Antihyperglycaemic, antihyperlipidaemic and β cell regenerative effects of Spondias pinnata (Linn.f.) Kurz. bark extract on streptozotocin induced diabetic rats

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Antihyperglycaemic, antihyperlipidaemic and β cell regenerative effects of *Spondias pinnata* (Linn. f.) Kurz. bark extract on streptozotocin induced diabetic rats

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Abstract

Introduction: In Sri Lanka, *Spondias pinnata* (Linn. f.) Kurz (Family: Anacardiaceae) is popular in traditional Ayurvedic medicine for the treatment of diabetes mellitus. This study investigated the effect of aqueous bark extract of *S. pinnata* on antihyperglycaemic, antihyperlipidaemic activities and β-cell regenerative potential in streptozotocin induced diabetic rats. Dose, long term effects and acute toxicity were also investigated.

Methodology: A series of experimental studies were conducted using *S. pinnata*. Diabetes was induced in rats by injecting them with streptozotocin (65 mg/kg, ip). Control rats were either healthy untreated, or diabetes induced untreated rats which only received distilled water. Diabetic rats were treated with different dose to identify the optimum effective dose of *S. pinnata*. having identified the optimum dose (1.00 g/kg) and glibenclamide (0.50 mg/kg) was given daily for 30 days. The assessment of biochemical parameters, histopathology and immunohistochemistry of the pancreas occurred at 30 days.

Results: The percentage of glycosylated hemoglobin decreased together with a concomitant increase in the concentration of serum insulin and C-peptide in *S. pinnata* treated diabetic rats ($p < 0.05$). Serum lipid parameters improved for *S. pinnata* treated diabetic rats ($p < 0.05$). Islet cell regeneration in *S. pinnata* treated diabetic rats was noted by the percentage increase in insulin secreting β-cells and increase in islet profile diameter in the pancreas. A significant ($p < 0.05$) dose dependent improvement in glucose tolerance with optimum effectiveness at 1.00 g/kg was shown for the *S. pinnata* treated diabetic rats. No toxicity was observed.

Conclusions: Results revealed that aqueous bark extract of *S. pinnata* exerted antihypelipidaemic and insulinotropic effects through increased biosynthesis of insulin in streptozotocin induced diabetic rats. Histopathology and immunohistochemistry confirmed that *S. pinnata* extract was able to induce β-cell regeneration in the pancreas of diabetic rats.

Introduction

Investigation of antidiabetic mechanisms of medicinal plant extracts has been considered as one of the most promising research avenues in search of novel drug candidates for the treatment of diabetes mellitus. A number of plants are currently used for their antidiabetic properties together with their active bio-molecules based on scientific investigations. However, despite the recent advances made in medicinal plant research, gaining more insight into the antidiabetic mechanisms represents a challenge in current ethnopharmacological research [1].

The pharmacological treatment of diabetes includes oral hypoglycaemic agents such as insulin releasers, insulin sensitizers and α-glucosidase inhibitors that have modest efficacy and limited mode of action. In addition, current antidiabetic drugs

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as (glyburide, glimepiride, glipizide) usually have adverse side effects, decreased efficacy over time, hypoglycaemia, gastrointestinal disturbances and ineffectiveness against long term use [2]. In contrast, plant products are generally considered to be less toxic with fewer side effects than synthetic drugs and low cost [3]. Consequently, plant derived materials have received attention as active agents in the therapy of diabetes mellitus.

Spondias pinnata (Linn. f.) Kurz (Family: Anacardiaceae) is a deciduous tree distributed in Sri Lanka, India and other South-East Asian countries. The bark of the tree is used for treating dysentery, muscular rheumatism and diabetes mellitus in traditional Ayurvedic medicine [4]. Hypoglycaemic activity of chloroform, methanol and water extracts of *S. pinnata* in normoglycaemic and alloxan induced diabetic rats has been reported [5]. The isolation of 24-methylene cycloartanone, stigma-4en-3one, lignoceric acid, β-sitosterol and its β-D-glucoside from *S. pinnata* have described previously [6]. In addition, the *in vitro* antioxidant activities of 70% methanol extract of *S. pinnata* have been described [7]. However, effect of the *S. pinnata* on antihyperglycaemic mechanisms and antihyperlipidaemic activity has not been tested on diabetic rats. Therefore, this study was aimed at investigating antidiabetic effects of bark extract of *S. pinnata* to investigate the potency of the extract to induce β-cell regeneration in the pancreas of streptozotocin induced diabetic rats.

Materials and methods

Chemicals

D-Glucose, glibenclamide and streptozotocin were purchased from Sigma–Aldrich Company (St. Louis, MO, USA).

Plant material

Stem bark parts of *S. pinnata* were collected during May–June 2013 from the Southern region of Sri Lanka. Botanical identity was determined by the descriptions given by Jayaweera [2] and confirmed by comparing authentic samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen was preserved at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (Attanayake/2011/01).

