Antidiabetic effects of some medicinal plant extracts in rats with chemically induced diabetes mellitus

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ABSTRACT

Medicinal plants have been recommended for the treatment of diabetes mellitus in Sri Lanka since antiquity. The study investigated the antidiabetic effects of selected medicinal plant extracts in alloxan induced and streptozotocin induced diabetic rats. Medicinal plants selected for the investigation were *Spondias pinnata* (Ambarella, Anacardiaceae), *Kokoona zeylanica* (Kokun, Celastraceae), *Coccinia grandis* (Kowakka, Cucurbitaceae), *Momordica charantia* (Wal-kariwila, Cucurbitaceae), *Sida alnifolia* (Kotikan-babila, Malvaceae), *Syzygium caryophyllatum* (Heen-dan, Myrtaceae), *Nyctanthes arbor-tristis* (Sepalika, Oleaceae), *Scoparia dulcis* (Wal-kottamalli, Scrophulariaceae), *Gmelina arborea* (Et-demata, Verbenaceae) and *Languas galanga* (Heen-aratta, Zingiberaceae). Aqueous extracts were used for all *in vivo* experiments.

The efficacy of acute hypolgycaemic and antihyperglycaemic activities of ten aqueous plant extracts for a range of doses (0.25-2.00 g/kg b.wt.) were evaluated by the improvement on glucose tolerance in healthy, alloxan induced and streptozotocin induced diabetic rats. The results revealed that the extracts of *S. pinnata*, *C. grandis*, *M. charantia*, *S. dulcis* and *G. arborea* possess significant hypoglycaemic effects in healthy rats ($p< 0.05$). In addition, all the ten plant extracts showed highly significant dose dependent antihyperglycaemic effects in diabetic rats ($p< 0.05$).

On the basis of results of the above investigations, the bark extract of *S. pinnata*, leaf extract of *C. grandis* and bark extract of *G.arborea* were selected at their optimum effective doses for the investigation of antidiabetic mechanisms in diabetic rats. An attempt was also made to study the toxicological effects of the three selected plant extracts at their optimum effective doses in healthy Wistar rats.

The effect of administration of the selected plant extracts of *S. pinnata*, *C.grandis*, *G. arborea* on the concentration of serum/blood glycaemic parameters, concentration of serum lipid parameters, concentration/activities of hepatic oxidative stress markers and regenerative potency of islet cells in the pancreas were investigated in alloxan and streptozotocin induced diabetic rats. Glibenclamide was used as the standard antidiabetic drug. The long term antihyperglycaemic effect was in the decreasing order of *C. grandis*, *G. arborea* and *S. pinnata* in diabetic rats. The leaf extract of *C. grandis* demonstrated a decrease in the percentage of glycated hemoglobin (35%, 33%), fructosamine (30%, 32%), an increase in insulin (72%, 78%), C-peptide (51%, 60%) in alloxan induced and streptozotocin induced diabetic rats respectively ($p< 0.05$). The increase in the concentrations of serum insulin and C-peptide and histopathology on H & E stained sections in plant extracts treated rats indicated the formation of functional islet cells, biosynthesis of insulin which was further confirmed through immunohistochemical assessments.

The results of this study confirm that the aqueous bark extract of *S. pinnata*, leaf extract of *C. grandis* and bark extract of *G. arborea* possess *in vivo* antioxidant activities. None of the extracts at their optimum effective dose produced toxic effects *in vivo*. Increased biosynthesis of insulin along with increased production of C-peptide coupled to β-cell regeneration in the pancreas of plant extracts treated diabetic rats suggest pronounced pancreatic mechanisms, although extra pancreatic effects cannot be ruled out. The secondary metabolites mainly as polyphenol compounds, flavonoids present in these plant extracts may be responsible for the antidiabetic effects in diabetic rats.

The study was performed at Dept. of Biochemistry and Dept. of Pathology, Faculty of Medicine, University of Ruhuna, Sri Lanka. The results were published in ten original research papers in peer reviewed indexed journals. In addition, 18 abstracts were presented in national and international forums. The study received four outstanding research awards including the Vice chancellor's Gold Medal for the excellent performance during the post graduate study in generating Knowledge that has accepted internationally.

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Introduction

Discovery of natural antidiabetic agents for the treatment of diabetes mellitus has been greatly intensified during the past few decades. Despite the great strides made in understanding and management of diabetes, the disease and related complications are increasing unabated due to multiple defects in its pathophysiology (1,2). However, the appreciation and scientific interest on the study of antidiabetic medicinal plants have gained much attention due to their scientifically proven health benefits (3).

