THE PROTECTIVE EFFECTS OF SWIETENIA MACROPHYLLA KING (SEEDS& ENDOCARPS) AQUEOUS-METHANOLIC EXTRACT ON PANCREATIC ISLETS HISTOLOGY IN STREPTOZOTOCIN–INDUCED DIABETIC RATS

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ABSTRACT

Objective: The oral antidiabetic drug, Glibenclamide, stimulates constantly the insulin producing β-cells through a harsh mechanism which eventually may reverse their endocrinofunction permanently. This study investigated the protective effects of the aqueous-methanol extract of Swieteniamacrophyllaseeds and endocarps on pancreatic islets in STZ-diabetic rats.

Methods: The experimental groups rendered diabetic by chemical combination of STZ (65 mg/kg bw, iv) and NAD (230 mg/kg bw, ip) in healthy adult rats. Diabetic rats were orally force-fed with Glibenclamide (5 mg/kg bw) or extracts (250 mg/kg bw) daily for three weeks. Body weight and FBG levels were determined at treatment intervals of 0, 7, 14 and 21 days, and, subsequently, Langerhans' islets were examined histologically.

Results: Photomicrographs of pancreatic islets shows that the administration of the extract has improved the cellular density, which suggests that the extract was capable of inducing β-cells recovery and/or regeneration, following the destructive effects of STZ. The findings indicate that Swieteniamacrophyllaking seeds and endocarps aqueous-methanolic extract exhibited protective effect on pancreatic islets and involved in the correction of altered biological parameters in diabetic rats.

Conclusion: this extract may serve as a candidate for developing a safe, compliance and promising nutraceutical product for the management of diabetes.

Keywords: Swieteniamacrophylla, Diabetes mellitus, Islet of Langerhans, STZ, Diabetic Rats.

INTRODUCTION

Diabetes mellitus is a complex heterogeneous metabolic disorder affecting nearly 4% of the population worldwide and is expected to increase by 5.4% in 2025 [1]. In experimental diabetes model, chemical induction with streptozotocin diminished insulin production and causes high levels of blood circulating glucose, which was similar as found in human diabetics [2]. The altered physiological function of the pancreas from the action of streptozotocin provides the signs of abnormalities in pancreatic islets function and morphology and is defended by an increased in oxidative stress markers reported in pancreatic islet of diabetic rats [3]. Antidiabetic medicinal plants are in general known to exert their rational means for the treatment of diabetes. Though, the action modeshall depended on the phytocomponents endowed in such plant [4] which are the natural bioactive components found in plants that work with nutrients and fibers to form an integrated part of defence system against various diseases and stress conditions [5]. Additionally, medicinal plants have the advantage over synthetic drugs due to the presence of antioxidant compounds which are important to modulate the level of oxidative stress [6]. Free radical scavenging molecules such as terpenoids, tannins, lignins, flavonoids, alkaloids, phenols and other metabolites are rich in antioxidant activity [7]. Some of these molecules are anticipated as potential hypoglycemic agent as well. Ethnopharmacological surveys indicate that more than 1200 plants are used worldwide in traditional medicine for their alleged antidiabetic activity and the lists include the Moraceae family of Swieteniamacrophyllaking [8]. Recent studies in animal models by Maiti et al. [9], Radhramani et al. [10] and Kalaiyanan&Pugalendi[11] have confirmed the antidiabetic efficacy of plant seed and endocarp separately in term of biochemical findings, yet efforts to define the histological studies and graphic evidences in this regard are not very far establish. Hence, the undertaken study aimed to investigate the protective effects of S.macrophyllaking seeds and endocarps aqueous-methanolic extract on pancreatic islets histologically in STZ-diabetic rats.

MATERIALS AND METHOD

Plant material
Swietenia macrophylla king’s seeds together with its endocarps were collected from the northern part of Malaysia where they are presumably in abundance. Subsequently, the plant parts were forwarded for taxonomical identification.

Chemicals
The solvents and other reagents used in the experiment were of analytical grade. Streptozotocin (STZ) for diabetic induction including injectable anaesthetics were procured from Sigma-Aldrich Co., USA. Glibenclamide tablets (Daonil™ Aventis) were purchased from a local pharmacy.