Preparation of the aqueous plant extract

The bark parts of *S. pinnata* were cut into small pieces, dried at 40 °C until a constant weight was reached and coarsely ground. Powdered plant material (50.00 g) was dissolved in 400.0 mL of distilled water and refluxed for 4 h. The mixture was strained and the final volume was adjusted to 50.0 mL. The concentrated *S. pinnata* extract was prepared. A single dose of 0.25, 0.50, 0.75, 1.00, 1.25 and 2.00 g/kg was administered orally to streptozotocin induced diabetic rats to evaluate the efficacy of inducing antihyperglycaemic activity. Acute toxicity screening was also carried out in healthy Wistar rats.

Animals

Healthy adult male rats of Wistar strain (200 ± 25 g, body weight) were purchased from Medical Research Institute (MRI), Sri Lanka and used to carry out the experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka (Tem 25 ± 2 °C, relative humidity 55–65% and 12 ± 1 h light/dark cycle). Rats were fed with standard diet (MRI rat formulae, Sri Lanka) with free access to water before and during the experiment. The rats were randomized into various groups and allowed to acclimatize for a period of seven days under standard environmental conditions before commencing the experiments. The animals described as fasting were deprived of food and water for 12 h *ad libitum*. All protocols used in this study were approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka guided by the Council for International Organization of Medical Sciences (CIOMS) international guiding principles of biomedical research involving animals.

Development of diabetes mellitus in Wistar rats

Streptozotocin dissolved in citrate buffer (0.1 M, pH 4.4) at a dose of 65 mg/kg was administered intraperitonially to rats fasted for 12 h. Thereafter, rats were maintained on 5% D-glucose solution for the next 24 h. Rats were allowed to stabilize for three days thereafter and on the 4th day, blood samples were drawn from tail vein to determine the blood glucose concentration in order to confirm the development of diabetes mellitus. Rats with fasting blood glucose concentration of 12.0 mmol/L or above were considered as hyperglycaemic and used for the experiments [8].

Three experimental studies were conducted.

Study 1 – The efficacy of antihyperglycaemic activity in diabetic rats

The first and second groups were untreated healthy and untreated diabetic rats ($n = 6$/group). Group three to eight consisted of six sub groups ($n = 6$/subgroup); streptozotocin induced diabetic rats which received different doses (0.25, 0.50, 0.75, 1.00, 1.25 and 2.00 g/kg) of the extract of *S. pinnata* orally. The ninth group was administered glibenclamide (0.50 mg/kg) which served as the positive control. The rats were given an oral dose of glucose (3.00 g/kg) 30 min after the administration of the *S. pinnata* extract and glibenclamide. Blood samples were collected in fasting (0) and at 1, 2, 3 and 4 h after the administration of the extract/drug subsequently. Blood glucose concentration was measured immediately by the glucose-oxidase method using a glucose assay kit based on the Trinder reaction [9]. The acute effect was evaluated over a 4 h period using area under the oral glucose tolerance curve [10]. The optimum effective antihyperglycaemic dose in diabetic rats was determined.

Study 2 – Acute toxicity study in healthy rats

Six groups containing healthy male Wistar rats ($n = 6$/group) received aqueous bark extract of *S. pinnata* at doses of 0.25,

0.50, 0.75, 1.00, 1.25 and 2.00 g/kg, orally while the untreated healthy control group received distilled water.

Animals were observed individually after dosing once during the first 30 min, periodically during the first 24 h and daily for a total of 14 days. Observations included changes in skin, fur, eyes and behavior pattern. Special attention was directed to the observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Study 3 – Experimental group design- long term treatment

Group one and two served as the healthy untreated, streptozotocin induced diabetic control rats initially received distilled water respectively ($n = 6$/group). Group three and four were streptozotocin induced diabetic rats, received the optimum effective dose of the extract *S. pinnata* (1.00 g/kg) and glibenclamide (0.50 mg/kg) daily for 30 days ($n = 6$/group).

At the end of the study (on the 30th day), animals were anaesthetized by diethyl ether and blood was collected by cardiac puncture. Serum was separated from blood of all fasted rats for the estimation of biochemical parameters. The pancreatic tissue was excised for the assessment of histopathology and immunohistochemistry.

Blood/serum glycaemic parameters in diabetic rats

Oral glucose tolerance test was performed for all groups on the 1st, 7th, 14th, 21st, 28th and 30th day. Blood glucose concentration was determined by using a spectrophotometric enzyme assay kit [9]. The antihyperglycaemic effect was evaluated over a 4 h period using area under the oral glucose tolerance curve [10]. The percentage of glycosylated hemoglobin (HbA$_{1C}$) and serum concentration of fructosamine were estimated in all rats using spectrophotometric enzyme assay kits [11,12]. Furthermore, the concentration of serum insulin and C-peptide in all rats were estimated using enzyme linked immune-sorbent assay methods [13,14].

Serum lipid parameters in diabetic rats

The concentration of serum total cholesterol (TC) high density lipoprotein cholesterol (HDL-C), triglyceride (TG) were estimated in all rats using spectrophotometric enzyme assay kits [15–17]. The concentrations of serum low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) were calculated using the Friedewald formulae [18].