The discovery and exploitation of active principle (s) of natural antidibetic agents is a key focus in scrutinizing therapeutic benefits of medicinal plant extracts and in developing novel pharmaceuticals for the treatment of diabetes mellitus.

Ethnopharmacological evidence has shown that, medicinal plant extracts are found to be useful against hyperglycaemia, dyslipidaemia and oxidative cellular damage, caused in diabetes mellitus (4,5). Besides, such medicinal plant extracts may offer a unique therapeutic option for the prevention and/or treatment of diabetic complications. The justification for the therapeutic use of natural antioxidants in diabetes is also emerging fast (6,7,8). Hence, there is a huge prospect in search of medicinal plant extracts with potential antihyperglycaemic, antihyperlipidaemic and antioxidative activities to combat diabetic complications. Medicinal plants selected for the investigation were *Spondias pinnata* (Ambarella, Anacardiaceae), *Kokoona zeylanica* (Kokun, Celastraceae), *Coccinia grandis* (Kowakka, Cucurbitaceae), *Momordica charantia* (Wal-kariwila, Cucurbitaceae), *Sida alnifolia* (Kotikan-babila, Malvaceae), *Syzygium caryophyllatum* (Heen-dan, Myrtaceae), *Nyctanthes arbor-tristis* (Sepalika, Oleaceae), *Scoparia dulcis* (Wal-kottamalli, Scrophulariaceae), *Gmelina arborea* (Et-demata, Verbenaceae) and *Languas galanga* (Heen-aratta, Zingiberaceae). The objective of the study was to investigate the antidiabetic effects of the selected plant extracts in rats with chemically induced diabetes mellitus.

Materials and methods

Chemicals

D-glucose, glibenclamide, alloxan monohydrate and streptozotocin were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). A UV visible spectrophotometer (Gallenkamp PLC, UK) and microplate reader (Mindray, China) were used for spectrophotometric and enzyme linked immunosorbant assay (ELISA) measurements respectively. Olympus CX 21(Japan) microscope was used in the assessment of histopathology and immunohistochemistry of the pancreatic tissues.

Selected plant parts were cut into small pieces, dried at 40°C until a constant weight was reached and coarsely ground. Powdered plant material (20.00 g) was dissolved in 60 mL distilled water and refluxed for four hours. The mixture was strained though a cheesecloth and adjusted the final volume to 10 mL to prepare the highest dose (2.0 g/kg). Plant extracts were prepared daily prior to the administration.

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 (Temperature 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 commencement of 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 Ethical Review 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.

Dosage and administration of the drugs

A graded range of doses 0.25, 0.50, 0.75, 1.00, 1.25, 2.00 g/kg b. wt. was selected including the extrapolated human therapeutic dose of each extract for screening of ten plant extracts for hypoglycaemic, antihyperglycaemic activities in healthy and diabetic rats (9). Glibenclamide at a dose of 0.50 mg/kg b.wt.was used as the standard drug in diabetic rats (10). A volume of 0.20 mL of the plant extract or glibenclamide was administered to a rat of average weight of 200 g orally via stomach tube.

Induction of diabetes mellitus in rats

Alloxan induced diabetic rat model

Alloxan monohydrate dissolved in sterile saline at a dose of 150 mg/kg b.wt. was administered intraperitonially to fasted rats (11).

Streptozotocin induced diabetic rat model

Streptozotocin dissolved in citrate buffer (0.1M pH 4.4) at a dose of 65 mg/kg body weight was administered intraperitonially to fasted rats (12).

The rats were maintained on 5% w/v D-glucose solution for the next 24 hours and fed with a standard diet with free access to water. Rats were allowed to stabilize for three days. Blood samples were drawn from tail tip on the fourth day and concentration of fasting blood glucose was estimated. Rats with fasting blood glucose 12.0 mmol/L or above were considered as hyperglycaemic and used for experiments (13).

Effect of plant extracts on oral glucose tolerance in healthy rats

Fasted healthy rats were randomly divided into seven groups (n=6/group). The first group served as the untreated healthy control group and received distilled water. Group two to seven consisted of six sub groups for the graded dose range (a-f); healthy rats received a dose range of 0.25-2.00 g/kg of plant extract. The procedure was carried out for ten aqueous plant extracts.

Effect of plant extracts on oral glucose tolerance in diabetic rats

The experiment was carried out in alloxan induced diabetic rats (150 mg/kg b.wt., ip) and streptozotocin induced diabetic rats (65 mg/kg, ip) separately.

