Effect of madecassoside in reducing oxidative stress and blood glucose in streptozotocin-nicotinamide induced diabetes in rats

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Abstract

Madecassoside is a triterpenoid constituent of *Centella asiatica* (L.) Urb., an ethnomedical tropical plant, extracts of which were shown to reduce blood glucose in experimental diabetes. This study examines madecassoside for its anti-hyperglycaemic effects and tests the hypothesis that it reduces the blood glucose in experimentally induced diabetic rats by protecting the β-cells. Diabetes was induced in overnight-fasted Sprague-Dawley rats using streptozotocin (60 mg/kg, i.v.) followed by nicotinamide (210 mg/kg, i.p.). Madecassoside (50 mg/kg, selected on the basis of preliminary experiments) was administered orally daily for four weeks, commencing 15 days after induction of diabetes; resveratrol (10 mg/kg) was used as a positive control. Fasting blood glucose, plasma insulin, HbA1c, liver and lipid parameters were measured, along with enzymatic and nonenzymatic activities, lipid peroxidation, histological and immunohistochemical studies. Madecassoside normalized the elevated fasting blood glucose levels. This was associated with increased plasma insulin concentrations. Treatment of diabetic rats with madecassoside alleviated oxidative stress by improving enzymatic antioxidants and reducing lipid peroxidation. Histopathological examination of the pancreas of madecassoside-treated rats showed significant recovery of islet structural degeneration and an increased area of islets compared to diabetic control rats. Immunohistochemical staining showed increased insulin content in islets of madecassoside-treated rats. The results demonstrate an antidiabetic effect of madecassoside associated with preservation of β-cell structure and function.

Keywords: madecassoside, resveratrol, diabetes, pancreatic beta cell, natural product

1. Introduction

Diabetes mellitus (DM) is a chronic disease that is characterised by hyperglycaemia, resulting from deficient production of insulin from the pancreatic islet β-cells and/or impaired actions of insulin (Maritim et al., 2003) on several tissues including the liver (Skov et al., 2012). β-cell dysfunction and insulin resistance are the hallmarks of type 2 diabetes. Diabetes is a major global cause of protracted ill health and premature mortality (Wou et al., 2019). The long-term microvascular and macrovascular complications of diabetes resulting from chronic hyperglycaemia and hyperlipidaemia are the principal causes of morbidity and mortality (Taskinen, 2002; Wu and Parhofer, 2014). Chronic hyperglycaemia results in oxidative stress (Rochette et al., 2014;

Yaribeygi et al., 2019) that impairs β-cell function (Gerber and Rutter, 2017) and insulin signalling (Eriksson, 2007).

Extracts of *Centella asiatica* (L.) Urb. have been shown to lower blood glucose in diabetic rats (Kabir et al., 2014). A principal constituent of this plant is madecassoside (Fig. 1), a triterpenoid, which is an antioxidant having anti-inflammatory actions (Li et al., 2009; Wang et al., 2014) and showing myocardial- (Bian et al., 2008; Li et al., 2007) and neuroprotective effects (Mamun et al., 2014). The effects of madecassoside on blood glucose have not previously been investigated, although a related compound from *Centella asiatica*, asiatic acid, lowered blood glucose in various experimental models (Liu et al., 2010; Ramachandran et al., 2014; Sun et al., 2017; Xue et al., 2015).

The present study investigated the potential anti-hyperglycaemic effects of madecassoside in a rat model of diabetes. Resveratrol, an antioxidant with known antidiabetic actions (Ahmad and Gani, 2021; Chang et al., 2012; Diker and Kutluay, 2021; Hamadi et al., 2012; Mozafari et al., 2015; Su et al., 2006; Yang and Kang, 2018) was used as a positive control.

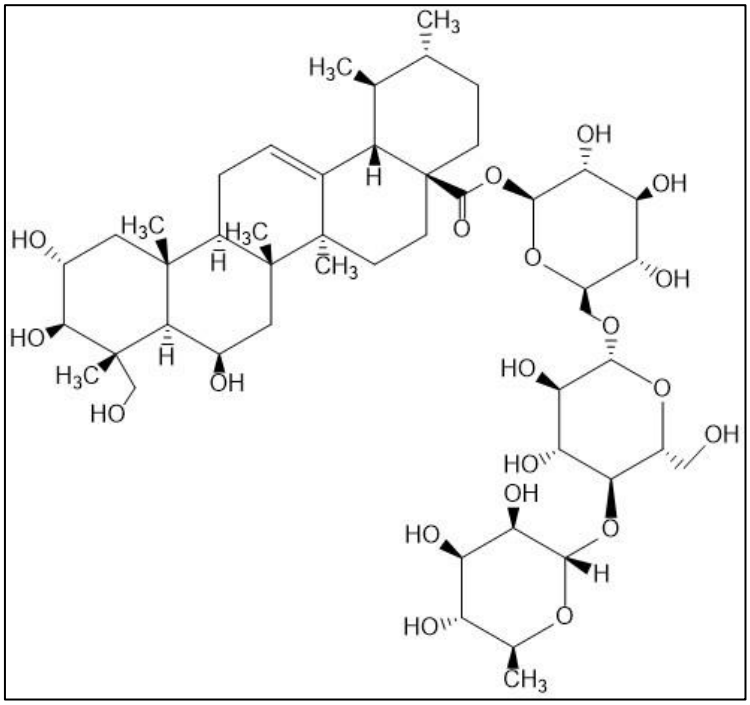

Figure 1. Chemical structure of madecassoside ($C_{48}H_{78}O_{20}$).