Histology of the pancreas through histopathology and immunohistochemistry in diabetic rats

Paraffin embedded tissue blocks of the pancreas were used for detailed assessment of histopathology and immunohistochemistry. The sections of the pancreatic tissues were stained with hematoxylin and eosin for the light microscopic examination of histopathology changes of the pancreatic tissue in all rats. Histopathology score was developed for the assessment of selected histological parameters of destruction of islet cells and regeneration of islet cells [19]. The criteria for scoring the islet

cell destruction are as follows. Score 0 (normal): normal number of islet cells, score 1 (mild): loss of 1/3 of islet cells, score 2 (moderate): loss of 1/3 to 2/3 of islet cells, score 3 (severe): loss of more than 2/3 of islet cells. The criteria for scoring the regeneration are as follows. Score 0 (none): no regeneration, score 1 (mild): regeneration of 1/3 of islet cells, score 2 (moderate): regeneration of 1/3 to 2/3 of islet cells, score 3 (prominent): regeneration of more than 2/3 of islet cells. Immunohistochemical staining was done to confirm the presence of insulin secreting cells in the islets of pancreas in all rats. Dako polyclonal guinea pig anti-insulin and Dako REAL^{TM} En Visison^{TM}/HRP, Rabbit/Mouse were used for immunohistochemical staining. Islets were observed on light microscopy (high power field).

Islets were defined as small, average and large with an islet diameter of ≤ 125 μm, 126–149 μm and ≥ 150 μm respectively [20]. Four islets of each size in each rat (72 islets for each group) were chosen randomly [21]. The percentage of insulin secreting β-cells in islets and islet profile diameter were estimated [21,22].

Statistical analysis

Results are expressed as mean ± SEM for biochemical estimations. The quantitative data were analyzed by ANOVA followed by Dunnett's multiple comparison tests. The Kruskal–Wallis test was used for the semi quantitative analysis of histopathology score values. Results were considered to be significant at $p < 0.05$.

Results

Study 1 – Efficacy of antihyperglycaemic activity in diabetic rats

The mean total area under the curve values of *S. pinnata* extract treated diabetic rats for the six doses ranging from 0.25 to 2.00 g/kg are shown in Table 1. The *S. pinnata* extract showed a dose dependent improvement on glucose tolerance in diabetic rats. The total area under the curve during the 4 h period was significantly increased ($p < 0.05$) in streptozotocin induced diabetic rats as compared to healthy rats (70.96 ± 3.98 *vs*. 25.88 ± 0.25 mmol/L h). The *S. pinnata* treated diabetic rats showed a statistically significant percentage improvement at the doses 1.00, 1.25 and 2.00 g/kg ($p < 0.05$). The extract of *S. pinnata* showed the optimum effectiveness at the dose of 1.00 g/kg in diabetic rats (50.39 ± 2.45 mmol/L h, improvement of 29%). Glibenclamide treated diabetic rats demonstrated an improvement of 41% on glucose tolerance in diabetic rats.

Study 2 – Acute toxicity study in healthy rats

There was no mortality or morbidity observed in the rats through the 14 day period following single oral administration of all selected doses of the extract of *S. pinnata*. The animals did not show any changes in general appearance during the three day period. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy and unusual behavior were observed.

Table 1
Total area under the glucose tolerance curve values in diabetic rats.

Treatment	Total area under glucose tolerance curve value (mmol/L. h)					
	Dose of administration of plant extracts (g/kg)					
	0.25	0.50	0.75	1.00	1.25	2.00
Healthy untreated	25.88 ± 0.25					
Diabetic untreated	70.96 ± 3.98					
Spondias pinnata		68.30 ± 1.34	67.66 ± 1.01	67.36 ± 2.12	50.39 ± 2.45[*]	48.34 ± 0.12[*]	46.48 ± 2.34[*]
Glibenclamide (0.50 mg/kg)	41.67 ± 0.76[*]					

The values are expressed as mean $\pm$ SEM ($n = 6$/group).

[*] Statistically significant from streptozotocin induced diabetic control rats at $p < 0.05$ (ANOVA followed by Dunnett's test).

Study 3 – Experimental group design – Long term treatment study

Blood/serum glycaemic parameters

The long term effect of the extract of *S. pinnata* (1.00 g/kg) on fasting blood glucose concentration in diabetic rats is shown in Fig. 1. The healthy animals were normoglycaemic throughout the experimental period. The fasting blood glucose concentration of *S. pinnata* treated diabetic rats was reduced significantly on the 7th day onwards for the period of 30 days ($p < 0.05$). The reduction in fasting blood glucose concentration with the administration of *S. pinnata* and glibenclamide was 37% and 42%