Fasted diabetic rats were randomly divided in to eight groups. The first group which served as the diabetic control group, received distilled water. Group two to seven consisted of six sub groups for the graded dose range (a-f); diabetic rats received a dose of 0.25, 0.50, 0.75, 1.00, 1.25, 2.00 g/kg b.wt. of the plant extract. The eighth group was administered glibenclamide (0.50 mg/kg b.wt.) which served as the positive control. The same procedure was carried out for selected ten plant extracts.

The rats of all test groups were given an oral dose of glucose (3.00 g/kg b.wt.) 30 minutes after the administration of the plant extract. Blood samples were collected just prior to administration of the extract/drug (FBG) first, second, third and fourth hours after the administration of glucose load subsequently. Blood glucose concentration was estimated immediately using a spectrophotometric enzyme assay kit method (14). The acute hypoglycaemic and antihyperglycaemic activities of medicinal plant extracts were evaluated by improvement on glucose tolerance over a four hour period using the area under the oral glucose tolerance curve (15,16).

On the basis of the results obtained, three medicinal plant extracts were selected at their optimum effective doses for further investigation of antidiabetic mechanisms in diabetic rats.

Effect of plant extracts on clinical signs of toxicity in healthy rats

Acute toxicity testing was performed for the aqueous bark extract of *S. pinnata*, leaf extract of *C. grandis* and bark extract of *G. arborea* following the Organization for Economic Cooperation and Development (OECD) guideline 420, fixed dose procedure (17).

Sub-acute toxicity assessment

Experimental design

Healthy male Wistar rats (200±25g, 10-12 weeks of age) were allotted to four groups (n=6/group), considering the average weight of animals. The first group served as the untreated healthy control group, received distilled water daily. Wistar rats in the second, third and fourth groups received the aqueous bark extract of *S. pinnata* (1.00 g/kg b.wt.), leaf extract of *C. grandis* (0.75 g/kg b.wt.) and bark extract of *G. arborea* (1.00 g/kg b.wt.) daily for 28 days. The fasted animals (12h) were sacrificed on the 28th day of the experiment. Blood samples were collected for biochemical and haematological assessments. The heart, lung, small intestine, liver, spleen, pancreas and kidney were excised for assessment of the relative weight of organs and histpathological changes on haematoxylin and eosin sections.

Effect of plant extracts on blood/serum glycaemic parameters in diabetic rats

The percentage of glycated haemoglobin; HbA_{1C}, concentration of serum fructosamine were estimated in all rats using spectrophotometric enzyme assay kits (18,19). Furthermore, the concentration of insulin and C-peptide were estimated in rats using enzyme linked immuno-sorbant assay methods (20).

Effect of plant extracts on serum lipid parameters in diabetic rats

The concentration of serum total cholesterol; TC, high density lipoprotein cholesterol; HDL-C, triacylglycerol; TAG were estimated in all rats using spectrophotometric enzyme assay kits (21,22,23). The concentration of serum low density lipoprotein cholesterol; LDL-C, very low density lipoprotein cholesterol; VLDL-C were calculated using the Friedewald formulae (24).

Effect of plant extracts on serum hepatic enzymes and hepatic antioxidative stress markers in diabetic rats

The serum activities of alanine aminotransferase; ALT, aspartate aminotransferase; AST, alkaline phosphatase; ALP in all rats were estimated using spectrophotometric enzyme assay kits (25, 26). The concentration of reduced glutathione; GSH, malonaldehyde; MDA in liver cell homogenate was estimated (27,28). In addition, concentration of glutathione reductase; GR, glutathione peroxidase; GPx and glutathione S-transferase; GST in liver cytosolic fraction of all rats were estimated (29,30). Furthermore, histomorphological changes on haemotoxylin and eosin stained sections of liver tissue of rats were determined using light microscopy.

Effect of plant extracts on histology of the pancreas through histopathological and immunohistochemcial assessments in diabetic rats

Paraffin embedded tissue blocks of the pancreas were used for detailed histopathological and immunohistochemical assessments. Immunohistochemical staining was done to confirm the regeneration/presence of insulin secreting β-cells in the islets of pancreas in all rats. Dako Polyclonal Guinea Pig anti-insulin and Dako REAL ™ En Visison™/HRP, Rabbit/Mouse were used for immunohistochemical staining (31).

Statistical data 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 histopathological score values. Results were considered to be significant at $p< 0.05$.

Results

Oral glucose tolerance test in healthy and diabetic rats

The results revealed that the extracts of *S. pinnata*, *C. grandis*, *M. charantia*, *S. dulcis* and *G. arborea* showed significant hypoglycaemic effects in healthy rats ($p<0.05$). The percentage improvement on glucose tolerance at the optimum effective dose was as follows; *M. charantia* (0.75g/kg: 9%), *C. grandis* (0.75 g/kg: 8%), *S. pinnata* (1.00 g/kg: 8%), *G. arborea* (1.00 g/kg: 8%) and *S. dulcis* (1.00 g/kg: 7%).