2. Materials & methods

2.1 Materials

2.1.1 Drugs

Madecassoside (M6949, Sigma, USA); Resveratrol (PI28587, PI Chemicals, Shanghai, China); Streptozotocin (CAS 18883-66-4, Santa Cruz Biotechnology Inc., USA); Nicotinamide (CAS 98-92-0, Santa Cruz Biotechnology Inc., USA)

2.1.2 Experimental work

Rodent chow pellets (Specialty Feeds, Western Australia); Rodent bedding (Chipsi Pet Bedding, Germany); Accu-Chek active glucometer (Roche Diagnostics, Germany); Accu-Chek active test strips (Roche Diagnostics, Germany); Yellow cap specimen bottles; Histology cassettes (Thermo Fisher Scientific, USA); Hirshmann® capillary tubes (Merck, Germany); BD Vacutainer® blood collection tubes (BD, USA); 10% Neutral buffered formalin (Thermo Fisher Scientific, USA); Methanol (Merck, Germany); Diethyl ether (Merck, Germany); Tissue ruptor probes (Qiagen, Germany)

2.1.3 Antioxidant assay kits

Malondialdehyde (MDA) (A003), Reduced glutathione (GSH) (A006-2), Glutathione peroxidase (GSH-Px) (A005), Superoxide dismutase (SOD) (A001-3) and Catalase (CAT) (A007-1) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2 Methodology

2.2.1 Experimental animals

Six- to eight-week-old male Sprague-Dawley rats (150-180 g) were obtained from Chenur Supplier (Malaysia) and maintained in a temperature (23 ± 2 °C) and light-controlled (12 h light/dark) room with 35-60% humidity in the animal holding facility (International Medical

University, Kuala Lumpur, Malaysia). The animals were acclimatized and provided with rodent chow pellet and water ad libitum throughout the study. The animal experimental protocols were performed in accordance with the animal experimentation guidelines set by IMU joint committee on research and ethics (IMU R 175/2015) and with internationally accepted principles for laboratory animal use and care.

2.2.2 Preliminary dose selection study

An oral glucose tolerance test (OGTT) study was performed to select an appropriate dose of madecassoside. Normal rats were treated with 12.5, 25 or 50 mg/kg, p.o. madecassoside for one week. OGTT was performed in the overnight-fasted rats on day 7. The normal animals received D-glucose (2.0 g/kg) by oral gavage. Blood glucose concentrations were measured in samples obtained from the tail vein before the glucose load and at 30, 60, 90, 120, 150 and 180 min after the glucose administration.

2.2.3 Induction of diabetes

Diabetes was induced by intravenous (i.v.) injection of freshly prepared STZ (60 mg/kg) dissolved in 0.1M citrate buffer (pH 4.5), followed by intraperitoneal (i.p.) injection of nicotinamide (210 mg/kg; dissolved in saline) 15 min after the STZ administration (Arya et al., 2015; Arya et al., 2012b). The development of hyperglycaemia was confirmed by elevations in blood glucose concentrations, determined at 72 h, and on the 7th day after induction; fasting blood glucose concentrations were determined in blood taken from the tail vein. Rats with fasting blood glucose concentrations of 8 - 15 mmol/L were considered to have diabetes: the blood glucose concentration in normal control rats averaged 6 mmol/L. Glucose measurement was performed using an Accu-check glucometer.

2.2.4 Experimental procedure

All rats were randomly divided into four groups, each group consisting of seven rats that were placed in individual cages.

Group 1: normal control rats treated with vehicle alone (5% Tween-80, p.o.)

Group 2: diabetic control rats treated with vehicle alone (5% Tween-80, p.o.)

Group 3: diabetic rats treated with madecassoside (50 mg/kg, p.o.)

Group 4: diabetic rats treated with resveratrol (10 mg/kg, p.o.)

Madecassoside was dissolved in water while resveratrol was dissolved in a vehicle containing 5% Tween-80 in distilled water. Treatment was started on the 15th day post induction, and it was given between 9:00 and 11:00 a.m. every day to all groups, via oral gavage once daily for 28 days. At the end of the experimental period, the rats were fasted overnight. Fasting blood glucose was determined in blood taken from the tail vein. Blood samples were then collected by retro-orbital sinus puncture using capillary tubes into heparinized blood collection tube under diethyl ether anaesthesia, followed by sacrificing via cervical dislocation. The blood samples were centrifuged at 4 °C at 3000 rpm for 15 minutes to separate plasma and erythrocytes. Then the supernatant containing plasma was aspirated and aliquoted into new labelled microcentrifuge tubes. They were stored at -80 °C for further biochemical analysis. Liver, kidney, pancreas, adipose tissue (abdomen), skeletal muscle (quadriceps) and heart were immediately dissected, washed in ice cold saline and snap frozen in liquid nitrogen before storage at -80 °C for biochemical analysis, or fixed in 10% neutral buffered formalin solution for histopathological examination.

2.2.5 Body weight, food and water intake

Changes in body weight of rats were noted, with the initial weight recorded on day 0 of treatment and at weekly intervals throughout the course of study.

2.2.6 Assessment of biochemical parameters

The blood glycated haemoglobin (HbA1c) was estimated by a DCA 2000 autoanalyzer device (Bayer, USA). Total cholesterol (CHOL), triglycerides (TG), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, aspartate transaminase (AST) and alanine transaminase (ALT) were determined using Dimension® clinical chemistry system (Siemens Healthcare Diagnostics Inc, USA).

2.2.7 Estimation of plasma insulin levels

Quantitative estimation of the plasma insulin concentration was undertaken using rat insulin ELISA kit (10-1250-01, Mercodia, USA) according to the manufacturers' instructions.

2.2.8 Estimation of antioxidants and lipid peroxidation levels

Tissue homogenates were used for estimation of malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT). Thiobarbituric acid reactive substances (TBARS) assay was used to measure lipid peroxidation end-product MDA, while GSH, GSH-Px, SOD and CAT were estimated as levels of endogenous antioxidants. All the colorimetric assay kits were conducted according to the manufacturers' instructions.

2.2.9 Histopathology

Pancreatic tissue was fixed in 10% formalin and processed with paraffin techniques. 5 µM thickness sections were cut and stained using haematoxylin and eosin. The observations were made, and pictures taken under Nikon Ti-U Microscope with NIS-Elements BR imaging software (Nikon Instruments Inc, Japan). The images obtained were further used for islets quantification using Image J Software.

