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RESEARCH ARTICLE

β-cell Regenerative Potential of Selected Herbal Extracts in Alloxan Induced Diabetic Rats

Anoja Priyadarshani Attanayake[1,*], Kamani Ayoma Perera Wijewardana Jayatilaka[1], Lakmini Kumari Boralugoda Mudduwa[2] and Chitra Pathirana[1]

[1]*Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Karapitiya, Sri Lanka and*
[2]*Department of Pathology, Faculty of Medicine, University of Ruhuna, Karapitiya, Sri Lanka*

Abstract: ***Background:*** Effective β-cell regeneration is a recognized therapeutic strategy in the treatment of type 1 diabetes mellitus. Regeneration of β-cells could be achieved via exogenous natural sources as medicinal plant extracts. Medicinal plants selected for the investigation were *Spondias pinnata* (Linn. f.) Kurz, *Coccinia grandis* (L.) Voigt and *Gmelina arborea* Roxb. The objective was to determine the β-cell re-generative potential of these plant extracts in alloxan-induced diabetic rats. Alloxan monohydrate was used to induce diabetes (150 mg/kg, ip).

Methods: Wistar albino rats were divided into six groups (n=6); healthy untreated rats (healthy control), alloxan-induced diabetic untreated rats (diabetic control), diabetic rats received the extracts (treatment groups) of *S. pinnata* (1.0 g/kg), *C. grandis* (0.75 g/kg), *G. arobrea* (1.00 g/kg) and diabetic rats received glibenclamide (0.5 mg/kg; positive control). The above treatment was continued for 30 days. On the 30th day, the rats were sacrificed and biochemical parameters were determined. In addition, histopathology and immunohistochemistry on the pancreatic tissue were done on the 30th day.

Results: According to the results obtained for biochemical parameters, there was a significant increase in the concentrations of serum insulin and C-peptide in plant extracts treated diabetic rats ($p < 0.05$). The extract of *C. grandis* produced the highest degree of β-cell regeneration demonstrated through an increase in the number of islets and percentage of the insulin-secreting β-cells (75%) in the pancreas of diabetic rats ($p < 0.05$) based on the histopathology and immunohistochemistry findings.

Conclusion: The results revealed that the selected extracts of *C. grandis* (0.75 g/kg), *G. arborea* (1.00 g/kg) and *S. pinnata* (1.00 g/kg) exerted β-cell regenerative potential in diabetic rats. The three plant extracts would be valued as natural agents of prompt-ing the β-cell regeneration *in vivo*.

*Address correspondence to this author at the Department of Bio-chemistry, Faculty of Medicine, University of Ruhuna, Karapitiya, Galle 80000, Sri Lanka; Tel: 94 71 4428121; Fax: 94 91 2222314; E-mail: anoja715@yahoo.com

1. INTRODUCTION

Diabetes mellitus is a heterogenic disease associated with a multitude of devasting complica-

tions. The pathophysiology of diabetes are multiple and therefore would require more than an active hypoglycaemic agent to reverse all or majority of harmful aspects of the disease. The effective therapeutic approach should be multimodal and in this regard, natural extracts have been preferred as it contains a plethora of active ingredients [1].

Type 1 diabetes mellitus is an auto-immune condition in which the immune system is activated to destroy the β cells in the pancreas which pro-duce insulin. The chronic hyperglycaemia, oxida-tive stress and inflammation consequently lead to β-cell dysfunction, apoptosis and necrosis. Loss of β-cell number and function underlies much of the pathophysiology of type 1 diabetes [2, 3]. There-fore, ways to preserve or uphold pancreatic β-cell mass and functions could be of great importance in type 1 diabetes mellitus. This strategy is inspiring as most of the current therapies for diabetes target on managing the symptoms of the disease rather than stimulating β-cell functions. Regeneration of β-cells could be achieved via exogenous natural sources as medicinal plant extracts. [4,5].

Medicinal plants are valued in indigenous systems of medicine for the treatment of various diseases including diabetes mellitus [6]. A number of Sri Lankan medicinal plants have been reported in the literature for their antidiabetic effects *in vivo* [7]. However, the mechanism is often not completely understood. Among the medicinal plants with Sri Lankan origin that has been investigated for diabetes, none has been reported to possess the regenerative property in β-cells *in vivo*.