The plant extracts exerted statistically significant improvement on glucose tolerance at the optimum effective dose in alloxan and streptozotocin induced diabetic rats as follows. *S.pinnata* (1.00 g/kg: 27%, 29%), *K. zeylanica* (1.00 g/kg: 18%, 19%), *C. grandis* (0.75 g/kg: 32%, 33%), *M. charantia*(0.50 g/kg: 37%, 39%), *S. alnifolia* (1.00 g/kg: 21%, 25%), *S. caryophyllatum* (1.00 g/kg: 19%, 20%), *N. arbortristis* (1.00 g/kg: 17%, 20%), *S. dulcis*(1.00 g/kg: 25 %, 25 %), *G. arborea* (1.00 g/kg: 29 %, 31%), *L. galanga* (1.25 g/kg: 18%, 1.00 g/kg: 19%). The improvement on glucose tolerance was 39% and 41% in glibenclamide treated alloxan induced and streptozotocin induced diabetic rats respectively.

Assessment of toxicity

The *acute toxicity study suggests that aqueous* bark extract of *S. pinnata*, leaf extract of *C.grandis* and bark extract of *G. arborea* are *safe in healthy Wistar rats up to a dose of 2.00 g/kg.* The oral administration of the above three extracts to rats for 28 days were not associated with adverse effects reflected in the general condition, growth, relative weight of organs, clinical biochemical, haematological values and more importantly did not result histopathological abnormalities. *Therefore, extracts of S. pinnata* (1.00 g/kg b.wt.), *C. grandis* (0.75 g/kg b.wt.) and *G. arborea* (1.00 g/kg b.wt.) were found to be toxicologically safe for further investigation of antidiabetic mechanisms in rats.

Effect of plant extracts on biochemical parameters in diabetic rats

The diabetic rats treated with the three plant extracts exhibited a remarkable glycaemic control as evident by a reduction in the percentage of HbA_{1C}. The reduction in the percentage of HbA_{1C} was in the decreasing order of *C. grandis* (35%, 33%), *G. arborea* (31%, 30%) and *S. pinnata* (29%, 25%) in alloxan induced and streptozotocin induced diabetic rats ($p< 0.05$). However, the glibenclamide treated diabetic rats demonstrated a fall in the percentage of HbA_{1C} in alloxan induced (42%) and streptozotocin induced (40%) diabetic rats ($p< 0.05$). The concentration of serum fructosamine, insulin and C-peptide were decreased significantly in a decreasing order of *C. grandis* (30%, 72%, 51%), *G. arborea* (25%, 44%, 44%) and *S. pinnata* (27%, 34%, 24%) in alloxan induced diabetic rats ($p< 0.05$). The order was similar in the plant extracts treated streptozotocin induced diabetic rats.

The extracts of *S. pinnata*, *C. grandis* and *G. arborea* treated alloxan induced diabetic rats showed a significant reduction in the concentration of serum TC (21%, 19%, 33%,), LDL-C (28%, 28%, 46%,), VLDL (39%, 17%, 25%,),TG (39%, 18%, 26%,) and an elevation in HDL-C (20%, 17%, 16%) on the 30th day of study ($p< 0.05$). Similarly, the streptozotocin induced diabetic rats administered with the three plant extracts demonstrated significant changes in the concentration of TC, HDL-C, LDL-C, VLDL-C, TG at the end of the study ($p< 0.05$). The concentration of serum TC, LDL-C, VLDL-C, TG were reduced by 36%, 43%, 40%, 38% and 32%, 38%, 49% and 48% in glibenclamide treated alloxan induced and streptozotocin induced diabetic rats respectively ($p< 0.05$). In contrast, there was no significant change in the concentration of serum HDL-C with the glibenclamide treatment in diabetic rats ($p>0.05$).

The 30 day treatment with the plant extracts restored the activities of hepatic enzymes, concentration of GSH, MDA and activities of antioxidant enzymes towards normal values in diabetic rats. The highest elevation of total antioxidant capacity was obtained by *G. arborea* extract treated alloxan induced and streptozotocin induced diabetic rats, indicated through an increase in the concentration of GSH (29%, 44%), activities of GR (49%, 49%), GPx (23%, 86%), GST (68%, 57%) and a reduction in the concentration of MDA (44%, 27%) ($p<0.05$). Further the extract of *G. arborea* reduced the hepatic enzyme activities of ALT (38%, 29%), AST (25%, 23%) and ALP (28%, 29%) markedly in alloxan and streptozotocin induced diabetic rats respectively ($p<0.05$). A significant reduction in hepatic concentration of GSH and activities of antioxidant enzymes in *S. pinnata* and *C. grandis* treated diabetic rats was also noted ($p< 0.05$).