2.2.10 Immunohistochemistry

Paraffin sections of 2-micron thickness were mounted on treated slides and dried in oven at 56 °C for 20 minutes. After progressive rehydration, the sections were then incubated with guinea pig anti-insulin polyclonal antibody (A0564, Agilent Dako, USA), and immunoreactivity was visualized as brown cytoplasmic staining using a Nikon Ti-U Microscope.

2.3 Statistical analysis

Data are expressed as mean ± standard error mean (SEM). Statistical comparisons were performed using the SPSS (Statistical Package for Social Science) by one-way analysis of

variance (ANOVA) followed by Dunnet's post-hoc test. Comparisons giving P values less than 0.05 were regarded as statistically significant.

3. Results

3.1 Preliminary dose selection study

OGTT was conducted to choose the appropriate dose for the main study. The area under the curve (AUC) of animals treated with 50 mg/kg madecassoside was significantly lower than control animals (Fig. 2), implying improved glucose tolerance compared to the control group. Therefore, 50 mg/kg was selected for further investigation in diabetic rats.

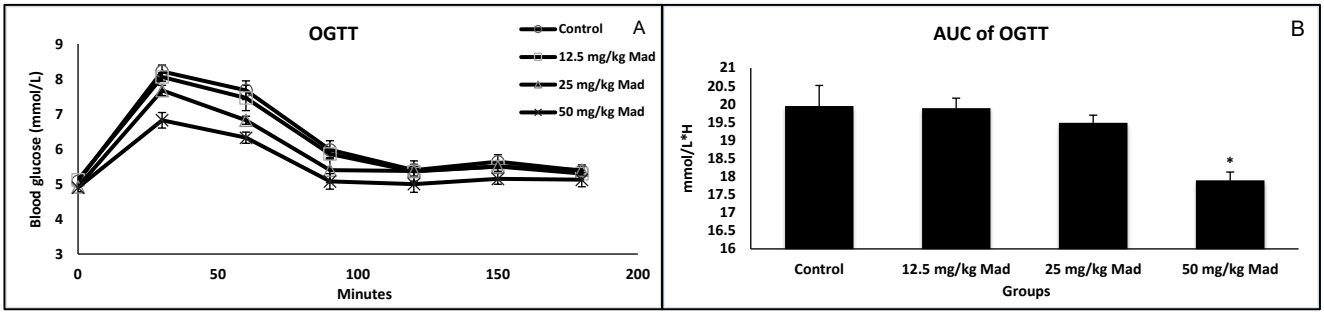

Figure 2. Effect of various madecassoside concentrations (12.5, 25 and 50 mg/kg) on OGTT. (A) OGTT curve (B) AUC of OGTT. Data are presented as mean ± SEM (n=7). * Denotes a significant difference compared to control (P < 0.001).

3.2 Body weight, food and water consumption

Table 1 shows the body weight of the treated groups compared to that of the diabetic control group. There was not much difference observed among the groups during the first three weeks. However, there was a reduction in body weight of the DC group compared to other groups in week 4, although this did not reach statistical significance. The cumulative food and water intake was significantly increased in the DC group (Fig. 3(A) and (B)), while madecassoside and resveratrol treated groups showed reduced food and water intake compared with the diabetic control group (P<0.05).

Table 1. Effect of madecassoside and resveratrol on body weight of normal and diabetic rats.

Treatment \ Week	W0	W1	W2	W3	W4
DC (5% Tween-80)	267.84 ± 15.07	282.83 ± 16.26	292.5 ± 13.80	292.49 ± 12.90	278.96 ± 8.54
MAD (50 mg/kg)	278.46 ± 10.89	293.94 ± 12.7	304.94 ± 8.29	315.61 ± 13.16	339.45 ± 17.02
RES (10 mg/kg)	260.59 ± 13.07	267.83 ± 23.64	295.24 ± 21.28	299.57 ± 22.92	354.23 ± 27.02
NDC (5% Tween-80)	233.21 ± 19.03	276.6 ± 11.07	284.6 ± 16.79	315.00 ± 14.30	335.67 ± 11.94

Effect of madecassoside and resveratrol on body weight of normal and diabetic rats. Data are presented as mean ± SEM (n=7). * Denotes a significant difference compared to diabetic control (P < 0.05). ǂ Denotes a significant difference compared to normal control group (P < 0.05).

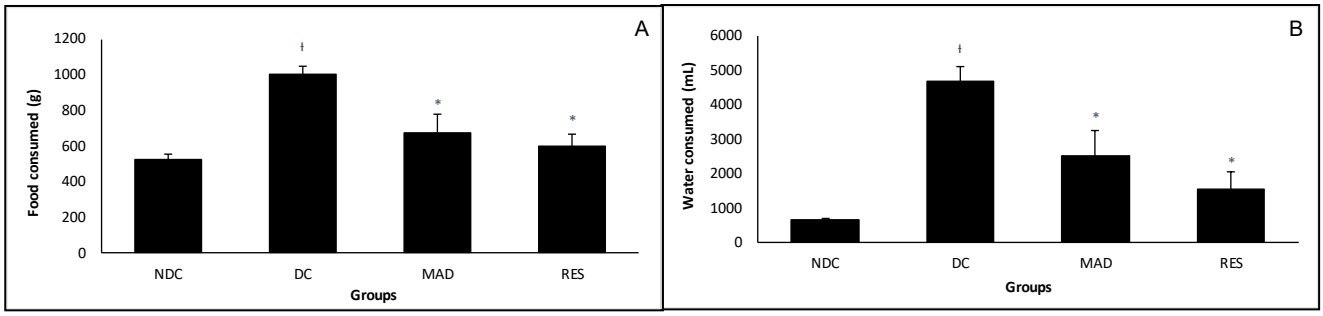

Figure 3. Effect of madecassoside and resveratrol on cumulative food consumption (A) and water consumption (B) of diabetic rats. Data are presented as mean ± SEM (n=7). * Denotes a significant difference compared to diabetic control (P < 0.05). ǂ Denotes a significant difference compared to normal control group (P < 0.05).