Extracts Preparation
The selected plant parts were dried, crushed in an electric grinder and pulverized into a coarse powder form. Out of this powder, 100 g were weighed. An aqueous-methanolic extraction was prepared by soaking 100 g of the coarse powder in a conical flask with a mixture solvent, consisting of 240 ml distilled water and 320 ml absolute methanol. The mixture was prepared according to De et al. [12] was kept in an incubator at 37 °C for 36 hours and stirred intermittently at 4 hours interval. It was then filtered and the filtrate was dried under low pressure and low temperature rotary evaporator fitted with vacuum pump. A final 23.75 g of powder was collected at the end of the process and was dissolved in normal saline in a fixed dose used for the treatment.

Preliminary Qualitative Phytochemical Screening
The qualitative phytochemical screening of the aqueous-methanol extracts from Swieteniamacrophyllaking combined seed and endocarp extracts, has been done using the method explained by Tiwari et al. [13], to reveal the presence of alkaloid, flavonoid, cardiac glycosides, reducing sugar, saponins, tannins and terpenoids.
Induction of Diabetes

Induction of type II diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted adult Sprague Dawley rats weighing (250-300)g by a single intravenous injection (i.v) of 65mg/kg STZ in citrate buffer (pH 4.5), 15 minutes after the ip administration of 230 mg/kg of nicotinamide in normal saline[14]. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 14 days after injection. The rats with permanent NIDDM (FBG > 170 mg/dl) were used for the anti-diabetic study.

Acute Oral Toxicity Studies

There were no deaths of rats with the extract treatment at doses of 2000 mg/kg, within both short and long outcomes. The different dose levels (125, 250, 500, 1000 and 2000) mg/kg of aqueous-methanolic extracts were given orally. The animals were observed for the initial 4 hours, alternately for the 60 min and once again at 24 hours following the drug administration. The LD_{50} was calculated to be greater than 2000 mg/kg.

Animal Treatment

The normoglycemic rats were randomly divided into four groups of six animals each. The extract treatments were force-fed daily for duration of 21 days at a fixed dose of 250 mg/kg. Diabetic control was done using the laboratory electrical balance and FBG level was made possible via AccuCheck Advantage blood glucometer (US, Roche Diagnostics).

At the end period of the treatment, animals were deeply anaesthetized, with an ip injection of a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg)[15], then the abdominal and chest cavities were cut opened, and the bile duct was clamped with a bulldog clamp at the Sphincter of Oddi where it enters the duodenum for the application of in situ ductal perfusion [16]. A 5ml syringe was inserted into the bile duct near the liver and 1–2 ml of distilled water slowly injected. The perfusion to all parts of the pancreas causes the inflation of the organ. At this time, it was excised with scissors and placed within a prepared Ringer’s solution. The bathed tissues were labelled accordingly and suspended in 10% buffered neutral formalin for histological processing.

Histological Examination

The specimens of pancreas were fixed in 10% formalin and processed with paraffin techniques prior to micromote sectioning (5 μm). Tissue sections were subjected to H&E staining. Histology slides observed under the microscope (Olympus DP 72) and micrographs were captured using Olympus CellSene Standard software.

Statistical Analysis

The results expressed as mean ± SEM. The significance of differences ofP value were confirmed by one-way ANOVA and multiple Dunnett’s tests, where P<0.05 is considered to have significant difference.

RESULTS

The phytochemical analysis of the aqueous-methanol extracts from SwieteniamacrophyllaKing combined seed and endocarps extracts revealed the presence of alkaloid, flavonoid, cardiac glycosides, reducing sugar, saponins, tannin and terpenoids (Table 1). The body weight measurement of animals was done using the laboratory electrical balance and FBG level was made possible via AccuCheck Advantage blood glucometer (US, Roche Diagnostics).