The medicinal plants used for the present inves-tigation were *Spondias pinnata* (Linn. f.) Kurz (Family: Anacardiaceae), *Coccinia grandis* (L.) Voigt (Cucurbitaceae), *Gmelina arborea* Roxb (Family: Verbenaceae). The three plant species are distributed in tropical Asia, commonly found in the Southern region of Sri Lanka. Every part of these plants is valuable in medicine and decoction of the bark of *S. pinnata, C. grandis* and *G. arborea* is successfully employed for the treatment of diabetes mellitus by Ayurvedic physicians in Sri Lanka [7, 8, 9]. Extensive research has been done for the investigation on phytochemicals, *in vivo* and *in vitro* antioxidant potentials by our research group [10, 11, 12]. In addition, the optimum effec-tive antihyperglycaemic doses of the bark extract of *S. pinnata*, leaf extract of *C. grandis* and bark

extract of *G. arborea* were found to be 1.00, 0.75 and 1.00 g/kg in alloxan-induced diabetic rats, re-spectively [13]. The objective was realized to de-termine the β-cell regenerative potential of the plant extracts in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Chemicals and Instruments

Alloxan monohydrate, D-glucose and glibenclamide were purchased from Sigma- Al-drich Company (St Louise, MO, and United States). Chemicals were of analytical grade and used without any purification. A UV visible spec-trophotometer (Gallenkamp PLC, UK) and micro-plate reader (Mindray, China) were used for spec-trophotometric and enzyme linked immunosorbent assay (ELISA) measurements, respectively. Olympus CX 21(Japan) microscope was used in the assessment of histopathology and immuno-histochemistry of the pancreatic tissues.

2.2. Plant Material

The stem bark of *G. arborea*, *S. pinnata*, leaves of *C. grandis* were collected during May–June 2013 from the southern region of Sri Lanka.

The botanical identity of all plants was deter-mined by the descriptions given by Jayaweera, [8] and confirmed by comparing with the samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen has been deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (FM/09/Attanayake/1-3).

2.3. Preparation of the Aqueous Plant Extract

The stem bark of *G. arborea*, *S. pinnata*, leaves of *C. grandis* was 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. A single dose of the bark of *G. arborea* (1.0g/kg) and *S. pinnata* (1.0g/kg), leaves of *C. grandis* (0.75g/kg) was administered orally to alloxan induced diabet-ic rats.

2.4. 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. 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.

2.5. Development of Diabetes Mellitus in Wistar Rats

Alloxan monohydrate dissolved in sterile saline at a dose of 150 mg/kg was administered intraperitoneally to fasted rats [14]. Rats with fasting blood glucose concentration of 12.0 mmol/L or above were considered as hyperglycaemic and used for the experiments [15].

2.6. Experimental Group Design

Group one and two served as the healthy untreated, alloxan-induced diabetic untreated control rats and received distilled water (n=6/group). Group three, four, five and six were alloxan-induced diabetic rats, received the optimum effec-tive dose (extrapolated value of the equivalent human therapeutic dose) of the hot water refluxed extract *G. arborea* (1.00 g/kg), *S. pinnata* (1.00 g/kg), leaves of *C. grandis* (0.75 g/kg) and glibenclamide (0.50 mg/kg) daily for 30 days (n=6/group). All treatments including the plant extracts and glibenclamide were administered oral-ly using an appropriate rat gavage.

At the end of the study (on the 30th day), animals were eu-thanized by an overdose of diethyl ether and blood was collected by cardiac puncture. The collected blood was used for biochemical assays. The pan-creatic tissue was excised for the assessment of histopathology and immunohistochemistry.

2.7. Blood/Serum Glycaemic Parameters in Diabetic Rats

The percentage of glycated haemoglobin (HbA$_{1C}$) and serum concentration of fructosamine were estimated in all rats using spectrophotometric enzyme assay kits [16, 17]. Furthermore, the con-centrations of serum insulin and C-peptide in all rats were estimated using enzyme-linked immune-sorbent assay methods [18, 19].