in streptozotocin induced diabetic rats at the end of the study respectively ($p < 0.05$). The total area under the curve values of *S. pinnata* extract treated diabetic rats showed a statistically significant improvement of 38% on the 30th day ($p < 0.05$, Fig. 2). Effect of *S. pinnata* extract on the percentage of HbA_{1C}, concentration of fructosamine, insulin and C-peptide in streptozotocin induced diabetic rats are shown in Table 2. The diabetic rats treated with the *S. pinnata* extract exhibited a remarkable glycaemic control as evident by a reduction in the percentage of HbA_{1C}. The reduction in the percentage of HbA_{1C}, fructosamine was 25% and 26% in diabetic rats respectively. However, glibenclamide treated diabetic rats demonstrated a fall of 40% and
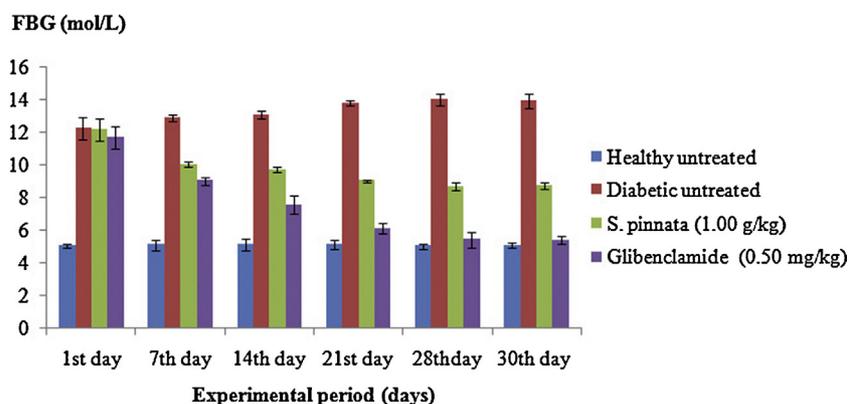
Fig. 1. Effect of aqueous bark extract of *Spondis pinnata* on fasting blood glucose concentration (FBG) in streptozotocin induced diabetic rats for 30 days. Data are expressed as mean $\pm$ SEM ($n = 6$/group).
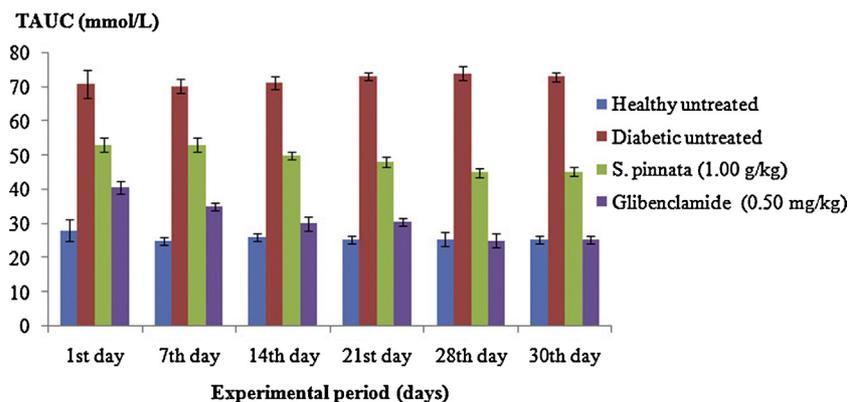
Fig. 2. Effect of aqueous bark extract of *Spondias pinnata* on total area under the curve (TAUC) values in streptozotocin induced diabetic rats for 30 days. Data are expressed as mean $\pm$ SEM ($n = 6$/group).

Table 2
Blood/serum glycaemic parameters in diabetic rats after 30 days of treatment.

Treatment	Glycosylated hemoglobin (%)	Fructosamine (μmol/L)	Insulin (μIU/mL)	C-peptide (ng/mL)
Healthy untreated	4.86 ± 0.10	221.88 ± 3.10	14.23 ± 0.44	9.53 ± 0.80
Diabetic untretaed	9.00 ± 0.09	405.39 ± 2.78	6.23 ± 0.09	5.75 ± 0.80
Spondias pinnata (1.00 g/kg)	6.75 ± 0.04*	303.51 ± 2.00*	8.81 ± 0.04*	6.78 ± 0.02*
Glibenclamide (0.50 mg/kg)	5.38 ± 0.06*	230.08 ± 0.99*	11.75 ± 0.02*	8.80 ± 0.01*

The values are expressed as mean ± SEM ($n = 6$/group).
* Statistically significant from streptozotocin induced diabetic control rats at $p < 0.05$ (ANOVA followed by Dunnett's test).

Table 3
Effect of *Spondias pinnata* on serum lipid parameters in streptozotocin induced diabetic rats after 30 days of treatment.