Effect of plant extracts on histopathological and immunohistochemical parameters in diabetic rats

The alloxan and streptozotocin induced untreated diabetic rats showed an extensive destruction of islet cells as compared with the sections of pancreas from healthy control rats (Plate 1). Immunohistochemical staining with anti-insulin antibody confirmed a

marked reduction in insulin secreting cells in small, average and large size islets in the two diabetic rat models ($p< 0.05$). There was an increase in the number of islets in plant extracts treated diabetic rats with compared to untreated diabetic control rats (Plate 1). 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 and streptozotocin induced diabetic rats. Further the *C. grandis* extract produced a significant increase in mean profile diameter in small (118%, 111%), average (10%, 6%), and large (13%, 16%) islets as compared with alloxan induced and streptozotocin induced diabetic control rats respectively. However, a statistically significant increase in the islet profile diameter was shown only in average (2%, 8%) and large (5%, 7%) islets in the *G. arborea* extract treated rats and large islets (5%, 5%) in *S. pinnata* extract treated diabetic rats ($p< 0.05$). There was a no change in the islet profile diameter of glibenclamide treated alloxan induced and streptozotocin induced diabetic rats ($p> 0.05$).

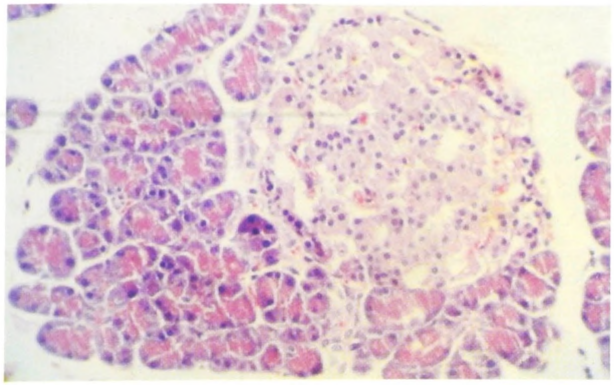
Discussion

Investigation of antidiabetic effects 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 the therapy of diabetes mellitus based on scientific investigations. However, despite the recent advances made in medicinal plant research, gaining more insight into the antidiabetic mechanisms of medicinal plant extracts, isolation of active agents from such extracts remain a challenge in current ethnopharmacological research (32). The study was aimed at screening of medicinal plants for hypoglycaemic and antihyperglycaemic activities and investigating the long term antidiabetic effects highlighting the potency of the extracts to induce β-cell regeneration in the pancreas of alloxan induced and streptozotocin induced diabetic rats.

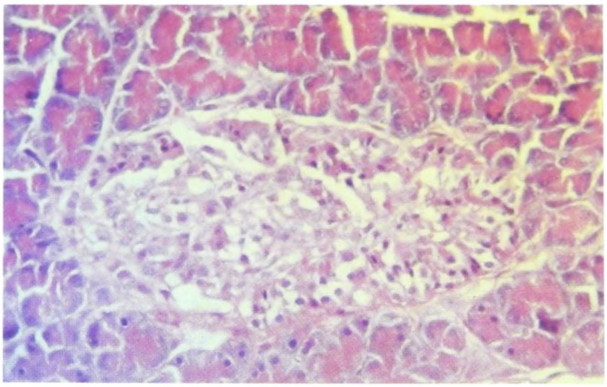
Animal models in diabetes research are very common where rodents are a good choice due to their smaller size, ease in handling, omnivorous nature and non-wild tranquil behavior. Chemically induced diabetic models are widely used in ethnopharmacological research due to low cost, wide availability, easy induction of diabetes and easy maintenance in laboratory settings. Laboratory animal models of diabetes mellitus allow investigation of mechanistic studies of antidiabetic agents, including the direct examination of tissue to assess pathology that is difficult or impossible to perform in human studies. Accordingly both alloxan and streptozotocin induced non- insulin dependent diabetic models were used in the present study.

The initial key event in drug discovery and development of new antidiabetic agents is determination of efficacy and safety in order to identify those that have an appropriately balanced safety-efficacy profile for a given indication. The plant extracts in a graded range of doses were used and hypoglycaemic and antihyperglycaemic effects were evaluated by the improvement on glucose tolerance for a period of four hours. The human therapeutic dose of each extract (which was used by the traditional Ayurvedic practioners) was extrapolated to rats in computing the dose range in the preliminary investigation (9). The range of doses was selected including the extrapolated human dose of each extract. The ten plant extracts investigated showed statistically significant dose dependent antihyperglycaemic effects and did not cause severe hypoglyacemic effects in diabetic rats ($p< 0.05$).