2.8. Histology of the Pancreas Through Histo-pathology and Immunohistochemistry in Dia-betic Rats

Paraffin-embedded tissue blocks of the pancre-as were used for detailed assessment of histo-pathology and immunohistochemistry. Histo-pathology score was developed for the assessment of selected histological parameters of the destruction of islet cells and regeneration of islet cells [20]. The criteria for scoring the islet cell de-struction 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 regenera-tion are as follows. Score 0 (none): no regenera-tion, 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. Immunohistochemi-cal staining was done to confirm the presence of insulin-secreting cells in the islets of the pancreas in all rats. Dako polyclonal guinea pig anti-insulin and Dako REAL ^{TM} En Visison^{TM}/HRP, Rab-bit/Mouse were used for immunohistochemical staining. Islets were observed under light micros-copy (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 [21]. Four islets of each size in each rat (72 islets for each group) were chosen randomly. The per-centage of insulin secreting β-cells in islets and islet profile diameter was estimated [22].

2.9. Statistical Analysis

Results are expressed as mean ± SEM for bio-chemical estimations. The quantitative data were analyzed by ANOVA followed by Dunnett's mul-tiple comparison tests. The Kruskal- Wallis test was used for the semi-quantitative analysis of his-topathology score values. Results were considered to be significant at $p<0.05$.

3. RESULTS

3.1. Blood/Serum Glycaemic Parameters

The effect of plant extracts on the percentage of HbA$_{1C}$, concentration of fructosamine, insulin and C-peptide in alloxan-induced diabetic rats is shown in Table **1**. The reduction in the percentage of HbA$_{1C}$ was in the decreasing order of *C.*

Table 1. Effect of plant extracts on blood/serum glycaemic parameters in alloxan induced diabetic rats after 30 days of treatment.

Treatment	Glycated haemoglobin (%)	Fructosamine (µmol/L)	Insulin (µIU/mL)	C-peptide (ng/mL)
Healthy rats	4.86±0.29	219.88±2.00	14.89±0.12	9.48±0.05
Diabetic rats	9.81±0.08	441.11±3.02	6.05±0.18	5.37± 0.16
Diabetic rats + *S. pinnata* (1.00 g/kg)	6.98±0.02*	320.76±2.79*	8.09±0.15*	6.67±0.11*
Diabetic rats + *C. grandis* (0.75 g/kg)	6.38±0.04*	308.43±3.01*	10.42±0.05*	8.10±0.15*
Diabetic rats + *G. arborea* (1.00 g/kg)	6.74±0.06*	331.00±2.95*	8.74±0.10*	7.76±0.07*
Diabetic rats + Glibenclamide (0.50 mg/kg)	5.60±0.05*	280.16±2.05*	11.00±0.03*	8.60±0.06*

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

grandis (35%)*, G. arborea* (31%) and *S. pinnata* (29%) in alloxan-induced diabetic rats ($p < 0.05$). However, the glibenclamide treated diabetic rats demonstrated a fall in the percentage of HbA1c in alloxan induced (42%) diabetic rats ($p < 0.05$). The concentration of serum fructosamine, insulin and C-peptide was decreased significantly in a de-creasing order of *C. grandis* (30%, 72%, 51%)*, G. arborea* (25%, 44%, 44%) and *S. pinnata* (27%, 34%, 24%) in alloxan-induced diabetic rats ($p < 0.05$).

3.2. Histology of the Pancreas Through Histopathological and Immunohistochemical Assessment

As shown in Fig. **1b**, alloxan induced untreated diabetic rats showed an extensive destruction of islet cells as compared with the sections of pancre-as from healthy control rats. Further, there was a definite reduction in the number of islets in diabet-ic rats, compared to healthy rats. However, haemorrhages were not observed and acinar cells were intact in pancreatic tissues of alloxan-induced diabetic control rats. Further, severe in-flammatory cell infiltrations in islets were also seen in the diabetic control group. As shown in Fig. **2**, immunohistochemical staining with anti-insulin antibody confirmed a marked reduction in insulin-secreting cells in small, average and large size islets in an alloxan-induced diabetic rat model ($p < 0.05$). There was an increase in the number of islets in plant extracts treated diabetic rats when compared to untreated diabetic control rats. As shown in Table **2**, the sections from *C. grandis*
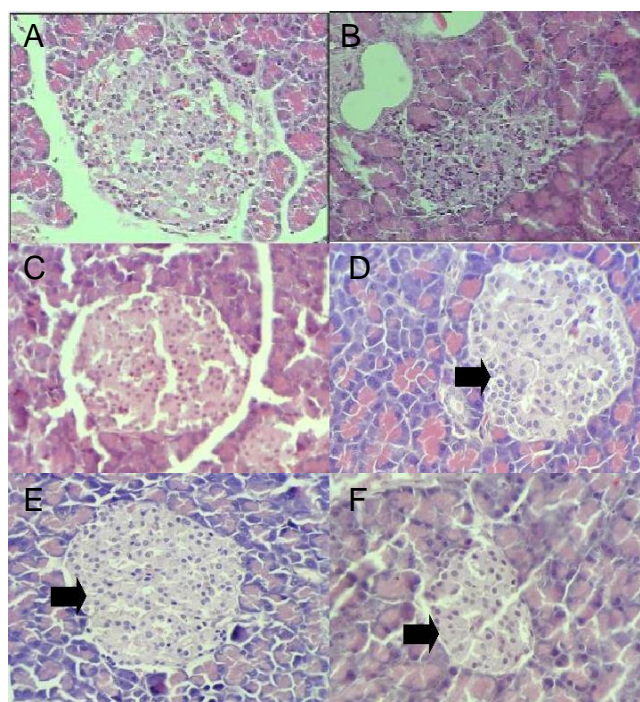