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Table 1: Phytochemical constitutes of Swietenias seeds and endocarps extracts

<table>
<thead>
<tr>
<th>Phytochemical Assay</th>
<th>Aqueous-Methanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) Present  (-) Absent

Table 2 and 3 showed the effects of the extract treatment on animal body weight and fasting blood glucose (FBG) level respectively. The body weight measurement of animals continued to decrease in diabetic rats (control group). The effect of aqueous-methanolic extract treatment was statistical significant on treatment interval 14 days onwards (P<0.05) compared to control. The indicated results in extract–treated group were better in managing body weight as compared to glibenclamide–treatment group. Thus, there were pattern of body weight increment in both; treatment of extract and glibenclamide, with an increment percentage of 10.1% and 6.9% respectively.

Table 2: Effect of Swietenias seeds and endocarps extracts treatment on animal body weight

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control (non-diabetic)</td>
<td>283.73±4.46</td>
</tr>
<tr>
<td>Control (diabetic)</td>
<td>270.02±7.68</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>273.30±7.82</td>
</tr>
<tr>
<td>Aqueous-Methanol Extract</td>
<td>271.48±8.82</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM; n = 6; *P<0.05 = significant; **P<0.001 = highly significant; ns = non-significant.

Result as compared with control (diabetic) group.

Table 3: Effect of Swietenia seed and endocarps extracts treatment on animal FBG level

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>FBG level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control (non-diabetic)</td>
<td>73.80±4.71</td>
</tr>
<tr>
<td>Control (diabetic)</td>
<td>185.76±4.78</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>177.12±5.74</td>
</tr>
<tr>
<td>Aqueous-Methanol E.</td>
<td>176.56±7.07</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SEM; n = 6; *P<0.001 = highly significant.

Result as compared with control (diabetic) group.
Results showed an elevation of FBG levels in studied groups (post-STZ administration). The effectiveness of aqueous-methanol extracts and glibenclamide administrations on diabetic rats were substantiated by decension of FBG levels measured on day 7, 14 and 21 ($P<0.001$). Though, the mean percentage of blood glucose levels drop between aqueous-methanol extract and glibenclamide treated groups, were 46.6% and 61.4% respectively.

The histomorphological sections of the pancreatic tissues dominantly on the islets of Langerhans slides were observed post hematoxylin-eosin (H&E) staining, (figure 1-4). The pancreatic islets of control rats appear normal throughout the 21 days of the study. The islets’ cells were seen embedded within the acinar cells. The cellular integrity and architecture were intact and no specific abnormalities in their structure were visible (Fig.1). On the contrary, STZ has led to quite severe damage on the pancreas, including reduction of pancreatic islet’s area. The acini around the islets, though seem to be in normal proportion, does not look typical. The diabetic islets were distorted from its classical round-shaped and atrophied, compared to the normal islets (Fig.2). Pancreatic islets of diabetic control group and diabetic rats treated with drug-glibenclamide showed relatively smaller sizes of islets in comparison with normal. Only few islets resembled as normal, but most of them were found to be deformed (Fig.3). Administration of Swietenia aqueous-methanolic extracts (250 mg/kg), orally fed in STZ diabetic rats, showed an improved area and the recognition of the islets of Langerhans were easier. The shrunken islets were infrequent and large proportions of intact islets cell were present with tolerable deformation, this may suggest a restoration of Langerhans islet (Fig.4).

DISCUSSION

This study has conducted to evaluate the protective effects of Swietenia macrophylla King (seeds and endocarps) aqueous-methanol extract on pancreatic islets of Langerhans histology. We are the first team who use this combination of Swietenia’s seeds and endocarps to evaluate their effect on diabetic state, even though few previous studies have led the way and mentioned to the hypoglycemic effect of swietenia’s seeds [9][11] and endocarps[10] but none have tested the effect of the combination of these parts before.

Streptozotocin (STZ), a structurally glucosamine derivative of nitrosourea, has been widely used for inducing experimental diabetes mellitus in rats [17]. STZ impairs glucose oxidation and decreases insulin biosynthesis and secretion [18][19][20]. It also generates reactive oxygen species that contribute to DNA fragmentation and evoke other deleterious changes in β-cells [21][22].