Treatment	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	VLDL-C (mmol/L)	TG (mmol/L)
Healthy untreated	3.70 ± 0.08	1.25 ± 0.03	2.21 ± 0.02	0.22 ± 0.01	1.10 ± 0.09
Diabetic untreated	5.80 ± 0.05	1.10 ± 0.01	4.27 ± 0.18	0.43 ± 0.00	2.14 ± 0.05
Spondias pinnata (1.00 g/kg)	4.67 ± 0.01*	1.17 ± 0.02*	3.18 ± 0.04*	0.32 ± 0.01*	1.62 ± 0.20*
Glibenclamide (0.50 mg/kg)	3.95 ± 0.06*	1.10 ± 0.09	2.63 ± 0.01*	0.22 ± 0.03*	1.12 ± 0.04*

The values are expressed as mean ± SEM ($n = 6$/group). TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, TG: Triglyceride.
* Statistically significant from streptozotocin induced diabetic control rats at $p < 0.05$ (ANOVA followed by Dunnett's test).

43% in the above parameters in diabetic rats. The concentration of serum insulin and C-peptide were increased significantly by 41%, 18% in *S. pinnata* extract treated diabetic rats respectively ($p < 0.05$). The concentration of serum TC, HDL-C, LDL-C, VLDL-C and TG in streptozotocin induced diabetic rats followed by the *S. pinnata* and glibenclamide treatments is shown in Table 3. The streptozotocin induced diabetic control rats had a significant elevation in the concentration of serum TC (57%), LDL-C (93%) VLDL-C (95%), TG (94%) and a reduction in HDL-C (12%) as compared with the untreated healthy control rats. The extract of *S. pinnata* treated streptozotocin induced diabetic rats showed a significant reduction in the concentration of serum TC (19%), LDL-C (26%), VLDL-C (26%), TG (26%) and an elevation in HDL-C (6%) on the 30th day of the study ($p < 0.05$). The concentration of serum TC, LDL-C, VLDL-C, TG were reduced by 32%, 38%, 49% and 48% in glibenclamide treated diabetic rats. In contrast, there was no significant change in the concentration of serum HDL-C with the glibenclamide treatment in diabetic rats ($p > 0.05$) (Table 4).

Histopathology and immunohistochemistry of the pancreas

Histopathological evaluation of the pancreas of streptozotocin induced diabetic rats showed an extensive destruction of

Table 4
Semi-quantitative analysis of pancreatic tissue on selected parameters in streptozotocin induced diabetic rats after 30 days of *Spondias pinnata extract* treatment.

Treatment	Destruction of islet cells	Regeneration of islet cells
Healthy untreated	0	N/A
Diabetic untreated	3	0
Spondias pinnata (1.00 g/kg)	0*	2*
Glibenclamide (0.50 mg/kg)	1*	1*

0 – none, 1 – mild, 2 – moderate, 3 – severe/prominent.
* Statistically different from streptozotocin induced diabetic control rats at $p < 0.05$ (Kruskal–Wallis test).

islets (Fig. 3B, score = 3). The healthy control rats had islets with no evidence of destruction (Fig. 3A, score = 0). There was a definite reduction of number of islets in diabetic rats compared to the number of islets in healthy rats. Although there was a significant destruction of islets by streptozotocin, there was no evidence that it caused any destruction of acinar tissue in diabetic rats compared to the healthy rats (Fig. 3B). A restoration of pancreatic islet cells with prominent islets were observed in *S. pinnata* treated diabetic rats (Fig. 3C, score = 2)

Immunohistochemical staining with anti-insulin antibody confirmed a marked reduction (more than 90% reduction) in insulin secreting cells in small, average and large size islets in diabetic control rats (Table 5 and Fig. 4B). The mean diameter of islets was also reduced in small (63%), average (8%) and large (13%) islets in diabetic control rats as compared with the normal control rats reflecting the significant reduction in the islet cell mass induced by streptozotocin.

Immunohistochemical staining of sections from *S. pinnata* extract treated diabetic rats with anti-insulin antibody, confirmed that there was a significant increase in the percentage of insulin secreting cells in the islets of all three sizes (small, average and large) compared to the diabetic controls (Fig. 4C). This increase was statistically significant (Table 5, $p < 0.05$). The number of islets was also increased in *S. pinnata* extract treated diabetic rats compared to diabetic control rats. Further the *S. pinnata* extract produced a significant increase in the mean profile diameter in large islets (6%) as compared to streptozotocin induced diabetic control rats ($p < 0.05$). Although the increase in the percentage of insulin secreting cells in islets and the increase in insulin secreting cell mass do not reach the insulin secreting cell mass in healthy normal rats, there is a significant increase in the insulin secreting cell mass reflecting a significant regeneration of insulin secreting cells induced by *S. pinnata* extract.