Fig. (1). Photomicrographs of H&E sections of the pancreatic tissue of alloxan induced diabetic rats after 30 days of plant treatment (x400); A: Healthy control rats Islets of Langerhans are normal with a normal islet cell population; B: Alloxan induced diabetic control rats Few preserved islet cells, fibrosis and infiltration by inflammatory cells s as a result of islet cell destruc-tion by alloxan; C: Alloxan diabetic rats + *S. pinnata* (1.00 g/kg), D: Alloxan diabetic rats + *C. grandis* (0.75g/kg), E: Alloxan diabetic rats + *G. arborea* (1.00 g/kg); C, D, E: Restoration of pancreatic islet cells and with no evidence of inflammation; F: Alloxan diabetic rats + Glibenclamide 0.50mg/kg) Pancreatic islet cells are restored with evidence of islet cell hyperplasia.

extract treated diabetic rats revealed a statistically significant score value for the regeneration of islet cells with some hyperplastic islets as compared with a diabetic untreated group (score value of 2 vs 0, *p*< 0.05). The extent of β-cell regeneration was in the decreasing order of *C. grandis, G. arborea, S. pinnata* reflected through the increased percentage of insulin secreting β-cells in alloxan-induced diabetic rats. Further, the *C. grandis* ex-tract produced a significant increase in mean pro-file diameter in small (118%), average (10%), and large (13%) islets as compared with diabetic con-trol rats, respectively (Table **3**). However, statisti-cally significant increase in the islet profile diame-ter was shown only in average (2%) and large (5%) islets in the *G. arborea* extract treated rats and large islets (5%) in *S. pinnata* extract treated diabetic rats (*p*< 0.05). Glibenclamide treated dia-betic rats showed a reduction in the number of in-flammatory cell infiltrations in islets as evident by the histopathological assessment with a mild in-crease in insulin-secreting β-cells with no islet cell hyperplasia. There was an improvement in the percentage of β-cells in glibenclamide treated dia-betic rats. However, the increment was less than that of plant extract treated diabetic rats. There was a no change in the islet profile diameter of glibenclamide treated alloxan-induced diabetic rats (*p*>0.05).

4. DISCUSSION

Natural products have a long history of being used as a medication and sources of drugs [23]. However, the problem in traditional medicine ap-

plications is that these have not scientifically scru-tinized and exact antidiabetic principle has not identified. The present study was aimed at investi-gating the potency of the bark extracts of *G. arborea* (1.0g/kg), *S. pinnata* (1.0g/kg), leaves of *C. grandis* (0.75g/kg) to induce β-cell regeneration in the pancreas of alloxan-induced diabetic rats.

Table 2. Semi-quantitative analysis of pancreatic tissue on selected parameters in alloxan induced dia-betic rats after 30 days of plant treatment.

Treatment	Destruc-tion of islet cells	Regenera-tion of islet cells
Healthy rats	0	N/A
Alloxan-diabetic rats	3	0
Alloxan-diabetic rats + *S. pinnata* (1.00 g/kg b.wt.)	0*	1*
Alloxan-diabetic rats + *C. grandis* (0.75 g/kg b.wt.)	0*	2*
Alloxan-diabetic rats + *G. arborea* (1.00 g/kg b.wt.)	0*	1*
Alloxan-diabetic rats + Glibenclamide (0.50 mg/kg b.wt.)	1*	1*

0-none, 1-mild, 2-moderate, 3-severe/prominent. *Statistically dif-ferent from alloxan induced diabetic control rats at *p* < 0.05 (Krus-kal-Wallis test).