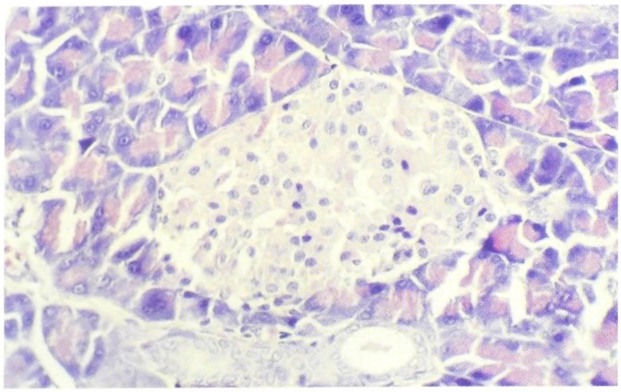
The bark extract of *S. pinnata*, leaf extract of *C. grandis* and bark extract of *G. arborea* were selected for further investigation of antidiabetic mechanisms in diabetic rats on the basis of the primary effectiveness against hyperglycaemia in diabetic rats, occurrence of relatively high *in vitro* antioxidant activities, presence of high polyphenol content together with considerable flavonoid content. More importantly, immunohistochemical assessments of the pancreas after the administration of these three plant extracts have not been studied earlier in diabetic rats. Although, the *M. charantia* showed highly significant hypoglycaemic and antihyperglycaemic effects in healthy and diabetic rats ($p< 0.05$), it was not selected for detailed investigations since most of its antidiabetic effects have been previously reported (33). Experimental evidence has shown that, medicinal plant extracts are found to be useful against hyperglycaemia, dyslipidaemia and oxidative cellular damage, caused in diabetes mellitus (34,35). In view of these facts, the present study focuses on the evaluation of antidiabetic effects in medicinal plant extracts on above three aspects in diabetes mellitus.



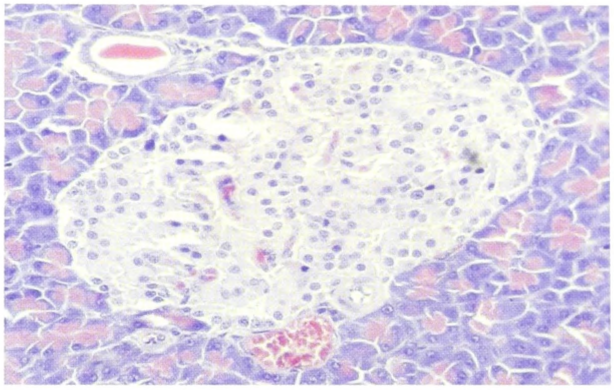
A: Healthy control rats



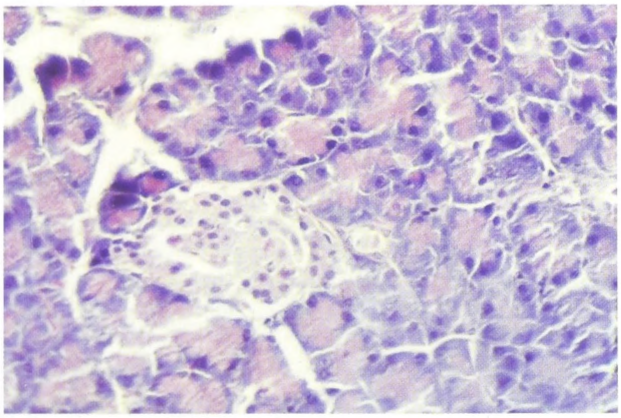
B: Streptozotocin induced diabetic control rats



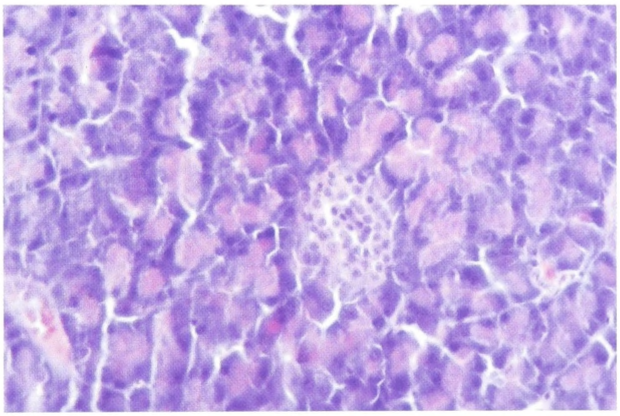
C: Streptozotocin diabetic rats + *S. pinnata* (1.00 g/kg)