There was an improvement in the percentage of β-cells in glibenclamide treated diabetic rats (Table 5 and Fig. 4D). However, the increment was less than that of *S. pinnata* extract treated
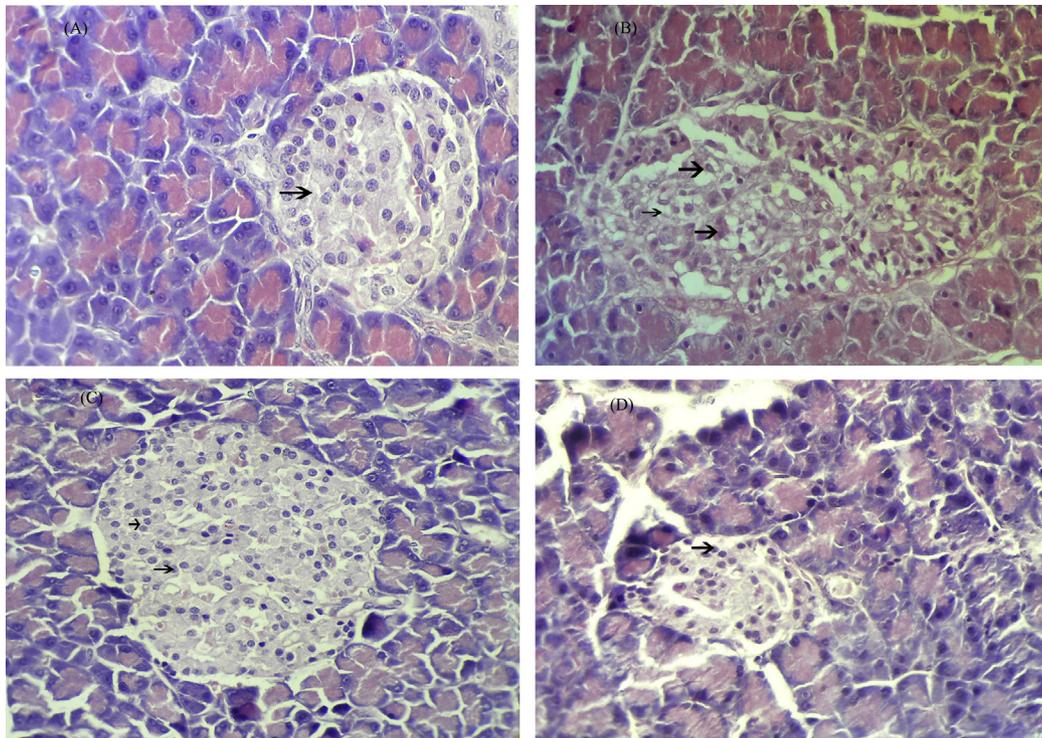
Fig. 3. Photomicrographs of histopathology of the pancreatic tissues, stained with hematoxylin and eosin (400×). (A) Healthy control rats, islets of Langerhans with normal islet cell population. (B) Diabetic control rats, an islet with few preserved islet cells, fibrosis and infiltration by inflammatory cells. (C) *Spondias pinnata* treated (1.00 g/kg) diabetic rats, restoration of pancreatic islet cells with prominent islets. (D) Glibenclamide treated (0.50 mg/kg) diabetic rats, reduced number of islet cells.
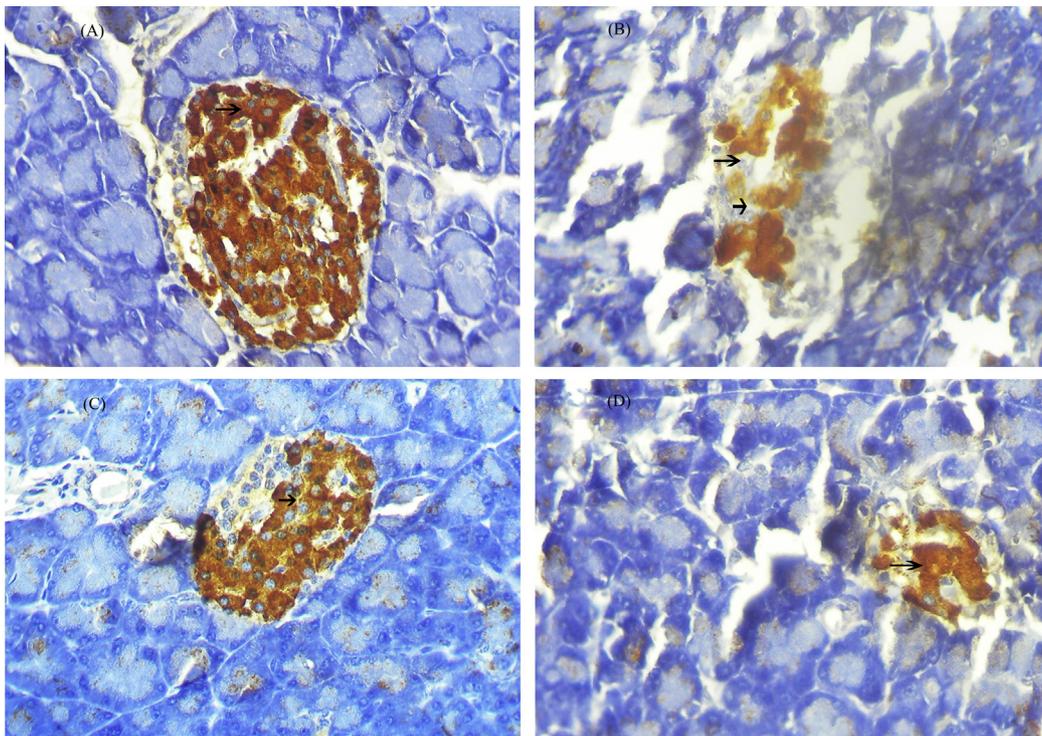
Fig. 4. Photomicrographs of insulin immunoreactivity in pancreatic islets with anti-insulin antibody (400×). (A) Healthy control rats, a normal islet composed predominantly of insulin secreting cells. (B) Diabetic control rats, marked reduction in the number of insulin secreting β-cells due to the destruction of islet cells by streptozotocin. (C) *Spondis pinnata* treated (1.00 g/kg) rats, an islet with a marked increase in insulin secreting β-cells. (D) Glibenclamide treated (0.50 mg/kg) rats, mild increase in insulin secreting β-cells.