The modern treatments for type II Diabetes Mellitus may consist of sulfonylureas such as glibenclamide which act mainly by stimulating β-cells to secrete insulin. Glibenclamide hence interacts on the β-cell plasma membrane to allow an abrupt release of preformed insulin adjacent to the plasma membrane [23]. However, emerging evidence suggested that forcing β-cells to secrete insulin at the time when it is struggling to cope with the demands of obesity and insulin resistance may cause the cells to become exhausted and die, secondarily activating autoimmunity. While the β-cells break apart in the process of cell
suicide, an overabundance of apoptotic bodies associated to the cells builds up in the body, triggering an immunoreactivity that attacks the still living β-cells, which may destroy them as well. Consequently, the stress of type II diabetes on β-cells may lead to the development of type 2 Diabetes Mellitus over a long run. On the contrary, the prepared extract was expected to protect and prolong the islets’ constitutive cells lifespan.

Micrographs of normal healthy rats’ pancreas showed maintained islet area with a normal lobular architecture of the pancreas. They were also seen embedded within the acinar cells with intact interlobular connective tissue. Conversely, the pancreatic sections of diabetic rats showed marked morphological changes. The border between the endocrine and exocrine region became indistinct. The diabetic islet showed retraction from its classical round-shaped and atrophied when compared to the normal islets. These may correspond to the effect by the circulating reactive oxygen species generated by STZ on β-cells.

Histological examination also revealed that the administration of the extract showed an improvement compared to the previous morphological changes, and the border between exocrine and endocrine portions became distinct. Many islets showed an increase in their total volume, suggesting that the extract could be capable of β-cells recovery and/or regeneration following the destructive effects of STZ. The increase of total volume of cell population can be due to increase of number of cells, increase of cellular volume, or a combination of both. Skau et al. [25] has postulated that an increase in total islet volume is caused entirely by the growth of existing islets; β-cells are primary sources for the new cells. This could be achieved by intra-islet β-cell mitosis, an event that definitely occurs based on the presence of mitotic figures in intra-islet β-cells. The new cells could be derived from intra-islet stem/progenitor cells.

Ideally, there were preservation of β-cell mass together with its insulin secretory granules in diabetic rats and this shall contribute to the even potentiated efficacy of extracts. For this reason, the improved architecture of β-cells was primarily due to the remained β-cells mass protected by S. macrophylla King extracts. The finding supports the histological report of retaining islets and few degranulations of pancreatic β-cells, as found in a previous study which has employed every close species of swietenia: S. mahagon{jaq, seeds extract [26].

Increased generation of circulating reactive oxygen species primarily from STZ, the metabolism of excessive glucose and/or free fatty acid have been identified as one of the major contributor to the deterioration of pancreatic β-cells function and structure [27][28]. Due to the relatively low expression of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in the pancreas [29], the pancreatic β-cells may be highly vulnerable to damage when exposed to oxidative stress. For that reason, oxidative stress may play a major role in β-cell deterioration frequently observed in type II Diabetes Mellitus[30]. Therefore, the mechanism for the suggested β-cells recovery or regeneration may be made available from the extract’s treatment, and that protection of the pancreas against oxidative stress will consequently contribute to its hypoglycemic effect. Antioxidants have been suggested to afford protection to the pancreas against oxidative stress in diabetes mellitus [31].

The abundance phytochemicals possessed within the seeds and endocarps of Swietenia macrophylla King were hypothesized as a potential antioxidant and may exhibit an anti-inflammatory activity. Hence, they were the candidates in the protection from progressive STZ induced damages of β-cells on the islets by clearing up circulating reactive oxygen species generated by STZ to destroy β-cells and then allowing other phytochemicals of the plant to induce recovery process and regenerative activities.

On the pancreatic islet histology of glibenclamide treated rats, there were no cellular density improvements observed. This was due to the mechanism of action of the drug itself, where glibenclamide only exhibits stimulatory effects upon pancreatic β-cells in order to forcefully enhance the endocrine release of insulin.

CONCLUSION

The overall histological studies have demonstrated a convincing outcome that S. macrophylla King (seed and endocarp) aqueous-methanol extract have shown functional protective effects on the pancreatic islet of Langerhans in STZ diabetic rats model. It advocates that the protective effects of the extract may be due to the scavenging of free radicals by its antioxidant nature. Though, the exact mechanism underlying this affair needs further validation.

REFERENCES