3.3 Biochemical parameters

The fasting blood glucose concentrations of NDC rats were not altered throughout the 28 days study period (Fig. 4A). On day 0 of treatment, the fasting blood glucose concentrations of the DC, MAD and RES groups were similar and significantly higher than that of the NDC group. 28-day treatment with either madecassoside or resveratrol produced a significant and marked reduction in fasting blood glucose compared with the diabetic control group (P<0.05). At the end of the study HbA1C values in the diabetic control group were significantly increased (P<0.05) compared to the non-diabetic control group. Treatment with either madecassoside or resveratrol significantly lowered the HbA1C values relative to the diabetic control group (P<0.001; Fig. 4B). Plasma insulin concentrations were significantly reduced in diabetic rats compared with the non-diabetic controls (P<0.05) (Fig. 4C), while treatment with either madecassoside or resveratrol produced a marked restoration (almost 2-fold) relative to the diabetic control rats (P<0.01). There were no significant differences in plasma concentrations of triglyceride (TRIG), total cholesterol (CHOL), low density lipoprotein cholesterol (LDL) or

high-density lipoprotein cholesterol (HDL) between normal and diabetic rats; moreover, these lipid parameters were not modified by treatment with either madecassoside or resveratrol (Fig. 4D). Plasma concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) were markedly elevated in diabetic rats compared with non-diabetic controls (P<0.001) (Fig. 4E). Treatment of diabetic rats with either madecassoside or resveratrol restored these liver function parameters to normal (Fig. 4E).

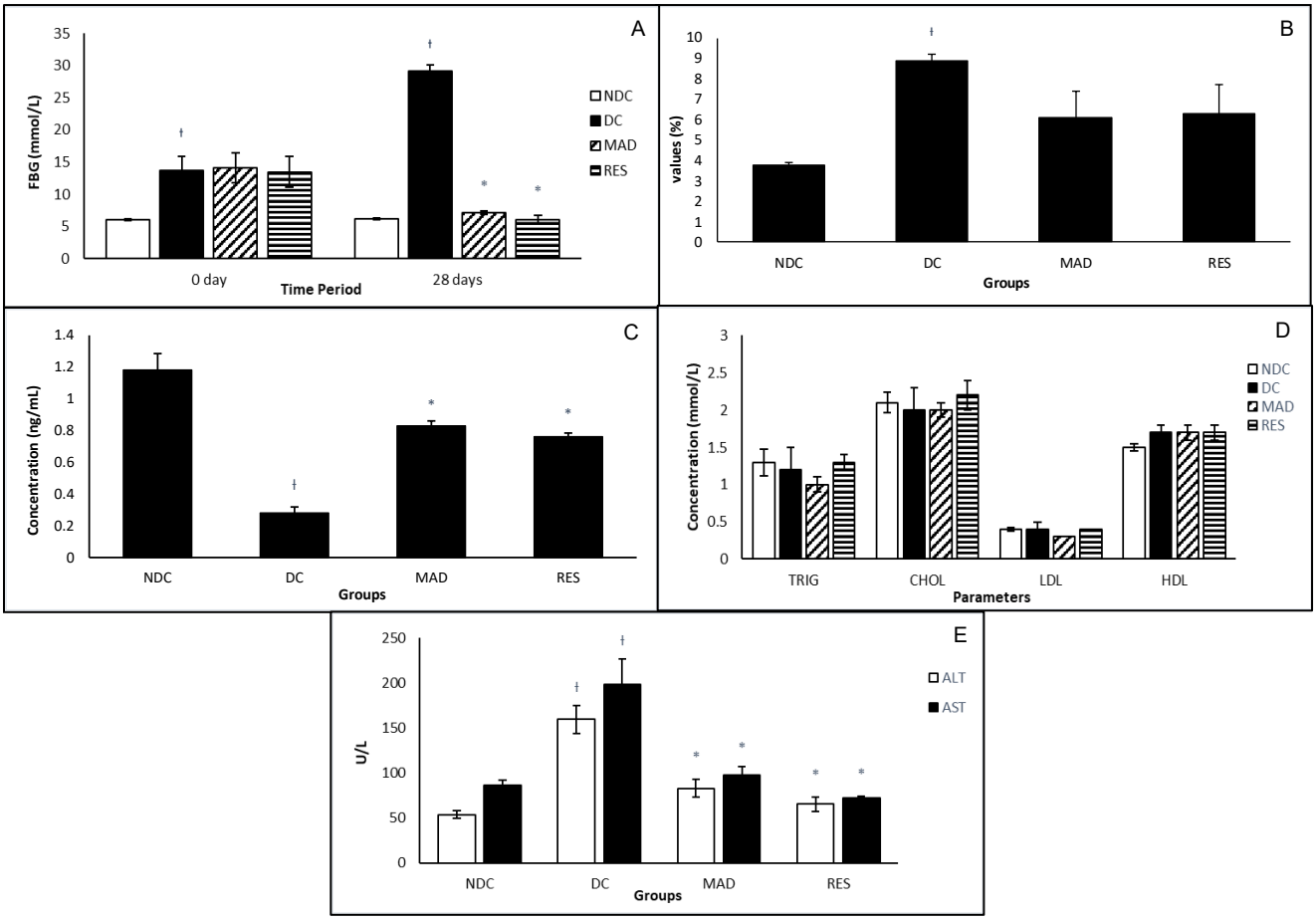

Figure 4. Effect of madecassoside and resveratrol on fasting blood glucose (A), HbA1C (B), plasma insulin concentrations (C), lipid parameters (D) and liver parameters (E). Data are presented as mean ± SEM (n=7). * Denotes a significant difference compared to diabetic control (P < 0.05). ǂ Denotes a significant difference compared to normal control group (P < 0.05).