Table 5

Effect of *Spondias pinnata* extract on percentage of insulin secreting β-cells and diameter of islets in the pancreas of streptozotocin induced diabetic rats after 30 days of treatment.

Treatment	Percentage area of insulin secreting cells in islets (%)			Diameter of islets (μm)		
	Small	Average	Large	Small	Average	Large
Healthy untreated	86.17 ± 3.54	72.00 ± 3.90	78.33 ± 7.53	86.80 ± 1.32	138.50 ± 5.57	173.16 ± 8.97
Diabetic untreated	9.17 ± 0.91	7.50 ± 1.23	6.83 ± 0.87	32.34 ± 1.55	127.43 ± 2.70	153.05 ± 0.37
Spondias pinnata (1.00 g/kg)	40.12 ± 3.23[*]	36.89 ± 5.34[*]	43.23 ± 3.12[*]	34.34 ± 2.4	126.89 ± 9.00	160.89 ± 2.78[*]
Glibenclamide (0.50 mg/kg)	33.33 ± 2.34[*]	10.00 ± 0.15[*]	7.17 ± 1.42[*]	36.10 ± 3.31	128.38 ± 1.99	154.08 ± 5.88

The values are expressed as mean ± SEM ($n = 6$/group).

[*] Statistically significant from streptozotocin induced diabetic control rats at $p < 0.05$ (ANOVA followed by Dunnett's test).

diabetic rats. There was a no change in the islet profile diameter of glibenclamide treated streptozotocin induced diabetic rats (Table 5, $p > 0.05$).

Discussion

The present study investigates the effect of aqueous bark extract of *S. pinnata* (1.00 g/kg) on serum/blood glycaemic parameters, serum lipid parameters and regenerative potential of islet cells in streptozotocin induced diabetic rats. The overall aim of this study was to investigate the possibility that *S. pinnata* extract might induce islet cell regeneration and biosynthesis of insulin in diabetic rats.

Refluxed aqueous extract was used in this study with the consideration that it may be more relevant nutritionally and medicinally to humans. This method of preparation is generally used by traditional Ayurvedic practitioners in Sri Lanka since antiquity.

Streptozotocin induced diabetes mellitus in rats is a well-recognized model for the screening of claimed antidiabetic agents [23]. Indeed, selection of a non-insulin dependent model is more suitable in assessing the efficacy of antidiabetic plants and the mechanism of action *in vivo* [24]. Diabetogenic action of streptozotocin occurs due to synergistic actions of DNA alkylation followed by fragmentation of DNA (deoxyribonucleic acid), activation of poly ADP (adenosine diphosphate)-ribose polymerase result in the inhibition of synthesis and secretion of insulin [25]. The blood glucose concentration in streptozotocin induced diabetic rats at the particular dose was in agreement with previously published reports [26]. Accordingly there was a significant difference in associated glycaemic parameters as HbA$_{1C}$, serum fructosamine, insulin, C-peptide and lipid parameters compared to healthy untreated rats. This is in congruence with other reports mentioning that streptozotocin induced diabetic rats are well recognized in representing distinct elevation in the above biochemical parameters [27,28].

Glibenclamide was used as the standard drug in the present study. It has been proposed that sulphonylureas produce antihyperglycaemic effects through secretion of insulin [29]. Glibenclamide has been widely accepted as a standard drug in diabetic animal experiments associated with mild or moderate hyperglycaemia [30].

The selection of a graded dose range (0.25–2.00 g/kg) was based on the extrapolated value of human therapeutic dose of the *S. pinnata* extract [31]. The extent of reduction in blood glucose concentration/improvement on glucose tolerance with the *S. pinnata* treatment over a period of four hours was evaluated using the total area under oral glucose tolerance curve as described previously [32]. In the present study, *S. pinnata* extract showed dose dependent percentage improvement on oral glucose tolerance and a reduction in blood glucose concentration in diabetic rats. The dose of *S. pinnata* extract at which showed the optimum improvement on glucose tolerance was selected for the investigation of antidiabetic mechanisms in diabetic rats.