The selection of medicinal plants for the pre-sent investigation was primarily based on the doc-umented effectiveness against diabetes mellitus, traditional use by Ayurvedic physicians and avail-

Table 3. Effect of plant extracts on percentage of insulin secreting β-cells and diameter of islets in the pancreas of alloxan induced diabetic rats after 30 days of treatment.

Treatment	Percentage of insulin secreting cells in islets (%)			Islet profile diameter (µm)		
	small	average	large	small	average	Large
Healthy rats	80.2±1.5	78.3±5.7	80.3±6.0	83.1±1.0	142.1±4.5	170.6±2.3
Diabetic rats	7.0±1.0	4.5±3.2	4.9±1.2	30.0±2.0	126.8±0.1	155.1±2.0
S. pinnata (1.00 g/kg)	35.1±4.7*	30.8±2.3*	33.2±5.2*	32.0±1.7	127.9±3.6	163.1±3.9*
C. grandis (0.75 g/kg)	75.3±2.0*	57.1±3.0*	51.8±4.0*	65.3±1.1*	139.0±1.2*	175.0±2.4*
G. arborea (1.00 g/kg)	43.2±3.3*	38.7±6.7*	39.0±6.8*	32.0±1.0	129.4±0.7*	163.4±1.7*
Glibenclamide	25.1±4.4*	9.0±1.9 *	7.1±1.1*	32.2±2.7	127.5±1.3	151.4±5.0

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

ability in the Sothern region of Sri Lanka. All these extracts are traditionally used in single prep-aration methods and as decoctions for the treat-ment of diabetes mellitus.
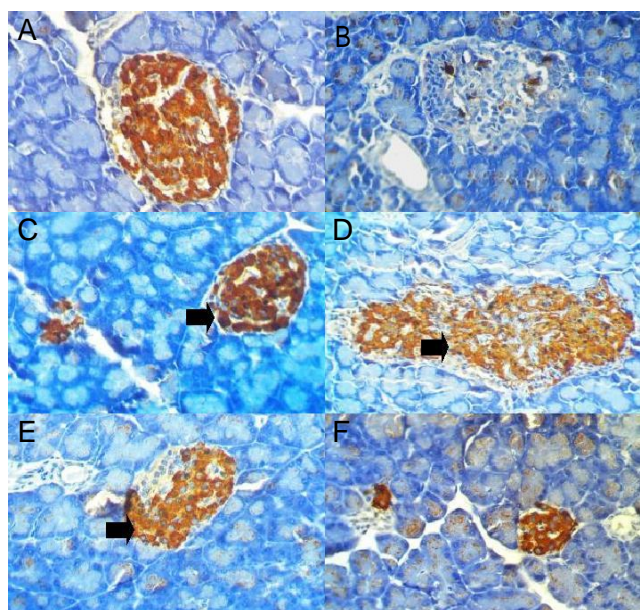

Fig. (2). Photomicrographs of insulin immunoreactivity in pancreatic islets of alloxan induced diabetic rats after 30 days of plant treatment (x400); A: Healthy control rats A normal islet composed predominantly of insulin secreting β-cells; B: Alloxan induced diabetic control rats Marked reduction in the number of β-cells due to the destruction of islet cells by alloxan; C: Alloxan di-abetic rats + *S. pinnata* (1.00 g/kg), D: Alloxan diabetic rats + *C. grandis* (0.75g/kg), E: Alloxan diabetic rats + *G. arborea* (1.00 g/kg); C, E,F: Increase in insulin se-creting β-cells in the islet; D: A hyperplastic islet with a marked increase in β-cells.

Alloxan-induced diabetic rat or mouse is one of the most currently used animal models for rapid screening of antidiabetic agents. A single intraper-itoneal injection of alloxan is well reported to induce insulin-dependent diabetes in rats [14]. Alloxan induces diabetes mellitus by destroying insulin-producing β-cells of the islets of Langerhans in the pancreas. The diabetogenic ef-fect of alloxan is initiated and maintained due to the excess production of reactive oxygen species leading to cytotoxicity in pancreatic β-cells which reduce the synthesis and release of insulin [24]. Our data showed that induction of diabetes by alloxan led to a significant increase of serum glu-cose concentration and a significant decrease in serum insulin and C-peptide concentrations com-pared to the healthy control rats. This was support-

ed by the assessment of histopathology in the pan-creas of diabetic rats. The histologic features of islets from the pancreas of diabetic animals in the present study are characterized by a decrease in the number of islets, inflammation and degranulation of the β-cells. This is in agreement with previous re-ports [25, 26].

