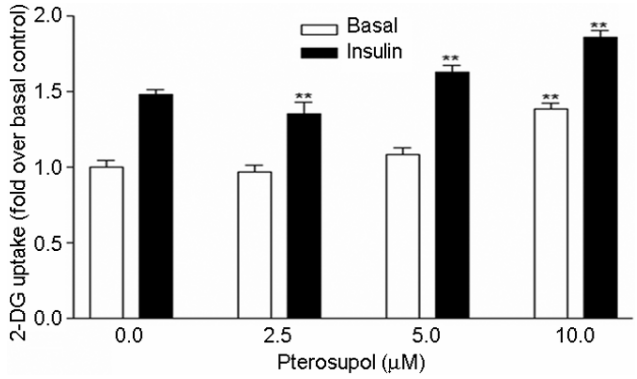

Fig. 7—Concentration-dependent effect of pterosupol on 2-deoxyglucose uptake by C2C12 cells

marsuposide, pterosupol and 2,6-dihydroxyphenyl-glucopyranosyl-C-glycoside isolated from n-butanol fraction of the ethanolic extract of heartwood of *P. marsupium* on glucose uptake by mouse skeletal muscle cells (C2C12). C2C12 cells efficiently take up 2-deoxy-glucose even in the absence of insulin in the media (basal) but addition of insulin in the media stimulated the 2-deoxy-glucose uptake to nearly 1.6 fold higher (insulin stimulated). Vijayoside showed dose dependent increase in both basal as well as insulin-stimulated 2-deoxy-glucose uptake by C2C12. The maximum stimulation could be achieved at 10.0 μM. Pteroside also increased both the basal as well as insulin stimulated glucose uptake in a dose dependent manner, however, the stimulatory effect of marsuposide on insulin stimulated 2-deoxy-glucose uptake was not observed dose dependent. Pterosupol stimulated the basal as well as insulin-dependent glucose uptake at 10.0 μM, only. 2,6-dihydroxyphenyl-glucopyranosyl-C-glycoside did not show any significant effect on 2-deoxy-glucose uptake by C2C12 cells.

Discussion

Results of the present study clearly indicate that both crude powder and ethanolic extract of *P. marsupium* have antihyperglycemic efficacy. The crude powder and the ethanolic extract of *P. marsupium* were found effective in lowering the blood glucose level of streptozotocin-induced diabetic rats and improved oral glucose tolerance in both normal as well as STZ-induced diabetic rats that may be triggered by increased insulin production from pancreatic β cells as reported earlier by Dhanabal *et al*[11].

The crude powder as well as ethanolic extract of *P. marsupium* heart wood declined the blood glucose levels of STZ-induced diabetic rats. Blood glucose lowering effect of *P.marsupium* heart wood has also been reported in alloxan-induced diabetic rat model. Streptozotocin cause selective toxic effect on pancreatic β cells, leaving less functional cells which results in hyperglycemia[18,19]. In most studies streptozotocin or alloxan was used for inducing diabetes[20,21]. One of the intracellular phenomenon for their cytotoxicity is through generation of free radicals[22,23]. Significant (P<0.01) decline in blood glucose by either crude powder or ethanolic extract of heart wood of *P.marsupium* may be because of their beta cell regenerative property of different constituents present in heartwood of *P. marsupium*.

One of the most common complications during diabetes mellitus is abnormalities in lipid profile[24, 25]. Acute insulin deficiency causes increase in free fatty acid mobilization from peripheral tissue, due to decrease activity of enzyme lipoprotein lipase. Insulin deficiency is related with hypercholesterolemia and hypertriglyceridemia[26-28]. It was observed in STZ-induced diabetic rats that the main reason for elevated cholesterol and triglycerides level could be insulin deficiency[29]. It was observed in the present study that there was an increase in triglycerides, cholesterol, LDL-cholesterol levels along with decrease in HDL-cholesterol level in sera of STZ-induced diabetic rats. Ethanolic extract of *P. marsupium* (100 mg/kg) was found effective in decreasing total serum cholesterol, triglycerides and LDL-cholesterol and increasing HDL-cholesterol levels. The antidyslipidemic efficacy of ethanolic extract of *P. marsupium* may be due to increase in

insulin secretion which finally led to a decrease in the synthesis of cholesterol and fatty acids.