There was a significant reduction in the percentage of HbA$_{1C}$ in *S. pinnata* treated diabetic rats. This may further confirm the potential long term antihyperglycaemic effect of the extract of *S. pinnata*. However, the reduction in the percentage of HbA$_{1C}$ in glibenclamide treated diabetic rats was superior to the reduction in *S. pinnata* extract treated diabetic rats at the end of the study period (30th day). The antihyperglycaemic effect of glibenclamide at the same dose in streptozotocin induced diabetic rats is similarly reported [33].

Diabetes mellitus is usually associated with prominent levels of serum lipids and such an increase causes a risk factor for coronary heart diseases [34]. A variety of alterations in metabolic and regulatory mechanisms, due to insulin deficiency may responsible for the observed accumulation of lipids [35]. Streptozotocin induced diabetic rats also developed hyperlipidaemia which is in agreement with previous observations [36,37]. In the present study, the *S. pinnata* extract significantly reduced the concentrations of TC, TG, LDL-C and VLDL-C together with an increase of HDL-C concentration in *S. pinnata* extract treated diabetic rats. This may be due to the indirect antihyperglycaemic potency and insulinotropic effects of the *S. pinnata* extract in diabetic rats.

The estimation of serum fructosamine is important in the screening of a claimed antidiabetic agent intervened for a sub-chronic period as it indicates the average blood glucose concentration over a one/two weeks [38]. The concentration of serum fructosamine directly depends on the concentration of serum glucose. A statistically significant reduction in serum fructosamine was noted in the *S. pinnata* extract treated diabetic rats in the present study ($p < 0.05$). This could be due to low concentration of serum glucose as a result of antihyperglycaemic effect of the *S. pinnata* extract in diabetic rats.

Several studies have provided evidence that loss of functional β-cell mass through apoptosis, impaired proliferation

consequent to hyperglycaemia is central to the development of both type 1 and type 2 diabetes mellitus [39,40]. Indeed, it has been considered as a hallmark of both types of diabetes mellitus. Regulation of functional β-cell mass has been considered as a critical therapeutic challenge in patients with the disease [41,42]. The most promising approaches for the replenishment of the β-cell mass in diabetes mellitus include ecto-pancreatic transplantation, islet transplantation and/or implantation, triggering of *in vivo* islet cell regeneration and preserving the existing mass of β-cells [43,44]. However, islet cell regeneration has gained much interest and been considered as a strategy to restore the loss of β-cell mass in diabetes mellitus [45]. The detailed histopathology and immunohistochemistry of the pancreas in the present study revealed that the *S. pinnata* extract showed different degrees of islet cell regeneration in different sizes of islets in streptozotocin induced diabetic rats. The pancreatic tissue is able to regenerate in order to maintain or increase its β-cell mass in response to metabolic demands [46]. In addition, increase in the number of islets in *S. pinnata* extract treated diabetic rats demonstrated that neogenesis has also occurred apart from β-cell regeneration. The distinct increase in the percentage of insulin secreting β-cells and the diameter of large islets contributed to the regenerative potency which may be responsible for the increased concentrations of serum insulin and C-peptide in *S. pinnata* treated rats. Regeneration of β-cells is a contributing factor for increase in β-cell mass [47,48], and it could be suggested that the treatment with the *S. pinnata* extract increased the β-cell mass in diabetic rats. We suggest that the islet cell regeneration in diabetic rats with the *S. pinnata* extract could be due to replication of existing islet cells and/or differentiation (or neogenesis) from ductal or intra-islet pancreatic precursor cells [48]. However, this fact merits further investigation. Similarly, signs of regeneration of islets have been reported with the administration of other crude medicinal plant extracts such as leaf extract of *Carica papaya* and fruit extract of *Terminalia catappa* in diabetic animal models [49,50]. The assessment of histopathology and immunohistochemistry showed that *S. pinnata* extract has a better effect on islet cell regeneration as compared to the glibenclamide. This may be due to the direct regenerative potency of phytochemicals such as polyphenol compounds, flavonoids present in the *S. pinnata* extract [2]. Indeed, these phytochemicals are well documented for islet cell regeneration and enhancement of β-cell function *in vivo* [51,52]. In contrast, the other serum glycaemic and lipid parameters did not express the similar effect. It has been well established that glibenclamide produce antihyperglycaemic effects through secretion of insulin from the existing pancreatic β-cells and enhancement of insulin action on target tissues as the predominant antihyperglycaemic mechanisms [29] rather than through β-cell regeneration in the pancreas. However, the detailed β-cell regenerative mechanism of glibenclamide has not yet been reported.

Conclusions

The results revealed that the aqueous bark extract of *S. pinnata* exerted insulinotropic effects through increased biosynthesis of insulin in the pancreas of streptozotocin induced diabetic rats. In addition, the *S. pinnata* extract exerted potent antihyperlipidaemic activities in diabetic rats. The quantitative data of histopathology and immunohistochemistry confirmed that *S. pinnata* extract was able to induce β-cell regeneration in the pancreas of streptozotocin induced diabetic rats. The β-cell regeneration potency of *S. pinnata* extract may be useful in the development of novel antidiabetic agents especially for the therapy of type 1 diabetes mellitus.

Conflict of interest

The authors report no conflict of interest.

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