The diabetic rats fed with the three extracts for 30 days exhibited a remarkable glycaemic control as demonstrated by a significant reduction in the percentage of HbA_{1c} in diabetic rats. This may further confirm the potential long-term antihyper-glycaemic effect of the extracts of *S. pinnata*, *C. grandis* and *G. arborea*. However, the reduction in the percentage of HbA_{1c} in glibenclamide treated diabetic rats was statistically significant to the re-duction in the plant extracts treated diabetic rats at the end of the study period (30th day). The antihy-perglycaemic effect of glibenclamide at the same dose in alloxan-induced diabetic rats is similarly reported by Borgohain *et al*. [27, 28].

Fructosamine, a glycated protein has a crucial role in the progression of many pathological con-ditions including diabetes mellitus [29]. The esti-mation of serum fructosamine is important in the screening of a claimed antidiabetic agent inter-vened for a sub-chronic period as it indicates the average blood glucose concentration over a one/two weeks [30]. The concentration of serum fructosamine directly depends on the concentration of serum glucose. A statistically significant reduc-tion in serum fructosamine was noted in the plant extracts treated diabetic rats in the present study ($P < 0.05$).

The histopathological and immunohistochemi-cal assessment of the pancreas in the present study revealed that the three extracts showed different degrees of islet cell regeneration in alloxan-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 [31, 32]. In addition, increase in the number of islets in plant extracts treated diabetic rats demonstrated that neogenesis has also occurred apart from β-cell regeneration. The leaf extract of *C. grandis* showed the highest degree of β-cell regeneration in diabetic rats as evident through the highest per-centage increase in the percentage of β-cells to-gether with the highest increase in islet cell profile diameter in the three types of islets. Accordingly,

the distinct increase in the percentage of insulin-secreting cells and diameter of small islets also contributed to the highest regenerative potency which may be responsible for the increased concentrations of serum insulin and C-peptide in *C. grandis* treated rats. A considerable percentage increase in insulin-secreting β-cells and increase in islet cell diameter in large (in the extracts of *S. pinnata* and *G. arborea*) average islets (in the extract of *G. arborea*) in diabetic rats are in agreement with the corresponding elevation in the concentration of serum C- peptide and insulin. Regeneration of β-cells is a contributing factor for the increase in β-cell mass and it could be suggested that the treatment with the three plant extracts increased the β-cell mass in diabetic rats [14]. Similar signs of regeneration of islets have been report-ed with the administration of other crude medici-nal plant extracts such as leaf extract of *Carica papaya* and fruit extract of *Terminalia catappa* in diabetic animal models [33, 34].

The topic of pancreatic β-cell regeneration is one of the most controversial topics of Type 1 dia-betes mellitus research and management. There is a possibility of regenerating the β-cells through the replication of pre-existing beta cells or ne-ogenesis from stem cells and progenitor cells in-side or outside the islets [35]. However, this needs to be clearly clarified in detail. Neogenesis can be originated from different cell types within the pan-creas: ∞-cells, δ-cells, duct epithelium, acinar cells, and centroacinar cells. However, this process depends on extra-pancreatic activators including hormones, growth factors, and others as suggested previously [35]. Therefore, further works on this issue are warranted. However, it was proved during body growth and after injury, beta cells can repli-cate to maintain glucose homeostasis [36]. The β-cell regenerative effects of the plant extracts are probably due to the presence of polyphenol compounds and flavonoids. The phytochemical analy-sis proved that the three plant sources are rich in above secondary metabolites. Indeed, these phyto-chemicals are well documented for islet regeneration and enhancement of β-cell function *in vivo* [37, 38].

CONCLUSION

The aqueous bark extract of *G. arborea* (1.0 g/kgb.wt.), leaf extract of *C. grandis* (0.75 g/kgb.wt.) and bark extract of *S. pinnata* (1.00 g/kgb.wt.) possess *in vivo* β-cell regeneration followed by increased synthesis of insulin in the pancreas of diabetic rats. The β-cell regeneration potency of *C. grandis, G. arborea* and *S. pinnata* extracts may be useful in the development of novel antidiabetic agents especially for the therapy of type 1 diabetes mellitus.