D: Streptozotocin diabetic rats + *C.grandis* (0.75g/kg)



E Streptozotocin diabetic rats + *G. arborea* (1.00 g/kg)



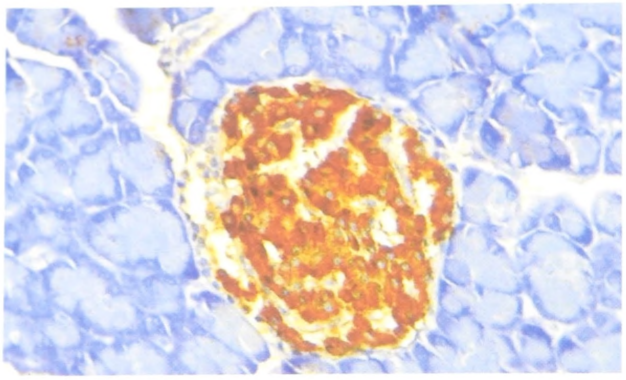
F: Streptozotocin diabetic rats + Glibenclamide 0.50mg/kg)

Plate 1 (A-F) Photomicrographs of H&E sections of the pancreatic tissue of streptozotocin induced diabetic rats after 30 days of plant treatment (x400)

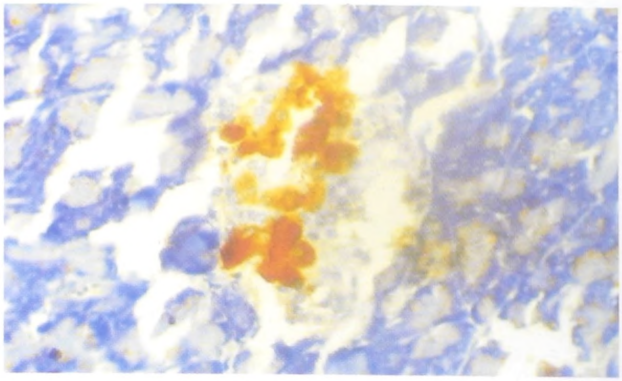
A: Islets of Langerhan are normal with a normal islet cell population B: Few preserved islet cells, fibrosis and infiltration by inflammatory cells s as a result of islet cell destruction by streptozotocin

C,E, F: Restoration of pancreatic islet cells and with no evidence of inflammation

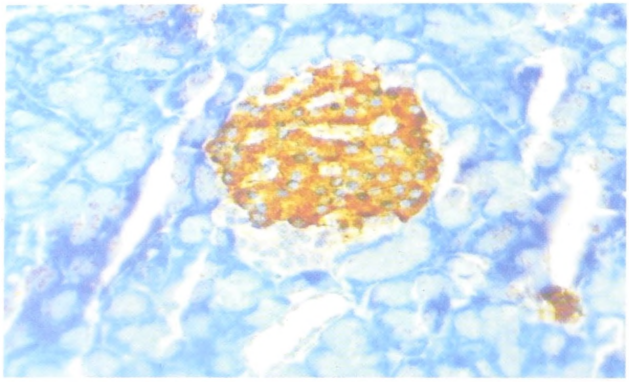
D: Pancreatic islet cells are resorted with evidence of islet cell hyperplasia



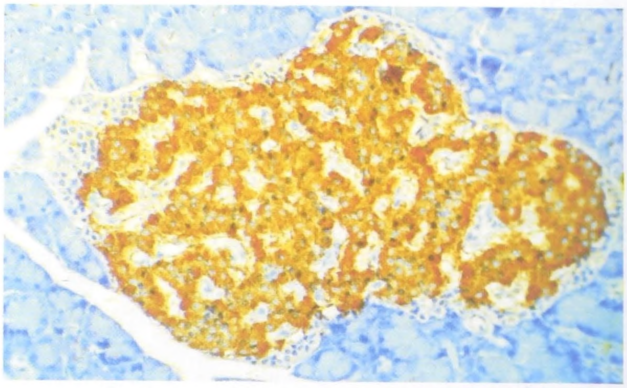
A: Healthy control rats



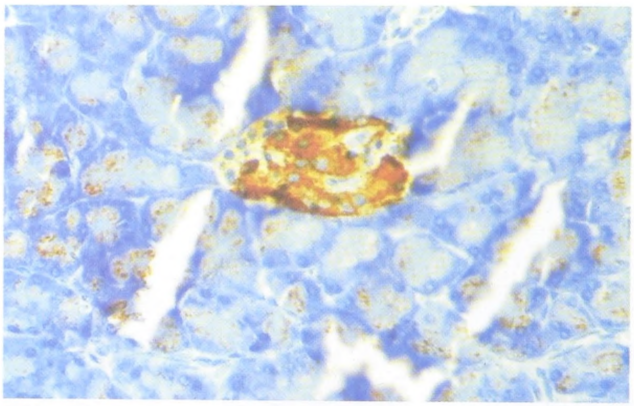
B: Streptozotocin induced diabetic control rats