Renal dysfunction is another most common complication during diabetes mellitus. Decrease in protein and increase in urea and creatinine levels in blood/serum are the markers of kidney dysfunction[30]. In the present study urea, uric acid and creatinine levels in serum of STZ-induced diabetic rats were found higher compared to normal rats. The STZ-induced diabetic rats treated with ethanolic extract of *P. marsupium* (100 mg/kg body weight) showed significant decline in their serum urea, uric acid and creatinine levels. These results are in accordance to that reported by Mahalingam and Kannabiran[31]. The increase in serum AST and ALT activities in STZ-induced diabetic rats indicated that chronic hyperglycemia may induce hepatic dysfunction. Liver is an insulin dependent tissue which plays important role in carbohydrate and lipid metabolism[32]. The ALT and AST activities are directly associated with the conversion of amino acids to keto acids. In the present study the rise in the activity of ALT is thought because of hepatocellular damage and is usually accompanied by a rise in AST. An increase in the activities of AST and ALT in the liver of diabetic animals has been frequently reported[33,34]. Curative effect by ethanolic extract of *P. marsupium* (100 mg/kg body weight) is at the moment advocated by way of restoration in the levels of liver function enzymes.

It is well established that fructose consumption induces insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertriglyceridemia and hypertension in rats and hamsters models[35]. Fructose feeding to normal rats and hamsters significantly increased their blood glucose, insulin and triglycerides levels. The results of the present study indicates that treatment with ethanolic extract of *P.marsupium* heart wood for 14 days to either fructose fed rats or hamsters significantly lowered their blood glucose and serum lipid profiles. These results suggest the usefulness of *P. marsupium* bark in improving glucose intolerance and lowering the raised lipid levels. These benefits of *P.marsupium* heart wood are presumed to be the single or synergistic effects of several antidiabetic ingredients like -epicatechin, pterosupin, marsupin and pterostiblene present in it.

Streptozotocin selectively destroys pancreatic β-cells, inhibits the synthesis and release of insulin, and causes the onset of diabetes mellitus[36]. In the neonatally-STZ-induced diabetic rats at adult ages, inflammatory changes, atrophy, decrease in numbers and destruction of pancreatic insulin-positive islets cells were observed[37]. However, treatment with ethanolic extract of *P. marsupium* raised the serum insulin level presumably by an increase in the number of β-cells of pancreas. A significant decrease in the blood glucose level and an increase of the serum insulin level in neonatally STZ-treated group fed the ethanolic extract of *P. marsupium* heart wood. These results indicated that the *P. marsupium* heart wood was effective in controlling hyperglycemia of the neonatally-STZ-induced diabetic model.

High fat fed Syrian golden hamsters have been reported as an ideal model for finding the antidyslipidemic efficacy of the drug/test substances[38,39]. Feeding of high fat diet for a longer period increases serum triglycerides, cholesterol, LDL-cholesterol and decreases HDL-cholesterol levels in the hamsters. The decline in the levels of serum triglyceride, cholesterol and LDL-cholesterol as well as an increase in HDL-cholesterol levels of hamsters treated with ethanolic extract of *P. marsupium* heart wood indicate the antidyslipidemic property of *P. marsupium* heart wood.

Glucose transport is the rate-limiting step in glucose utilization in insulin targeted skeletal muscle cells. This transport is mediated by major glucose transporter proteins present in skeletal muscle cells[40]. Impaired glucose transport with reduced Glut-4 translocation and disturbance in insulin signalling cascade are the major defects in insulin resistance and type-2 diabetes mellitus. Skeletal muscle cells are considered a well-established *in vitro* model to study the regulation of glucose transport, since skeletal muscle are also the major site for primary glucose disposal and glucose utilization[41,42]. Results of the present study demonstrated that four out of the five phenolic-C-glycosides stimulates the glucose utilization process in mouse skeletal muscle cells i.e. C2C12.

In conclusion, it may be stated that the results of the present study demonstrate the usefulness of heartwood of *P. marsupium* for improving overall glycemic control and thereby reducing the risk of diabetic complications. Further pharmacological and biochemical investigations are underway to elucidate the exact mechanism of action of phenolic glycosides present in heart wood of *P. marsupium.*

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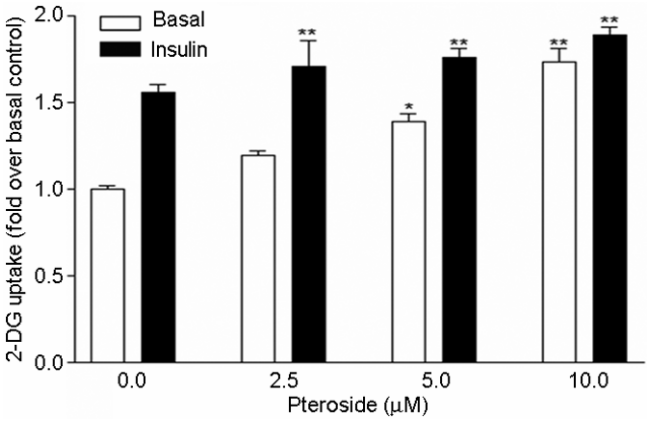

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