LIST OF ABBREVIATIONS

CIOMS = Council for International Organization of Medical Sciences

HbA_{1C} = glycated haemoglobin

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All protocols used in this study were approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka.

HUMAN AND ANIMAL RIGHTS

Animals were used for the study that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENT

REFERENCES

[1]   Akpaso MI, Atangwho IJ, Akpantah A, Fischer VA, Igiri AO, Ebong PE. Effect of combined leaf extracts of *Vernonia amygda-lina* (Bitter Leaf) and *Gongronema latifolium* (Utazi) on the pan-creatic β-cells of streptozotocin-induced diabetic rats. Br J Med Res 2011; 1: 24-34.

[2]   Ashcroft FM, Rorsman P. Diabetes mellitus and the β cell: The last ten years. Cell 2012; 148: 1160-71.

[3]   Ferrannini E. The stunned beta cell: A brief history. Cell Metab 2010; 11(5): 349-52.

[4]   Vetere A, Choudhary A, Burns SM, Wagner BK. Targeting the pancreatic β-cell to treat diabetes. Nat Rev Drug Discov 2014; 13: 278-89.

[5]   Hosseini A, Shafiee-Nick R, Ghorbani A. Pancreatic beta cell pro-tection/regeneration with phytotherapy. Braz J Pharm Sci 2015; 51: 1-16.

[6]   Coman C, Rugina OD, Socaciu C. Plants and natural compounds with antidiabetic action. Not Bot Horti Agrobo 2012; 40(1): 314-25.

[7]   Ediriweera ERHSS, Ratnasooriya WD. A review on herbs used in treatment of diabetes mellitus by Sri Lankan Ayurvedic and tradi-tional physicians. Ayu 2009; 30(4): 373-91.

[8]   Jayaweera DMA. Medicinal Plants (indigenous and exotic) used in Ceylon. Sri Lanka: National Science Foundation in Sri Lanka 1982.

[9]   Tiwari P, Ahmad K, Baig MH. *Gymnema sylvestre* for diabetes: From traditional herb to future's therapeutic. Curr Pharm Des 2017; 23(11): 1667-76.

[10]   Attanayake AP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. Phytochemical screening and *in vitro* antioxidant potentials of ex-tracts of ten medicinal plants used for the treatment of diabetes mellitus in Sri Lanka. Afr J Tradit Complement Altern Med 2015; 12(4): 28-33.

[11]   Attanayake AP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. Antioxidant activity of *Gmelina arborea* Roxb. (Verbenaceae) bark extract: *In vivo* and *in vitro* study. J Med Nutr Nutraceut 2015; 4(1): 32-38.

[12]   Attanayake AP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. Potential antioxidant activities of *Spondias pinnata* (family: Ana-cardiaceae) bark extract in rats with streptozotocin induced diabetes mellitus. Int J Pharmacogn 2015; 2: 166-72.

[13]   Attanayake AP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. Study of antihyperglycaemic activity of some Sri Lankan medicinal plants in alloxan induced diabetic rats. Anc Sci life 2013; 32(4): 193-8.

[14]   Ahmed MF, Kazim SM, Ghori SS, *et al.* Antidiabetic activity of *Vinca rosea* extracts in alloxan-induced diabetic rats. Int J Endo-crinol 2010; 2010: 841090.

[15]   Vasconcelos CF, Maranhao HM, Batista TM, Carneiro EM, Fer-reira F, Costa J. Hypoglycaemic activity and molecular mecha-nisms of *Caesalpinia ferrea Martius* bark extract on streptozotocin-induced diabetes in Wistar rats. J Ethnopharmacol 2011; 137(3): 1533-41.

[16]   Abraham EC, Huff TA, Cope ND, Wilson JB Jr, Bransome ED, Jr Huisman TH Determination of the glycosylated haemoglobins (HbA$_1$) with a new micro column procedure. Suitability of the technique for assessing the clinical management of diabetes melli-tus. Diabetes 1978; 27: 931-7.

[17]   Johnson RN, Metcalf PA, Baker JR. Fructosamine: A new ap-proach to the estimation of serum glycosylprotein. An index of dia-betic control. Clin Chim Acta 1982; 127(1): 87-95.

[18]   Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME. Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 1993; 39(4): 578-82.