3.4 Antioxidant activity in liver, kidney and pancreas

Catalase activity was markedly reduced in the pancreas, but not in the liver or kidney of untreated diabetic rats (P<0.05) (Table 2). Treatment with either madecassoside or resveratrol significantly elevated pancreatic catalase activity (P<0.05). There was a significant (P<0.05) reduction in glutathione peroxidase in liver and kidney from diabetic rats; the reduction seen in the pancreas was not statistically significant. However, treatment with either

madecassoside or resveratrol significantly increased glutathione peroxidase in all three tissues. GSH was markedly reduced in liver of diabetic rats compared to the non-diabetic control ($P<0.05$); treatment with madecassoside but not resveratrol restored hepatic GSH (3.7-fold increase compared with the diabetic control group, $P<0.05$). However, GSH levels were not significantly reduced in either kidney or pancreas of diabetic rats, although resveratrol produced a slight but significant increase in the pancreas. SOD levels were significantly reduced in the liver and pancreas of diabetic rats ($P<0.05$) but not in the kidney. Treatment with madecassoside significantly increased the SOD content in the pancreas of diabetic rats.

Table 2. Effect of madecassoside and resveratrol on antioxidants in normal and diabetic rats.

Enzymes analysed (unit of activity)	NDC	DC	MAD	RES
Catalase (U/mgprot)				
Liver	80.4 ± 2.5	69.9 ± 3.8	72.6 ± 3.4	73.8 ± 2.9
Kidney	33.9 ± 1.6	26.9 ± 1.3	34.2 ± 3.4	27.8 ± 1.8
Pancreas	5.2 ± 0.4	1.4 ± 0.1ǂ	3.3 ± 0.6*	3.2 ± 0.5*
Glutathione peroxidase (Unit)				
Liver	163.2 ± 7.4	140.6 ± 2.6ǂ	238.2 ± 3.8*	208.0 ± 5.8*
Kidney	138.7 ± 5.4	119.0 ± 4.2ǂ	151.2 ± 6.7*	142.3 ± 5.5*
Pancreas	12.1 ± 0.5	10.9 ± 0.8	14.7 ± 1.6*	14.5 ± 0.5*
Reduced glutathione (Umol/gprot)				
Liver	23.1 ± 0.8	4.8 ± 0.2ǂ	22.7 ± 0.4*	6.4 ± 0.2
Kidney	16.5 ± 0.8	14.6 ± 0.8	16.5 ± 0.6	16.8 ± 1.0
Pancreas	26.1 ± 0.9	23.6 ± 0.8	26.2 ± 1.4	28.0 ± 0.8*
Superoxide dismutase (U/mgprot)				
Liver	1.3 ± 0.0	1.0 ± 0.1ǂ	1.2 ± 0.1	1.1 ± 0.1
Kidney	1.6 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
Pancreas	4.8 ± 0.5	2.5 ± 0.1ǂ	4.3 ± 0.5*	3.0 ± 0.5

Effect of madecassoside and resveratrol on catalase, glutathione peroxidase, reduced glutathione and superoxide dismutase activity in liver, kidney and pancreas of diabetic rats. Data are presented as mean ± SEM (n=7). * Denotes a significant difference compared to diabetic control ($P < 0.05$). ǂ Denotes a significant difference compared to normal control group ($P < 0.05$).

3.5 Malondialdehyde (MDA) assay

The MDA content was markedly and significantly elevated in the liver of diabetic rats (Fig. 5A); this increase was significantly prevented by treatment with either madecassoside or resveratrol. While the increase in MDA content in the kidney was not significant (Fig. 5B), treatment with either madecassoside or resveratrol significantly reduced the MDA content in this tissue. No significant changes in MDA content were seen in the pancreas (Fig. 5C)

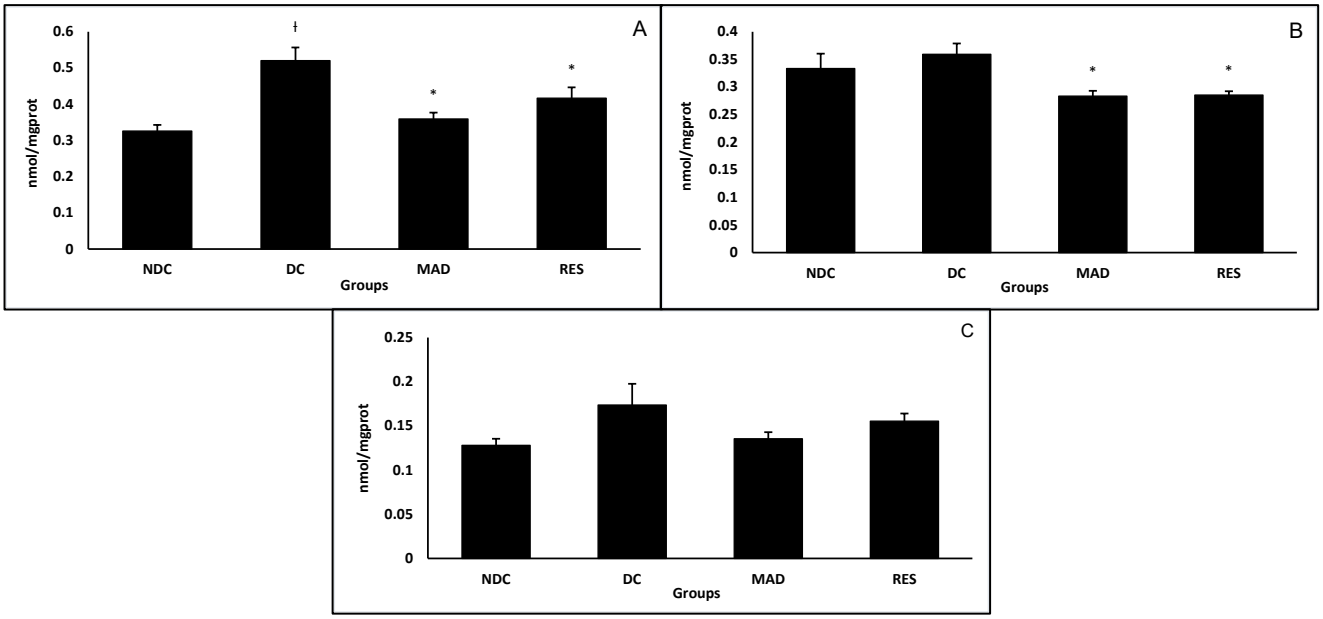

Figure 5. Effect of madecassoside and resveratrol on malondialdehyde activity of liver (A), kidney (B) and pancreas (C) of diabetic rats. Data are presented as mean ± SEM (n=7). * Denotes a significant difference compared to diabetic control ($P < 0.05$). ǂ Denotes a significant difference compared to normal control group ($P < 0.05$).