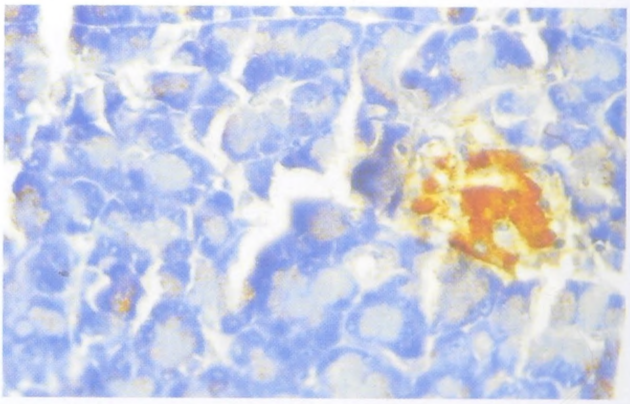
C: Streptozotocin diabetic rats + *S. pinnata* (1.00 g/kg)



D: Streptozotocin diabetic rats + *C. grandis* (0.75 g/kg)



E Streptozotocin diabetic rats + *G. arborea* (1.00 g/kg)



F: Streptozotocin diabetic rats + Glibenclamide 0.50g/kg)

Plate 2 (A-F) Photomicrographs of insulin immunoreactivity in pancreatic islets of streptozotocin induced diabetic rats after 30 days of plant treatment (x400)

A: A normal islet composed predominantly of insulin secreting β-cells B: Marked reduction in the number of β-cells due to the destruction of islet cells by streptozotocin C, E,F: Increase in insulin secreting β-cells in the islet D: A hyperplastic islet with a marked increase in β-cells.

Several studies have prompted clinical trials with antioxidants, antioxidant supplements in diabetic individuals and found beneficial effects against oxidative stress in diabetes mellitus (36). The results of the present study revealed that the selected plant extracts reduced oxidative stress and increased the concentration of antioxidant enzyme activities in diabetic rats.

The histopathological and immunohistochemical assessment of the pancreas in the present study revealed that the three extracts showed different degrees of islet cell regeneration in alloxan and 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 (37, 38). In addition, increase in the number of islets in plant extract 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 percentage increase in percentage of β-cells together with the highest increase in islet cell profile diameter in the three types of islets. The reduction in HbA_{1C} and FBG throughout the study period was higher in the glibenclamide treated rats as compared to plant extracts treated group. This could be due to antihyperglycaemic effects and glycaemic control by the well- established insulin secretary effects in glibenclamide. Although, the maximum regeneration of the pancreas was observed through histopathological and immunohistochemical assessments in plant extracts treated rats, the serum concentration of insulin, in plant extracts treated rats were lower than that in the glibenclamide treated rats. This could be due to the fact that the plant extracts were not able to induce insulin secretion or regenerated β-cells were not mature enough to show the optimum insulin secretary effects in plant extracts treated diabetic rats. The β cell maturity depends on the direction of division in β cell replication and intrinsic and extrinsic forces during the cell cycle (39). 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 increase in β-cell mass (40,41) and it could be suggested that the treatment with the three plant extracts increased the β-cells mass in diabetic rats. The islet cell regeneration in diabetic rats with the plant treatments could be due to replication of existing islet cells and differentiation (or neogenesis) from ductal or intra-islet pancreatic precursor cells (42).

Final conclusions

- The extracts of *S. pinnata*, *C. grandis*, *M. charantia*, *S. dulcis* and *G. arborea* showed significant hypoglycaemic effects in healthy rats.
- All the ten plant extracts showed highly significant dose dependent antihyperglycaemic effects in diabetic rats.
- *The extracts of S. pinnata* (1.00 g/kgb.wt.), *C. grandis* (0.75g/kgb.wt.) and *G. arborea* (1.00 g/kgb.wt.) were found to be toxicologically safe in healthy rats.
- 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* antidiabetic activities in synergistic mechanisms including increased antioxidant potential, increased biosynthesis of insulin, islet cell regeneration in the pancreas in diabetic rats. In addition, the plant extracts exerted potent antihyperlipidaemic activities in diabetic rats.

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