[19]   Ashby JP, Frier BM. Circulating C-peptide: Measurement and clinical application. Ann Clin Biochem 1981; 18: 125-30.

[20]   Li QG, Sun R, Gao FZ. Effect of Shen Di Jiang Tang granules on diabetic rats. China J Chin Mater Med 2001; 26: 488-90.

[21]   Mac Gregor RR, Williams SJ, Tong PY, Kover K, Moore WV, Stehno-Bittel L. Small rat islets are superior to large islets in *in*

[22]   Elayat AA, El-Naggar MM, Tahir M. An immunocytochemical and morphometric study of the rat pancreatic islets. J Anat 1995; 186: 629-37.

[23]   Ghorbani A. Best herbs for managing diabetes: A review of clinical studies. Braz J Pharm Sci 2013; 49(3): 413-22.

[24]   Sakurai K, Ogiso T. Effect of ferritin on lambda DNA strand breaks in the reaction system of alloxan plus NADPH cytochrome P450 reductase: Ferritin's role in diabetogenic action of alloxan. Biol Pharm Bull 1995; 18(2): 262-6.

[25]   Adeyemi DO, Komolafe OA, Adewole OS, Obuotor EM, Abiodun AA, Adenowo TK. Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with extracts of *Annona muricata.* Folia Morphol 2010; 69(2): 92-100.

[26]   Aralelimath VR, Bhise SB. Anti-diabetic effects of *Gymnema sylvester* extract on streptozotocin induced diabetic rats and possi-ble β-cell protective and regenerative evaluations. Dig J Nanomater Biostruct 2012; 7(1): 135-42.

[27]   ADA-American Diabetes Association. Standards of medical care in diabetes. Diab Care 2013; 36**:** 11-66.

[28]   Borgohain R, Lahon K, Das S, Gohain K. Evaluation of mechanism of anti-diabetic activity of *Terminalia chebula* on alloxan and adrenaline induced diabetic albino rats. IJPBS 2012; 3: 256-66.

[29]   Salgado JM, Mansi DN, Gagliardi A. *Cissus sicyoides*: Analysis of glycaemic control in diabetic rats through biomarkers. J Med Food 2009; 12(4): 722-7.

[30]   Marca MC, Loste A, Ramos JJ. Effect of acute hyperglycaemia on the serum fructosamine and blood glycated haemoglobin concen-trations in canine samples. Vet Res Commun 2000; 24(1): 11-16.

[31]   Ackermann AM, Gannon M. Molecular regulation of pancreatic beta-cell mass development, maintenance and expansion. J Mol Endocrinol 2007; 38(1-2): 193-206.

[32]   Mellado-Gil JM, Cobo-Vuilleumier N, Gauthier BR. Islet β-cell mass preservation and regeneration in diabetes mellitus: Four fac-tors with potential therapeutic interest. J Transplant 2012; 2012: 230870.

[33]   Juarez-Rojop IE, Diaz-Zagoya JC, Ble-Castillo JL, *et al.* Hypogly-caemic effect of *Carica papaya* leaves in streptozotocin-induced diabetic rats. BMC Complement Altern Med 2012; 12: 236.

[34]   Nagappa AN, Thakurdesai PA, Venkat-Rao N, Singh J. Antidiabet-ic activity of *Terminalia catappa* Linn fruits. J Ethnopharmacol 2003; 88(1): 45-50.

[35]   Bouwens I, Rooman I. Regulation of pancreatic beta-cell mass. Physiol Rev 2005; 85(4): 1255-70.

[36]   Avolio F, Pfeifer A, Courtney M, Gjernes E, Ben-Othman N, Vieira A, Druelle N, Faurite B. and Collombat P. From pancreas morphogenesis to β-cell regeneration. Curr Topics Develop Biol 2013; 106: 217-38.

[37]   Ansarullah, Bharucha B, Umarani M, Dwivedi M, Laddha NC, Begum R. *Oreocnide integrifolia* flavonoids augment reprogram-ming for islet neogenesis and β-cell regeneration in pancreatecto-mized BALB/cMice. J Evid Based Complementary Altern Med 2012; 2012: 260467.

[38]   Babujanarthanam R, Kavitha P, Pandian MR. Quercitrin, a biofla-vonoid improves glucose homeostasis in streptozotocin-induced di-abetic tissues by altering glycolytic and gluconeogenic enzymes. Fundam Clin Pharmacol 2010; 24(3): 357-64.