3.6 Histopathology study

3.5.1 Histology - Pancreas

Pancreatic islets in diabetic rats (Fig. 6B) were irregular in shape, and reduced in size, number and distribution as compared to the non-diabetic controls (Fig. 6A). In the pancreas

of rats treated with either madecassoside (Fig. 6C) or resveratrol (Fig. 6D) there was evidence of β-cells preservation, also with less destructive changes in the acinar cells.

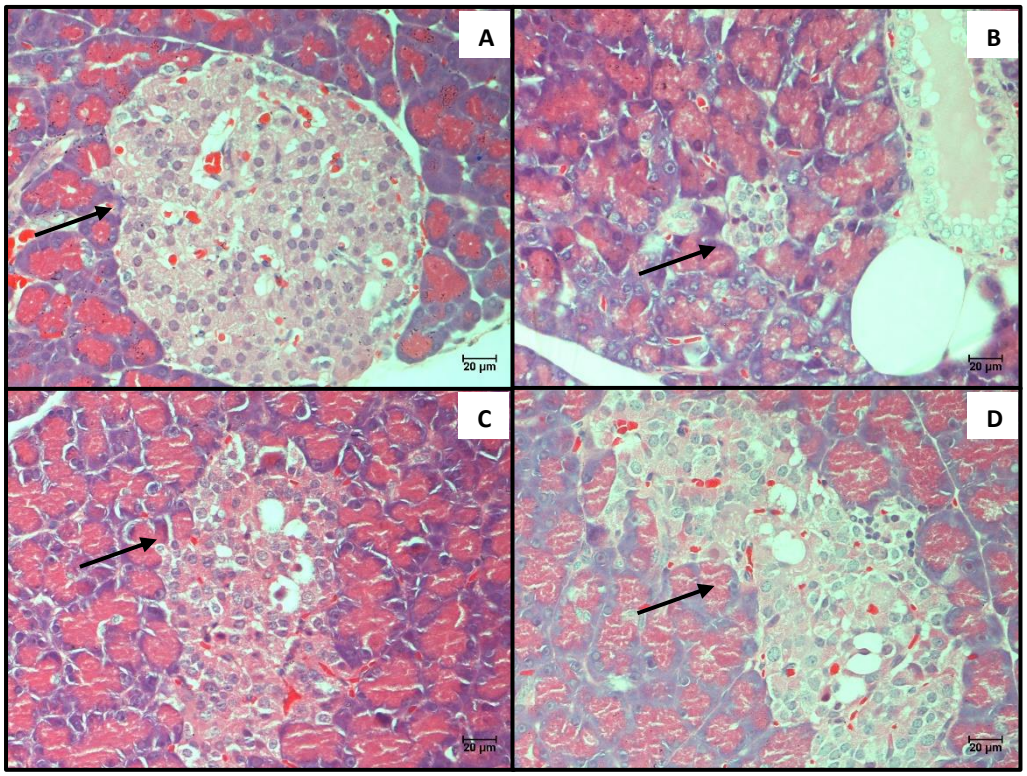

Figure 6. Photomicrograph of pancreatic sections of NDC rat (A), DC rat (B), MAD treated rat (C) and RES treated rat (D) rat under H&E stain at 100x magnification.

Quantitative analysis showed that the islet area in the pancreas of diabetic rats was significantly ($P<0.01$) reduced in comparison to that of normal pancreas (Fig. 7). The islet area in the pancreas from either madecassoside or resveratrol-treated animals was not significantly

different from that of the normal controls and significantly greater than that of the diabetic control rats. .

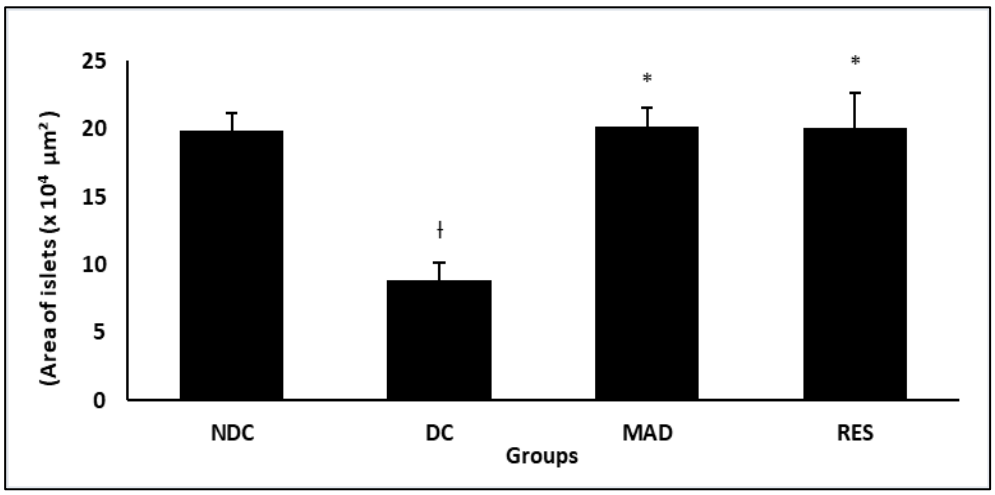

Figure 7. Quantitative analysis of islet areas. Data are presented as mean ± SEM (n=7). * P < 0.01 vs DC. ł P < 0.001 vs NDC.

3.7 Immunohistochemistry of pancreatic islets

Immunostaining of pancreatic islets for insulin in non-diabetic control rats demonstrated large, well-rounded insulin-positive β cells in the form of brown stained granules (cytoplasmic and membrane) (Fig. 8). The islets of diabetic rats showed a marked reduction in the number of β-cells with weak immunostaining for insulin. The sections from the pancreas of rats treated with either madecassoside or resveratrol showed increased numbers of β-cells in comparison to the diabetic group. The staining also highlighted the ability of the treatments to preserve islet β-cells. The mean density and mean intensity of the staining is shown in Table 3.

Table 3. The Allred Scoring System for IHC staining. The two parameters of interest were staining intensity (SI) and staining density (SD).

IHC	NDC	DC	MAD	RES
Mean density	3	1	2	2
Mean Intensity	4	1	3	3

Staining intensity (SI) was scored according to the following scale: no visible staining = 0, weak staining = 1+, moderate staining = 2+, and intense staining = 3+.

Staining density (SD) for each antibody was semi-quantified into five main categories based on the percentage of cells being stained positive:

0 = no cells stained positive, 1 = <10% of the cells stained positive, 2 = 10-50% stained positive, 3 = 50-90% stained positive and 4= > 90% of the cells stained positive. The respective grades given to both SI and SD were tabulated and averaged from n=7.

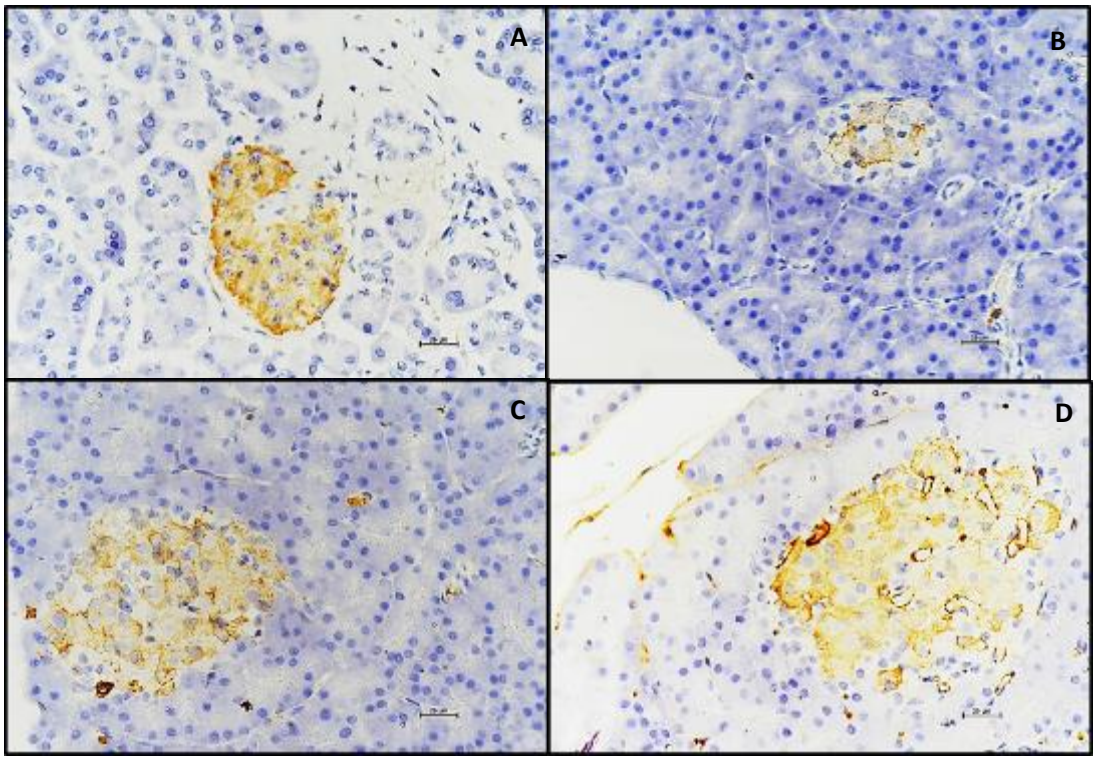

Figure 8. Photomicrographs of immunohistochemical staining of insulin in pancreatic islets of NDC rat (A), DC rat (B), MAD treated rat (C) and RES treated rat (D). Magnification: 400x.

4. Discussion

We used the streptozotocin-nicotinamide rat diabetes model. This is regarded as a model for insulin-deficient type 2 diabetes without significant insulin resistance (Furman, 2021; Nakamura et al., 2006; Tahara et al., 2008) . The diabetic rats showed marked hyperglycaemia associated with a reduction in body weight. Surprisingly, despite the marked hyperglycaemia, no significant changes were seen in the blood triglyceride, total cholesterol or LDL cholesterol in the diabetic rats; the most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Mitra et al., 1995). Loss of body weight, although not statistically significant in this study, despite significantly increased consumption of food and water is a classical feature of insulin-deficient diabetes mellitus (Pupim et al., 2005) resulting from dehydration and catabolism of fat and proteins, leading to muscle wastage (Arya et al., 2012b). Oral treatment with either madecassoside or resveratrol prevented the weight loss and reversed the increased food and water consumption seen in the diabetic animals, as reported elsewhere for treatment with extracts of antidiabetic medicinal

plants (Arya et al., 2012a; Arya et al., 2012b; Nagarajan et al., 2005; Pari and Saravanan, 2004) , including *Centella asiatica* (Kabir et al., 2014). This was associated with marked reductions in blood glucose, decreases in HbA1C and elevation in plasma insulin concentrations suggesting a marked antidiabetic effect. Our observations confirm previous findings for resveratrol (Su et al., 2006) and demonstrate for the first time the antidiabetic of madecassoside. We hypothesize that the antidiabetic effect of madecassoside is mediated through its reported antioxidant actions and lipid peroxidation reducing effects (Bian et al., 2012; Ling et al., 2017; Lu et al., 2014; Wang et al., 2018; Zhou et al., 2020) as discussed below. Resveratrol has previously been shown to attenuate hyperglycaemia-mediated oxidative stress in streptozotocin-nicotinamide induced diabetic rats (Palsamy and Subramanian, 2010).

Oxidative stress caused by persistent hyperglycaemia has been demonstrated to play a major role in the pathogenesis of T2DM (Sepici-Dincel et al., 2007); it accelerates ROS formation by glycating and inactivating the antioxidant defence enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) (Cotter and Cameron, 2003). Elevated ROS levels not only alter antioxidant defence systems, but also increase lipid peroxidation (Bansal et al., 2012), and contribute to insulin resistance (Balasubashini et al., 2004). The present study showed decreases in CAT, GSH-PX, GSH and SOD in various tissues in streptozotocin-nicotinamide diabetic rats, confirming other studies (Kakkar et al., 1995; Maritim et al., 1999; Ramar et al., 2012; Saxena et al., 1993). However, administration of madecassoside and resveratrol significantly restored the endogenous antioxidant activities in one or more tissues. Malondialdehyde (MDA) is often used as an index of tissue damage, as it is produced by degradation of lipids by ROS and causes damage to membrane components of cells and results in cell necrosis and inflammation (Evans et al., 2003; Evans et al., 2002; Nain et al., 2012). Significantly increased lipid peroxidation levels in diabetic rats and diabetic patients have been reported (Sato et al., 1979). This was observed in the present study, where MDA levels were significantly increased in the liver of diabetic rats. Oral administration of madecassoside or resveratrol effectively reduced the MDA levels in liver and kidney. These results suggest *in vivo* antioxidant activities of both madecassoside and resveratrol. This is supported by the effect of madecassoside and resveratrol in preventing the diabetes-induced elevation in plasma alanine transaminase and aspartate transaminase, further indicating the liver protective effects of madecassoside and resveratrol. The effects of resveratrol are consistent with previous studies (Ku

et al., 2011; Palsamy and Subramanian, 2010; Sadi et al., 2015; Su et al., 2006). It is postulated that elevate plasma ALT and AST indicate hepatic damage caused by diabetes induced oxidative stress (Bedi et al., 2019; Harris, 2005). To what extent these effects of madecassoside are due to a direct antioxidant action or are secondary to the markedly reduced blood glucose, which would itself reduce oxidative stress, remains to be determined.

Impairment of β-cell function in diabetes is caused at least in part by chronic oxidative stress and inflammation as a result of glucotoxicity and lipotoxicity (Montane et al., 2014). The pancreatic islets of diabetic control rats showed obvious β-cell damage with degenerative changes, along with reduced numbers. This finding is consistent with other studies which reported shrunken islets, as well as degenerated and reduced β-cells in diabetes induced by STZ in rats (Arya et al., 2014; Fujita et al., 2008; Motshakeri et al., 2014; Palsamy and Subramanian, 2011; Roat et al., 2014). Interestingly, treatment with madecassoside for 28 days substantially increased the numbers of pancreatic islets as evidenced by visual microscopic inspection; moreover, the total islet area in pancreas from madecassoside or resveratrol-treated diabetic rats was restored to that of normal controls. Histopathology examinations also revealed reduced β-cell degeneration with lesser destructive changes in the acinar cells. Furthermore, immunohistochemical analysis of islets showed enhanced insulin immunoreactive expression provides additional evidence for reduced β-cell degeneration and β-cell preservation in the treated diabetic rats. Thus, there is considerable support for the hypothesis that madecassoside may protect and preserve pancreatic β-cells while possibly also stimulating their regeneration, resulting in augmented insulin secretion. Whether these effects on the islets are related to antioxidant effects remains to be determined. The increased levels of the antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase in the pancreas of madecassoside-treated diabetic rats compared with the diabetic controls, may not be reflected in corresponding changes in the islets, as islet tissue makes up a relatively small percentage of the pancreas.

5. Conclusion

The present investigation shows that madecassoside improves glycaemic control, increases plasma insulin concentrations and improves pancreatic β-cells structure and function in STZ–nicotinamide diabetic rats. The protective effect of madecassoside on pancreatic β-cells is

associated with the ability of madecassoside to enhance antioxidant status and protect against lipid peroxidation. These findings support madecassoside as a potential antidiabetic agent. The mechanism underlying the effect of madecassoside in increasing glucose clearance in normal rats remains to be determined.

Author Contributions

Conceptualization; Methodology; Funding acquisition - Ramkumar Rajendran, Subrat Kumar Bhattamisra, Purushotham Krishnappa, Amalraj Fabian Davamani, Ebenezer Chitra, Stephen Ambu, Brian Furman and Mayuren Candasamy; **Data curation; Formal analysis; Project administration; Supervision; Investigation**; **Validation** – Swee Ching Tan, Ramkumar Rajendran, Subrat Kumar Bhattamisra, Purushotham Krishnappa and Mayuren Candasamy; **Writing - original draft** – Swee Ching Tan; **Writing - review & editing** - Swee Ching Tan, Brian Furman and Mayuren Candasamy. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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List of Abbreviations

DM	Diabetes mellitus
β-cells	Beta cells

USA	United States of America
Inc	Incorporation
BD	Becton, Dickinson and Company
MDA	Malondialdehyde
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
SOD	Superoxide dismutase
CAT	Catalase
h	Hour
°C	Degree Celsius
i.v.	Intravenous
STZ	Streptozotocin
M	Molar
i.p.	Intraperitoneal
min	Minutes
%	Percent
p.o.	per oral
rpm	rotations per minute
HbA1c	Glycated haemoglobin
CHOL	Total cholesterol
TG	Triglycerides
LDL	Low density lipoprotein
HDL	High density lipoprotein
AST	Aspartate transaminase
ALT	Alanine transaminase
TBARS	Thiobarbituric acid reactive substances
µM	Micrometre
EM	Standard error mean
SPSS	Statistical Package for Social Science
ANOVA	One-way analysis of variance
Fig.	Figure
DC	Diabetic control group
NDC	Non-diabetic control group
MAD	Madecassoside treated group

RES Resveratrol treated group